PROGRAM

Immuno2023

XLVII CONGRESS OF THE BRAZILIAN SOCIETY OF IMMUNOLOGY

OURO PRETO - MINAS GERAIS | OCTOBER 02-06

XLVI CONGRESS OF BRAZILIAN SOCIETY OF IMMUNOLOGY

PROGRAM

Immuno 2023

#Immuno2023

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1st OCTOBER

Pre Course in Advanced Immunology

ROOM – Ouro Preto Theater

09:00 - 12:00 **Pre-Course 1: Flow cytometry**

- *Flow cytometry with imaging*

Andrea Teixeira (Fiocruz-MG)

- *Multiparametric analysis in flow cytometry*

Kenneth Gollob (Hospital Israelita Albert Einstein)

- *Applications of flow cytometry for diagnostics*

Olindo Assis Martins-Filho (Fiocruz-MG)

12:00– 14:00 **Lunch**

14:00 - 17:00 **Pre-Course 2: Transcriptome and imaging**

- *How to plan a fluorescence experiment: in vivo or ex vivo!*

Gustavo Menezes (UFMG)

- *Data Storytelling for Immunology*

Helder Nakaya (Hospital Israelita Albert Einstein)

2nd OCTOBER

08:50 – 14:30 **Satellite Meeting – Fio Mucosa**

ROOM – Ouro Preto Theater

08:50: Opening
09:00: The role of Translational Network of FIOCRUZ (Me. Ana Paula Cavalcanti)
09:15: Challenges for Fio-Mucosa translational network (Dr. Herbert Guedes)
09:30: Round-table for “mucosal studies” with Dr. Ana Caetano, Dr. Alda Cruz, Dr. Maria de Lourdes and Dr. Rodrigo Correa
10:40: Coffee break
11:00: Conference 1- Dr. Bernardo Reis (Rockefeller University) “Revealing Diverse Functions of γδ T Cells in Colon Cancer Pathogenesis”
Chair: Thais Moreira (Harvard University)
12:00 Questions/discussion
12:20: Lunch

14:00: Conference 2 - Dr. Angélica Viera (UFMG) " The interface between diet, gut microbiota and infections diseases "
14:40: Questions/discussion
14:50: Conference 3 - Dr. Robson Coutinho (UFRJ) "Purinergic signaling in context of mucosal inflammation"
15:30: Questions/discussion
16:00: Closing

14:00 - 17:00 **Course to Ouro Preto Community**
ROOM - Auditorium São João Del Rey

Promovendo a saúde: A importância da Vacinação na Comunidade
14:00 - 14:45 - Introdução, História da Vacinação
14:45 - 15:40 - Impactos e Importância da Vacinação
15:40 - 16:00 - Coffee-break
16:00 - 17:00 - Abordagens para maior adesão à vacinação e combate à desinformação
16:30 - 17:00 - Encerramento com atividade Interativa

18:30 – 19:00 **Opening Cerimony and Lifetime Achievement Award**
ROOM – Ouro Preto Theater

19:00 – 20:30 **Opening Conference**
ROOM – Ouro Preto Theater
Breadth of IgG Fc-mediated effector function delineates grades of clinical immunity against P. falciparum malaria
Faith Osier (Imperial College London, UK)
Chair: Ana Maria Caetano Faria (UFMG, MG, Brazil)

21:00 – 00:00 **Opening Cocktail**
ROOM – Mariana

3rd OCTOBER

08:00 – 09:00 **BD Biosciences Course**
ROOM – São João Del Rey

- Spectral and convencional flow cytometry: Discover the main differences and the flexibility to choose between them in a single equipment

Nayane Galdino

08:00 – 09:00 **Beckman Course**

ROOM – Tiradentes II

- Concept & Recent Scientific Impact of Exosomes and Extracellular Vesicles (EVs)

Anis Larbi

- Biofluids as a source of EVs and non-EVs components. Why do we want to stain EVs? Can we explore different Side Scatters?

Alfonso Blanco

09:00 – 10:00 **Distinguished Lecture 1**

ROOM – Ouro Preto Theater

Chairperson: Dario Zamboni (USP-RP)

Nuclear arms races in host-pathogen interactions

Russell Vance (University of California, USA)

10:00 – 10:20 **Coffee Break**

10:20 – 12:20 **Symposium 1: Immune response to Trypanosomatid infections**

ROOM – Ouro Preto Theater

Chairperson: Juliana Assis Gomes (UFMG, MG, Brazil)

- Exploring New Immunosweet Horizons: *Trypanosoma cruzi* Neoglycoconjugates as Vaccines and Biomarkers for Chagas Disease

Igor Almeida (University of Texas at El Passo, USA)

- Spying out T cell specificity in patients with chronic Chagas disease: towards novel T. cruzi vaccine candidates

Karina Gomez (CONICET, Argentina)

- Serological and Molecular Assessment of potential Trypanosoma cruzi infections in hospitalized parturient women on the U.S-Mexico Border

Rosa Maldonado (University of Texas at El Passo, USA)

Short Talks

ROOM – Ouro Preto Theater

- DO05 - Correlation Between Circulating Immune Molecules And Their Cardiac Gene Expression In Chronic Chagas Cardiomyopathy

Thaiany Goulart de Souza e Silva

- DO06 - SUCNR1-mediated response in CD11c+ macrophages is essential for the statement of resistance to cutaneous leishmaniasis

Stella Francy Vicente de Assunção

10:20 – 12:20 Symposium 2: Infectious diseases I

ROOM – Auditorium São João Del Rey

Chairperson: Chair: Maria Bellio (UFRJ, RJ, Brazil)

- Oxygen matters in neutrophil survival and antimicrobial functions

Benoit Marteyn (University of Strasbourg, Inserm-France)

- Type I interferon exhaustion

Elina Zuniga (UCSD- USA)

- CRUZIVAX Project. R&D+i of a prophylactic and therapeutic vaccine based ona chimeric proteinand the latest generation adjuvant cyclic-di-AMP

Emilio Luis Malchiodi(Universidad de BuenosAires, Argentina)

10:20 – 12:20 Symposium 3: Neuroimmunology

ROOM – Tiradentes I

Chairperson: Alexandre Basso (UNIFESP, SP, Brazil)

- Role of sleep disorders in immune outcomes

Daniela S. Rosa (UNIFESP, Brazil)

- Neuro-immune interactions in the gut

Daniel Mucida (Rockefeller Univ, USA)

- Immunoregulation In Sepsis: Role Of The Sympathetic Splanchnic Nerves

Alexandre A. Steiner (ICB – USP, Brazil)

- Emotional and social aspects of inflammation: molecular studies in mice

David Engblom (Linkoping University, Sweden)

12:20 – 15:00 Lunch

14:00 – 15:00 BD Symposium

ROOM – São João Del Rey

- Innovative applications in image-based and high dimensional spectral flow cytometry

Robert Balderas (VP Biological Science)

14:00 – 15:00 Art & Science

ROOM – Auditorium Teatro

- Art and Immunology: A long-life loving relationship

Marta de Menezes

15:00 – 17:00 Workshop 1: Antiviral Immunity

ROOM – Ouro Preto Theater
Chairperson: Juliano Bordignon (Fiocruz-Curitiba, Brazil)

- Changes in neutrophil and dendritic cell function during infection with Dengue and Zika virus

Pryscilla Wok (Fiocruz-Curitiba, Brazil)

- Usutu virus pathogenesis in mice: from neurological disease to pregnancy

Rafael Elias (CNPEM, SP, Brazil)

- Flavivirus overcome translational stress to enhance replication

Daniel Mansur (UFSC, Brazil)

- Immunity to Emerging Viruses: SARS-CoV-2 and MPOX

Carolina Lucas (Yale University, USA)

15:00 – 17:00 Workshop 2: Infectious Disease II

ROOM – Auditorium São João Del Rey
Chairperson: Ken Gollob (IAE-SP, Brazil)

- γδ T cell dichotomy on murine cutaneous leishmaniasis

Herbert Guedes (FIOCRUZ-RJ, Brazil)

- Chimeric Polyepitop Vaccine Administered By Microneedles Induced Central, Effector And Resident Memory And Protected Against Visceral Leishmaniasis

Alexandre Reis (UFOP, MG, Brazil)

- Interleukin 4-induced gene 1 (IL-4i1) in cutaneous leishmaniasis

Ramona Hurdayal (Cape Town University, South Africa)

- Pathogenesis and prognosis markers of post-acute syndrome in Chikungunya

Viviane Boaventura (FIOCRUZ-BA, Brazil)

15:00 – 17:00 Workshop 3: Inflammation and Tissue Repair

ROOM – Tiradentes I
Chairperson: Larissa Cunha (FMRP-USP, SP, Brazil)

- Handling the corpse: how metabolism shapes dendritic cell activation during efferocytosis of MRSA-infected

Alexandra I. Medeiros (UNESP, Brazil)

- Mechanisms of necrotic cell debris clearance from injury sites

Pedro E. Marques (Universiteit Leuven, Belgium)

- Effector responses to cell death: principles of resolving and non-resolving injuries

Sourav Ghosh (Yale University, USA)

- Remodeling of tissue-resident macrophage upon metabolic dysfunction requires BLIMP-1/Prdm1

Gislaine Martins *(Cedars-Sinai Medical Center, USA)*

15:00 – 17:00 Workshop 4: Immunological Axis

ROOM – Tiradentes II
Chairperson: Gustavo Menezes (UFMG, MG, Brazil)

- The MerTk receptor is important in regulating lung immune cell populations following acute exposure to cigarette smoke and for determining the outcome of pneumococcal infection

Alessandra Filardy (UFRJ, Brazil)

- Defining the role and potential for therapeutic targeting of microbiomes in respiratory disease

Philip M. Hansbro (University of Technology Sydney, Australia)

- Key roles of gamma-delta T cells in regulating the gut-brain axis in health and disease

Rafael Rezende (Harvard University, USA)

Short Talks
ROOM – Tiradentes II

- 1427 DO07 - Th17 axis activation in the immune response against Cryptococcus gattii and the influence of IL-22 and IL-23 cytokines in the systemic experimental infection

Israel Diniz Lima

- 1910 PD02 - Comparing the impact of different diets in the gut-lung axis by evaluating changes in the microbiota and macrophages's function

Izabela Galvão

17:00 – 17:20 Coffee Break

17:20 – 18:00 Bright Sparks in Immunology 1

ROOM – Ouro Preto Theater

Chairperson: Carlos Rodrigo Zarate Blades (UFSC, SC, Brazil)

- 1303 IC01 - Inflammatory monocyte recruitment and profiling in experimental models susceptible and resistant to cerebral malaria

Mônica Lucas Ribeiro de Almeida

- 1503 PD03 - A potential metabolic and immunoregulatory role of antigen B lipoprotein in Echinococcus granulosus biology: similarities and differences with plasma HDL

Maite Folle

- 1953 IC02 - Implications of eosinophil deficiency in severe experimental malaria: increased susceptibility associated to uncontrolled parasitemia and imbalanced cytokine profile

Luiza Pinheiro Silva

- 1440 IC08 - Immunological Tour: A Play About ImmunityAgainst Viruses

Artur Vitor Cavalcante Menezes

- 1867 DO13- Putative role of non-canonical autophagy machinery in the control of HiF-1α function

Douglas dos Santos

- IC17 - 1320 - Application of the Chagas-Flow ATE IgG1 serology for post-therapeutic monitoring and genotype-specific diagnosis of Chagas disease

Carolina Malheiros Araújo Silvestrini

17:20 – 18:00 Bright Sparks in Immunology 2

ROOM - Auditorium São João Del Rey
Chairperson: Heitor Affonso De Paula Neto (UFRJ, RJ, Brazil)

- 1470 DO08 - The antigen effect over immune response modulation by IFN-γ-stimulated-FRCs derived from human lymph nodes

Bianca de Oliveira Ferreira

- 1475 DO09 - Ultra-Fast Protocol For Car-T Cell Generation

Luiza de Macedo Abdo

- 1795 DO10 - Single-cell atlas reveals immunophenotypes associated with clinical outcome and enrichment of CD8 memory cells in adjacent normal tissue of pancreatic cancer

Gabriel Francisco Pozo de Mattos Pereira

- 1653 ME01- Functional Characterization Of Anti-Cd19 Humanized Cars

Clara de Oliveira Andrade

17:20 – 18:00 Bright Sparks in Immunology 3

ROOM – Tiradentes I

Chairperson: Leonardo Travassos Corrêa (UFRJ, RJ, Brazil)

- 1379 DO11 - Role Of Ikaros-Hdac Complex In Regulating Micro-Rna And Apoptosis Pathway In Murine B-1 Cells

Lucas Vasconcelos Soares Costa

- 1246 ME02 - Genetic screening of autoinflammatory genes in Latin American pediatric patients with Multisystem Inflammatory Syndrome in Children (MIS-C) associated with SARS-CoV-2 infection

Raquel Bispo de São Pedro

- 1557 PD04 - Role Of Death-Associated Protein 6 In Chromatin Remodeling Mechanisms In Intestinal Cells

Nathalia Vitoria Pereira Araujo Hall

- 1633 ME03 - Molecular Characterization of Antibodies against the Spike Protein in Hyperimmunized Horses

Yala Sampaio

17:20 – 18:00 Bright Sparks in Immunology 4

ROOM – Tiradentes II

Chairperson: Letícia Carneiro (UFRJ, RJ, Brazil)

- 1755 PD06 - Differential immunomodulatory effects of iron in regulatory t (Treg) and Th17 cells

Eduardo Pereira Duarte da Silva

- 1610 DO12 - Pak-2 is involved in the activation of several inflammasomes

Amanda de Matos Becerra

- 1567 IC03 - Influence Of Cd4 T Lymphocytes During The Development And Maturation Of The Central Nervous System

Letícia Barros de Souza

- 1229 PD05 - Macrophages-Myoblasts Interplay: Roles Of Gdf11 Signaling In Myogenesis In Culture

Rafaella Ferreira Reis

18:00 – 18:50 Distinguished Lecture 2

ROOM – Ouro Preto Theater

Chairperson: Lis Antonelli (FIOCRUZ, MG, Brazil)

Type 2 circuitries maintaining tissue resident macrophages as a replicative niche for Leishmania

David L. Sacks (National Institutes of Health)

19:00 – 21:00 Poster Section

DO01 to DO95

ME01 to ME141

4th OCTOBER

08:00 – 09:00 BD Biosciences Course

ROOM – Auditorium São João Del Rey

- Panel Design: Improving experiments in Classical and Spectral Flow Cytometry

Iris Castro

08:00 – 09:00 Beckman Course

ROOM - Tiradentes II

- CytoFLEX as the choice for NanoFLow. What is the difference compared to traditional flow cytometers?

Anis Larbi

- EVs Isolation Methods & How to prepare EVs for NanoFlow

Alfonso Blanco

09:00 – 10:00 Distinguished Lecture 3

ROOM – Ouro Preto Theater

Chairperson: Angélica Thomaz (UFMG, MG, Brazil)
Microbiome, Metabolites and Modulation of Immune Function

Kate McCoy (University of Calgary, Canada)

10:00 – 10:20 Coffee Break

10:20 – 12:20 Symposium 4: T cells and NK cells

ROOM – Ouro Preto Theater
Chairperson: Simone Fonseca (UFG, GO, Brazil)

- Cytotoxic CD4 T cell, a new player in Chagas Disease

Maria Bellio (UFRJ, Brazil)

- Role of CD73 and adenosine in the differentiation of exhausted and precursor exhausted CD8+ T cells

Daniela Mahaluf (Universidad do Chile, Chile)

- Posttranslational protein modifications: the link between metabolism and immune function

Luciana Berod (Johannes Gutenberg University Mainz, Germany)

Short Talks

ROOM – Ouro Preto Theater

- 1550 PD07 - The role of mitochondrial inheritance in the early rise of asymmetric T cell fates

Mariana Borsa

- 1671 DO14 - Impact Of Sting Signaling Pathway On The Differentiation And Function Of Regulatory T Cells

Caroline Vitória de Oliveira

10:20 – 12:20 **Symposium 5: Mucosal Immunology**

ROOM - Auditorium São João Del Rey

Chairperson: Marcelo Bozza (UFRJ, RJ, Brazil)

- Immune crosstalk through shared lymph node drainage in the digestive system

Daria Esterhazy (University of Chicago, USA)

- A poison for the cure: Could type 2 immunity prevent allergic diseases?

Denise Fonseca (USP, Brazil)

- Sensing of the intestinal state by gd T cells

Bernardo Reis (Rockefeller University, USA)

Short Talks

ROOM - Auditorium São João Del Rey

- 1443 DO15 - Impact Of Intestinal Inflammation On The Kidneys Of Mice Submitted To An Experimental Model Of Chronic Colitis

José Arimatéa de Oliveira Nery-Neto

- 1762 PD08 - SARS-CoV-2 infection promotes cellular and humoral responses in the human airways

Mariana De Oliveira Diniz

10:20 – 12:20 Symposium 6: Tumor Immunology

ROOM – Tiradentes I

Chairperson: Cristina Bonorino (UFCSPA, RS, Brazil)

- Costimulation and cell interactions modulate PD-1+ CD8 T cells

Alice Kamphrost (Mount Sinai Shcool of Medicine, USA)

- Endocrine Therapy Primes Antitumor Immunity Opening the Door to Immune Checkpoint Blockade in Breast Cancer Luminal Tumors

Mariana Salatino (CONICET, Argentina)

- The malignant rolesof tumor-expressed CD43 (Sialophorin)

Yvonne Rosenstein (Universidad Autonomade Mexico, Mexico)

Short Talks

ROOM – Tiradentes I

- 1609 PD09 - Immune mechanisms involved in the activation of BCG-induced systemic antitumor response

Nina Mari Gual Pimenta de Queiroz

- 1803 DO16 - Intratumoral delivery of mRNA-TRAIL via Ionizable Lipid Nanoparticles reduces Colon Cancer and Drive Robust Immune Response

Walison Nunes Da Silva

12:20 – 15:00 Lunch

12:30 – 13:30 Tissue Gnostics – Symposium

ROOM - Tiradentes I

AI Powered Tissue Image Cytometry - Applications in Immunology Research

Rupert ECKER - CEO TissueGnostics & Adj. Prof. QUT (Australia)

13:00 – 15:00 Thereza Kipnis Award

ROOM - Auditorium São João Del Rey

Chairperson: Cláudia Brodskyn (Fiocruz-Bahia, Brazil)

- DO01 - The Sympathetic Nervous System Modulates The Metabolic Reprogramming Of Macrophages

Beatriz Marton Freire

- PD01 - Recombinant BCG expressing SARS-CoV-2 chimeric protein protects k18-hACE2 mice against viral challenge

Fábio Mambelli Silva

- DO02 - Ketogenic Diet Modifies The Microbiota Promoting Long-Term Immune Consequences In The Lungs

Marina Caçador Ayupe

- DO03 - Hyperglycemia impairs glycolysis activation in efferocytic CD11c+ cells during MRSA skin infection

Letícia de Aquino Penteado

- DO04 - Persisting Thromboinflammation in COVID-19 survivors

Remy Martins Goncalves

14:00 – 15:00 Illumina - Symposium

ROOM – Auditorium São João Del Rey

Single Cell Applications and the Growth of Multiomics in NGS.

Nelson Junior

15:00 – 17:00 **Workshop 5: INCT Investigação em Imunologia: COVID-19 – Immunology and Inflammation**

ROOM – Ouro Preto Theater

Chairperson: Edecio Cunha (USP, SP, Brazil)

- Unraveling Inflammatory Mechanisms of Severe COVID-19 in Individuals with Diabetes and Prediabetes

Natália Tavares (Fiocruz-BA, Brazil)

- Profile of SARS-CoV-2 T and B cell epitope responses in convalescents and vaccinated individuals

Keity Santos (INCOR-USP, Brazil)

- Development of a nasal spray vaccine against SARS-CoV-2

Jorge Kalil (INCOR-USP, Brazil)

Short Talks

ROOM – Ouro Preto Theater

- 1720 DO18 - Nasal administration of anti-CD3 mAb (Foralumab) downregulates NKG7 and increases TGFB1 and GIMAP7 expression in T cells in subjects with COVID-19

Toby Lanser

- 1787 PD11 - SREBP induced lipid droplet biogenesis and inflammasome activation during SARS-CoV-2 infection

Vinicius Cardoso Soares

15:00 – 17:00 **Workshop 6: INCT - Vacinas**

ROOM - Auditorium São João Del Rey

Chairperson: Ricardo Gazzinelli (UFMG/FIOCRUZ-MG, Brazil))

- The Butantan Dengue vaccine phase 3 trial

Maurício Nogueira (FAMERP-SP, Brazil)

- CRISPR-attenuated parasites and RNA vaccines as novel strategies for parasitic diseases

Santuza Teixeira (UFMG, Brazil)

- Clinical development of SpiN-Tec: a T cell-based Covid-19 vaccine

Helton Santiago (UFMG, Brazil)

- Hiltonol as a vaccine adjuvant and stand alone therapy for cancer and infectious diseases

Andrew J G Simpson (Director, Orygen Biotecnologia)

15:00 – 17:00 **Workshop 7: INCT in dengue and host parasite-interactions – Mediators of resolution of inflammation**

ROOM – Tiradentes I
Chairperson: Mauro Teixeira (UFMG, Brazil)

- Treating the host in arboviral infections: Pro-resolving molecules as a novel therapeutic paradigm

Vivian Costa (UFMG, Brazil)

- Annexin A1/SOCS2 axis is crucial during Trypanosoma cruzi infection promoting modulation of inflammation and microbiota composition

Fabiana Machado(UFMG, Brazil)

- Investigating mechanisms for resolving joint Inflammation and reducing bacterial burden in septic arthritis

Flavio Amaral(UFMG, Brazil)

- Resolution of inflammation and pain.

Waldiceu Verri Jr (UEL, PR, Brazil)

15:00 – 17:00 **Workshop 8: INCT- Doenças Tropicais**

ROOM – Tiradentes II
Chairperson: Edgar Carvalho (UFBA/FIOCRUZ-BA, Brazil),

- IL-10 produced by B cells enhances Leishmania braziliensis replication and contributes to the pathology of Cutaneous Leishmaniasis

Augusto Carvalho (FIOCRUZ-BA, Brazil)

- Cutaneous leishmaniasis: the control of lesions development

João S. da Silva (FIOCRUZ-FMRP, Brazil)

- MMP-2 and MMP-9 and their contribution as possible caspase-1-independent pathway for IL-1b activation in Trypanosoma cruzi immunity

Juliana Assis Estanislau (UFMG, Brazil)

Short Talks

ROOM – Tiradentes II

- 1693 DO17 - Sand fly yellow salivary proteins modulate neutrophil and macrophage responses to Leishmania parasites

Jullyanna Oliveira da Silva

- 1354 PD10 - Microbial translocation and its association with high neutrophil count and severity in Yellow Fever Virus infection

Mateus Vailant Thomazella

17:00 – 17:20 Coffee Break

17:20 – 18:00 Bright Sparks in Immunology 5

ROOM – Ouro Preto Theater
Chairperson: Pryscilla Fanini Wowk (ICC/Fiocruz, PR, Brazil)

- 1382 IC04 - Corynebacterium Diphtheriae Extracellular Vesicles Characterization And Its Effects On Mouse Macrophages

Luiza Souza

- 1578 PD12 - Asymptomatic malaria is associated with an IFN-γ-induced program on adaptive immunity

Gregório Guilherme Almeida

- 1407 DO19 - HIF-1α interaction with HIF-1A antisense long non-coding RNA shift response in macrophages during Leishmania infantum infection

Jonathan Miguel Zanatta

17:20 – 18:00 Bright Sparks in Immunology 6

ROOM - Auditorium São João Del Rey
Chairperson: Alexandra I. Medeiros (UNESP, Brazil)

- 1448 IC05 - Omega-3 decreases TNF-α and NO production, alters the glucose usage by macrophages, and leads to better clearance of Pseudomonas aeruginosa.

Ana Clara Moreira

- 1546 ME05 - Role of inflammasomes in the immune response to Legionella longbeachae

Rhanoica Oliveira Guerra

- 1374 DO36- Ubiquitin Ligase Smurf1 Regulates The Inflammatory Response In Macrophages And Attenuates Systemic Inflammation During Murine Hepatic Coronavirus Infection

Luiz Pedro de Souza Costa

- 1233 ME04 - Neutrophil extracellular vesicles promote proinflammatory effects of on primary human peripheral blood mononuclear cells

Camila Couto do Espírito Santo

17:20 – 18:00 Bright Sparks in Immunology 7

ROOM – Tiradentes I
Chairperson: Gustavo Menezes (UFMG, Brazil)

- 1252 DO20 - Vaccination With Recombinant Listeria Monocytogenes Induces An Effective Anti-Tumor Immune Response In Necroptosis- And Pyroptosis-Deficient Mice

Abolaji Samson Olagunju

- 1364 DO21 - Humoral Responses Among Individuals Infected With Wild-Type Yellow Fever Virus And Vaccinated with 17DD Yellow Fever Vaccine

Carolina Argondizo Correia

- 1500 DO24- Therapeutic effect of roflumilast, a selective PDE4 inhibitor, in a murine model of coronavirus infection

Vinícius Amorim Beltrami

- 1909 PD13 - Fast and Efficient Monoclonal Antibody Isolation from Human Memory B cells

Luciana Conde Rodrigues Maia

17:20 – 18:00 Bright Sparks in Immunology 8

ROOM – Tiradentes II
Chairperson: Alexandre Reis (UFOP, MG, Brazil)

- 1654 ME06 - Analysis Of Chimerism Of Regulatory T Cells In Patients Recipients Of Kidney And Liver Transplants

Tamires Moreira Gomes

- 1415 DO23 - Cannabinoid Receptor Type 2 Agonist Gp1a Attenuates Macrophage Activation Induced By M. Bovis-Bcg By Inhibiting Nfkb Signaling

Jessica Do Prado Valeriano

- 1980 DO25 - Temporal dynamics of murine brain lymphocytes distribution during life

Danillo Pereira Dantas

- 1500 DO24 - Therapeutic effect of roflumilast, a selective PDE4 inhibitor, in a murine model of coronavirus infection

Vinícius Amorim Beltrami

18:00 – 18:50 **Distinguished Lecture 4**

ROOM – Ouro Preto Theater

Chairperson: Patricia Bozza (FIOCRUZ, RJ, Brazil)

Exploring how inflammasomes shape the macrophages' and astrocytes' ability to fight infections

Karina Bortolucci (UNIFESP, Brazil)

19:00 – 21:00 **Poster Section**

DO96 to DO191
PD01 to PD103
PR01 to PR34
DO260
IC39

5th OCTOBER

08:00 – 09:00 **Beckman Course**

ROOM – Ouro Preto Theater

- Rigor and Excellence to do NanoFlow: challenges we must overcome

Raquel Ferraz Nogueira

- Exosomes and extracellular vesicles analysis and Conclusion

Alfonso Blanco

09:00 – 10:00 **Distinguished Lecture 5**

ROOM – Ouro Preto Theater

Chairperson: Silvia Boscardin (USP, SP, Brazil)

Antibody responses to infection and vaccines

Michel Nussenzweig (Rockefeller University, USA)

10:00 – 10:20 **Coffee Break**

10:20 – 12:20 Symposium 7: Immunology of Nutrition

ROOM – Ouro Preto Theater

Chairperson: Andrea Teixeira Carvalho (FIOCRUZ-MG)

- Loss of tolerance in a protein-free diet is associated with DC impairment and microbiome dysbiosis

Thaís Moreira (Harvard University, USA)

- Obesity as a susceptibility factor to Leishmania major infection

Tatiani Maioli (UFMG, Brazil)

- The role of fatty acids in skin immune responses

Hosana Gomes Rodrigues (UNICAMP, Brazil)

Short Talks

ROOM – Ouro Preto Theater

- 1244 DO26 - Undernutrition Alters Expression Of Splenic Extracellular Matrix Components And Modifies Splenocytes Location In Mice Infected With Leishmania Infantum

Renata Azevedo do Nascimento

- 1899 DO27 - Reconstruction Of Pasteurized Donor's Milk Microbiota As An Immune Interventional Strategy For Premature Newborns: Molecular Benefits Revealed By A Multiomic Approach Of A Clinical Trial

Lucas Soveral

10:20 – 12:20 Symposium 8: Innate immunity

ROOM - Auditorium São João Del Rey

Chairperson: Sérgio Costa (USP, SP< Brazil)

- cGAS-like receptor-mediated immunity in Drosophila flies

Jean-Luc Imler (University of Strasbourg, France)

- A signaling hub controlling plant immunity and growth

Elizabeth Fontes (UFV, MG, Brazil)

- Antiviral resistance in vector mosquitoes

João Marques (UFMG, Brazil)

Short Talks

ROOM – Ouro Preto Theater

- 1296 DO28 - Targeting adrenergic receptors to mitigate iNKT-induced acute liver injury

Michelangelo Bauwelz Gonzatti

10:20 – 12:20 Symposium 9: Immunosenescence

ROOM – Tiradentes I

Chairperson: Olindo M. Filho (FIOCRUZ-MG, Brazil)

- Strategies to Enhance Immunity During Ageing

Arne Akbar (University of London, UK)

- Fingerprinting of senescent T cells in the immunopathology of American Tegumentary Leishmaniasis

Daniel Gomes (UFES, Brazil)

- Premature immunosenescence in rheumatoid arthritis – relevance to clinical progression

Moisés Bauer (PUC-RS, Brazil)

Short Talks

ROOM – Tiradentes I

- 1248 DO29 - B-1 Cell Progenitor As A New Candidate To Explain B-1 Cell Accumulation In Aging And Disease

Olivia Fonseca Souza

- 1717 DO30 - Comprehensive characterization of the immune profile in short telomere syndromes

Willian Robert Gomes

12:20 – 15:00 Lunch

12:30 – 13:30 Beckman Symposium

ROOM – Tiradentes II

- Welcome to the Nanosorting World

Alfonso Blanco

13:00 – 15:00 General Assembly SBI

ROOM – Ouro Preto Theater

13:00 – 15:00 BD Award

ROOM – São João del Rey

Categoria Jovem Talento

- Papel do eixo oro-intestinal no agravamento da colite murina

André Luiz Amorim da Costa (IMPG/UFRJ)

- Efeito da suplementação com probiótico selenizado em modelo experimental de colite ulcerativa agravada por infecção

Gabriele Manamy Baba Rodrigues (ICB IV - USP)

- Caracterização da Senescência e Exaustão Celular em Crianças Vivendo com HIV em Regime de Terapia Antirretroviral

Mônica Pereira Coelho (FMUSP)

Categoria Pesquisador SBI

- Avaliação e Caracterização de Resposta Celular no Contexto de Infecções e Vacinas

Cássia G. Terrassani Silveira (FMUSP)

- Impacto da citometria de fluxo multiparamétrica no diagnóstico precoce e análise de resposta imune intratumoral no câncer pediátrico

Cristiane de Sá Ferreira Facio (IPPMG/UFRJ)

- Identificação das microvesículas relacionadas a exaustão celular em pacientes com hanseníase.

Katherine Kelda Gomes de Castro (IOC/FIOCRUZ)

15:00 – 17:00 Workshop 9: Inflammatory diseases

ROOM – Ouro Preto Theater

Chairperson: Tatiani Maioli (UFMG, MG, Brazil)

- A Novel Role for A20-Regulated CNS Endothelial ICOSL in Immune Cell Adhesion and Autoimmune Neuroinflammation

Ari Waisman (University of Mainz, Germany)

- Microbial recognition by intestinal T lymphocytes

Angelina Bilate (Rockefeller University, USA)

- Interferon epsilon: an estrogen-dependent type I interferon that is uniquely exploited by Neisseria gonorrhoeae

Douglas Golenbock (University of Massachusetts, USA)

Short Talks
ROOM – Ouro Preto Theater

- 1728 DO31- Plasmodium berghei NK65 malaria induces neuroinflammation and promotes cognitive deficits in mice

Flaviane Vieira Santos

15:00 – 17:00 **Workshop 10: Biology of B Cells**

Auditorium São João Del Rey
Chairperson: Camila Indiani Oliveira (FIOCRUZ-BA, Brazil)

- Regulation of antibody production by specialized TFH subsets

Luís Graça (IMM, Portugal)

- Exploring the Unique Molecular Traits of Horse Antibodies and Their Fascinating Contrasts with Humans

Liza Felicori (UFMG, Brazil)

-

Short Talks
Auditorium São João Del Rey

- 1295 - Unraveling The Significance Of N-Glycolylneuraminic Acid Evolutionary Loss In B Cell Dynamics And Its Correlation With The Intestinal Microbiota

Philippe Caloba Oliveira de Mattos Cruz

- 1577 - Phenotypic rhythm, integrative networks, and profile of T and B-cell subsets associated with distinct clinical outcomes of severe COVID-19 patients

Gabriela de Oliveira

15:00 – 17:00 **Workshop 11: Immunology of allergic diseases**

ROOM – Tiradentes I

Chairperson: Momtchilo Russo (USP, SP, Brazil)

- B cell memory of allergic responses

Maria Curotto-Lafaille (Mount-Sinai School of Medicine, USA)

- T cells specific to food proteins and their role in allergic diseases

Daniel Lozano-Ojalvo (Mount-Sinai School of Medicine, USA)

- Is specific immunotherapy useful in atopic dermatitis?

Luisa Karla Arruda (USP-RP, Brazil)

- TING activation induces neutrophilic asthma in response to house dust mite allergen

Dieudonnée Togbe(University of Orleans, France)

15:00 – 17:00 **Workshop 12: Immunotherapy**

ROOM – Tiradentes II

Chairperson: João Viola (INCA, RJ, Brazil)

- Personalized medicine apporach toward increasing access to checkpoint inhibitors therapy in melanoma

Ken Gollob (Albert Einstein Hospital, SP, Brazil)

- PI3Kdelta as a target for immunotherapy

Klaus Okkenhaug (University of Cambridge, UK)

- CAR-T initiatives under development in Rio de Janeiro

Martin Bonamino (INCA, RJ, Brazil)

Short Talks

ROOM – Tiradentes II

- 1869 DO35 - Targeting Polycomb Repressive Complex 2 (PRC2) as a strategy to improve the antitumor activity of CAR-T cells

Maria Leticia Rodrigues Carvalho

- 1820 DO34 - Distinct Exhaustion Marker Profiles Reveal Time-Dependent Immune-Related Adverse Events During Immunotherapy

Guilherme Ferreira de Britto Evangelista

17:00 – 17:20 **Coffee Break**

17:20 – 18:00 Bright Sparks in Immunology 9

ROOM – Ouro Preto Theater
Chairperson: Joana Amaral (UFOP, MG, Brazil)

- 1945 DO39 - CD38 increased activity may account for NAD+ metabolism imbalance in the brain of neonate mice during Zika virus infection

Georgia do Nascimento Saraiva

- 1684 DO37 - ATP-P2X7 signaling in the pathophysiology of COVID-19

Nayara Carvalho Barbosa

- 1734 DO38 - Evaluation of the role of butyrate in the migration of plasmacytoid dendritic cells during experimental COVID-19

Jéssica Assis Pereira

- 1939 IC06 - Sex-related immune responses and gastrointestinal (GI) commitment converge to long COVID-19 sequelae in female patients

Jackeline Marino Lucas

17:20 – 18:00 Bright Sparks in Immunology 10

ROOM - Auditorium São João Del Rey
Chairperson: Rafael Elias Marques (CNPEM, SP, Brazil)

- 1758 PD14 - Scale-Up Production Of A Low-Cost Anti-Cd19 Car-T Cell For Leukemia Immunotherapy

Sabrina Alves dos Reis

- 1477 ME07 - Evaluation Of The Immunomodulatory Activity Of A Kunitz-Type Protease Inhibitor Present In The Saliva Of The Tick Amblyomma Sculptum

Gretta Huamanrayme Bustamante

- 1791 DO40 - Intensity Of Toll-Like Receptors Expression Per T Cells Correlated With Clinical Parameters Of Multiple Sclerosis

Marcos Octávio Salvaterra Dutra Cafasso

- 1932 ME08 - The Role Of Sting In The Develop Of Kidney Injury In Lap-Deficient Mice

Daniel Leonardo Alzamora Terrel

17:20 – 18:00 Bright Sparks in Immunology 11

ROOM – Tiradentes I

Chairperson: Daniel S. Mansur (UFSC, SC, Brazil)

- 1281 IC07 - Identification of genes associated with immune infiltration and tumor regression in melanoma

Vinícius Gonçalves de Souza

- 1348 DO41 - The role of DPP-IV inhibition in regulating inflammation during acute colitis and colitis-associated colorectal cancer: combining in sílico and experimental approaches

Eloisa Martins da Silva

- 1634 DO43 - Systemic Immunological Profile from Children with B-Cell Acute Lymphoblastic Leukemia in the Brazilian Amazon

Fábio Magalhães-Gama

- 1629 DO42 - BCG immunotherapy depends on TLR2-MyD88 signaling to control melanoma in mice

Vinicius Martins Borges

17:20 – 18:00 Bright Sparks in Immunology 12

ROOM – Tiradentes II

Chairperson: Alessandra Filardy (UFRJ, RJ, Brazil)

- 1974 DO44 - Aryl Hidrocarbon Receptor Immunometabolic Role During A Murine Betacoronavirus Experimental Infection

Fernando Roque Ascenção

- 1632 ME09 - Development of a mRNA vaccine for Dengue virus serotypes 2 and 3

Sarah A. R. Sérgio

- 1771 PD15 - An IgG fusion vaccine strategy increases RBD immunogenicity and protects against SARS-CoV-2

Mariangela de Oliveira Silva

18:00 – 18:50 **Distinguished Lecture 6**

ROOM – Ouro Preto Theater

Chairperson: Gustavo Amarante-Mendes (USP, SP, Brazil)

Some aspects in the Pathogenesis od Cutaneous Leishmaniasis and Alternative Therapeutic Approaches for Treatment of the Disease

Claudia Brodskyn (FIOCRUZ-BA, Brazil)

19:00 – 21:00 **Poster Section**

DO192 to DO281:
IC01 to IC130:
PR35 to PR51

6th OCTOBER

09:00 – 10:00 **Distinguished Lecture 7**

ROOM – Ouro Preto Theater

Chairperson: Verônica Coelho (USP, SP, Brazil)

Clonal dynamics of the recall antibody responses

Gabriel Victora (Rockefeller University, USA)

10:00 – 11:00 **Closing Conference**

ROOM – Ouro Preto Theater

Chairperson: Helder Nakaya (Albert Einstein Hospital, SP, Brazil)

The circadian cycle and immunometabolism in malaria

Ricardo Gazzinelli (UFMG/FIOCRUZ, Brazil)

11:00 – 12:00 **Closing Ceremony and Awards**

ROOM – Ouro Preto Theater

BD Award
Teresa Kipnis Award
SLB Award

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Dear Participants,

On behalf of the Brazilian Society of Immunology (SBI) and the Organizing Committee, we would like to welcome you to the **XLVII Meeting of the Brazilian Society of Immunology**. This is a special meeting since we will celebrate the 50th anniversary of SBI and for the occasion the project “History of Immunology in Brazil” will be launched with small samples of the series of interviews with pioneer immunologists that helped to build our society and the field of Immunology in Brazil.

The Scientific Program is composed of symposia, workshops, and keynote lectures on the recent advances in different fields of immunology, including lymphocyte development and function, immune responses to pathogens, mucosal immunology, vaccines, immunotherapy, tumor immunology, autoimmune and inflammatory diseases, allergy, immunosenescence, and much more. The conference will gather renowned scientists from Brazil and several other countries with substantial participation of young investigators.

As part of SBI tradition, we are giving special attention to the sections in which undergraduate and graduate students, as well as post-doctoral fellows, will present their ongoing work during oral and poster sessions. In this booklet you will find the abstracts of all posters and oral presentations that will be part of these sessions.

This year, we have some novelties: a special childcare program to facilitate the participation of researchers with kids, and a section on art & science to highlight the many bonds of science to other forms of human creativity.

Finally, we would like to acknowledge the invited speakers, SBI members, colleagues, and students for supporting and attending this meeting. We are particularly grateful to the Secretariat that oversaw the organization of the meeting, Mrs **Ana Paula Lopes Vidal** (SBPZ) and Mrs **Vilma de Araújo Andrade** (SBI, SBPZ), and to **Rafaela Garcia and Paula Vinhas** who are doing a terrific work taking care of all congress communication activities. We are thankful to all the SBI members and foreign speakers as well who kindly helped us in the Award Committees, Poster and Oral presentation sections. We would also like to acknowledge the financial support received from CNPq, CAPES, FAPEMIG, The Company of Biologists (UK), Immunology Letters, The Journal of Leukocyte Biology and the private sponsors that have been our partners in many editions of the congress. A special acknowledgement to Luis Andrade and LABA for their splendid work with the interviews in the project on the history of Immunology in Brazil.

We welcome you all to our meeting, hoping that the scientific program covers your expectations and stimulates the high-level discussions that have always characterized our meetings.

Ouro Preto, October 2nd, 2023

Ana M. Caetano Faria
President of SBI
and the SBI board of directors

DO-001 - **THE SYMPATHETIC NERVOUS SYSTEM MODULATES THE METABOLIC REPROGRAMMING OF MACROPHAGES**

FREIRE, B.M.[1]; GONZATTI, M.B.[1]; LITH, S.C.[2]; DE MELO, F.M.[3]; GUERESCHI, M.G.[4]; MARICATO, J.T.[1]; VIEIRA, P.M.M.M.[5]; KELLER, A.C.[1]; DE VRIES, C.J.[2]; BASSO, A.S.[1]. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, UNIVERSIDADE FEDERAL DE SÃO PAULO SÃO PAULO - SP - BRASIL; 2. AMSTERDAM UMC, AMSTERDAM UMC AMSTERDAM - HOLANDA; 3. UNIVERSITY OF CALIFORNIA - UCLA, UNIVERSITY OF CALIFORNIA - UCLA SÃO PAULO - SP - BRASIL; 4. INSTITUTO ADOLFO LUTZ, INSTITUTO ADOLFO LUTZ SÃO PAULO - SP - BRASIL; 5. UNIVERSIDADE ESTADUAL DE SÃO PAULO, UNIVERSIDADE ESTADUAL DE SÃO PAULO CAMPINAS - SP - BRASIL.

The effector function of macrophages is distinctly variable, and these cells play fundamental roles within different tissues, in both health and disease. Hence, modulating the effector function of macrophages can be beneficial for achieving or restoring organ homeostasis. The expression of adrenergic receptors on leukocytes enables the direct modulation of immune responses by the sympathetic nervous system (SNS). The beta-2 adrenergic receptor (β2AR) is the most expressed one by macrophages. It is knowledgeable that engagement of β2AR in macrophages induces the cell towards an anti-inflammatory phenotype. However, little is known about the effects of adrenergic signaling in cell metabolism, which potentially dictates its effector function. By using bone-marrow-derived cells (BMDM), we demonstrated that β2AR signaling in classical activated BMDM sustains oxidative phosphorylation (OxPhos), preventing its classical glycolysis enhancement and its pro-inflammatory by-products, for instance the production of nitric oxide (NO), reactive oxygen species (ROS), and HIF1-α stabilization. Gene silencing of arginase 1 (Arg1) abrogated the effects of β2AR in limiting glycolysis rates. Concordantly, we observed that β2AR engagement in classical activated BMDM culminated with a massive increase in Arg1 expression and activity. We identified the orphan nuclear receptor Nur77 (Nur77) as the inducer of Arg1 upon β2AR engagement. Hence, BMDM deficient for Nur77 is no longer capable to sustains OxPhos and preventing glycolysis upregulation. Finally, taking advantage of conditional knockout mice lacking the expression of β2AR in monocytes, we confirmed the importance of the SNS in controlling macrophage-induced inflammation in a model of neuroinflammation. Altogether, our data reveal a new neuro-immune-metabolism axis mediated by b2AR-Nur77-Arg1, that could be further explored as a potential therapeutic target. **Keywords:**MACROPHAGES;METABOLISM;NEUROIMMUNE.

DO-002 - **KETOGENIC DIET MODIFIES THE MICROBIOTA PROMOTING LONG-TERM IMMUNE CONSEQUENCES IN THE LUNGS**

AYUPE, M.C.[1]; SALGADO, C.L.[1]; GONÇALVES, L.M.[1]; MENEZES-SILVA, L.[1]; PIZZOLANTE, B.C.[1]; DA SILVA, G.W.[1]; OLIVEIRA, B.D.C.[1]; RODRIGUES, G.M.B.[1]; MOREIRA, F.[1]; DE ARAUJO, M.V.[1]; DE SOUZA FILHO, A.F.[1]; SILVA-PEREIRA, T.T.[1]; SANTOS, G.D.A.[2]; DE OLIVEIRA, E.E.[1]; LIMA, G.D.M.[1]; SILVA, C.R.[3]; SOMESSARI, E.S.R.[3]; RIBEIRO, M.S.[3]; RODOVALHO, V.D.R.[4]; VINOLO, M.A.R.[4]; ALVES-FILHO, J.C.[5]; HEINEMANN, M.B.[1]; HAND, T.W.[6]; HIRATA, M.H.[1]; GUIMARÃES, A.M.D.S.[1]; LIMA, M.R.D.[1]; DA FONSECA, D.M.[1]. 1. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO SÃO PAULO - SP - BRASIL; 2. UNIVERSITY OF VIRGINIA, UNIVERSITY OF VIRGINIA VIRGINIA - ESTADOS UNIDOS DA AMERICA; 3. IPEN, IPEN SÃO PAULO - SP - BRASIL; 4. UNIVERSIDADE DE CAMPINAS, UNIVERSIDADE DE CAMPINAS SÃO PAULO - SP - BRASIL; 5. UNIVERSIDADE DE SÃO PAULO-RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO-RIBEIRÃO PRETO SÃO PAULO - SP - BRASIL; 6. UPMC CHILDREN´S HOSPITAL UNIVERSITY OF PITTSBURGH, UPMC CHILDREN´S HOSPITAL UNIVERSITY OF PITTSBURGH PITTSBURGH - ESTADOS UNIDOS DA AMERICA.

Mucosal tissues are endowed with a specialized immune system designed to respond to an enormous range of regional challenges while maintaining tissue homeostasis. The gut-lung communication mediated by commensals, metabolites and immune cells, also called the ‘gut-lung axis’, contributes to both, protection and development of inflammatory diseases, depending upon the context. In the intestine, dietary components are essential for the proper functioning of the gut-associated immune system often by influencing the resident microbiota. However, little is known about how diet specifically impacts distal mucosal sites and the respective resident microbiota. Here, we hypothesized that the unbalanced carbohydrate-deficient ketogenic diet may cause long-term consequences for the lung immunity, particularly by interfering in the gut-lung axis. We found that feeding mice with a ketogenic diet promoted a persistent neutrophilic infiltrate in the lung that was dependent on increased recruitment of IL-17A-producing Tγδ lymphocytes. This effect was dependent on high levels of ketone bodies and the IL-6 production by inflammatory monocytes, possibly driven by diet-induced changes in the lung microbiota. Indeed, both lung and gut microbiota are impacted by the dietary changes and germ-free mice are uncapable to recapitulate the phenotype found in SPF animals. As a consequence of the changes in the lung immune tone, mice fed a ketogenic diet became susceptible to immune-mediated lung diseases, such as COVID-19 and asthma. Therefore, in the context of the gut-lung axis, the diet-modified microbiota shapes lung immunity and defines the outcome of inflammatory dieases. **Financial support:** FAPESP, CAPES, CNPq. **Keywords:** ketogenic diet;neutrophils;gut-lung axis.

DO-003 - **Hyperglycemia impairs glycolysis activation in efferocytic CD11c+ cells during MRSA skin infection**

PENTEADO, L.D.A.[1]; PEREIRA, L.D.S.[2]; SALINA, A.C.G.[3]; RAGONESE, F.[2]; RAIMUNDO, B.V.B.[2]; VIEIRA, P.M.M.D.M.[4]; SEREZANI, C.H.[3]; DE MEDEIROS, A.I.[2]. 1. UNIVERSITY OF SAO PAULO, UNIVERSITY OF SAO PAULO RIBEIRÃO PRETO - SP - BRASIL; 2. SAO PAULO STATE UNIVERSITY, SAO PAULO STATE UNIVERSITY ARARAQUARA - SP - BRASIL; 3. VANDERBILT UNIVERSITY MEDICAL CENTER, VANDERBILT UNIVERSITY MEDICAL CENTER TENNESSEE - ESTADOS UNIDOS DA AMERICA; 4. UNIVERSITY OF CAMPINAS, UNIVERSITY OF CAMPINAS CAMPINAS - SP - BRASIL.

Diabetic patients are susceptible to Methicillin-resistant *Staphylococcus aureus* (MRSA) skin infections. Previous work showed that hyperglycemia in diabetic mice impairs Langerhans cell CCR7 expression and migration to lymph nodes, resulting in poor defense against subcutaneous (s.c.) MRSA infection. Given that metabolism governs cell function, we sought to unveil the impact of hyperglycemia on the metabolism of skin-resident immune CD11c$^+$ cells during MRSA infection. RNA-seq analysis from skin-isolated CD11c$^+$ cells from vehicle (CT) or STZ-treated mice after 18h of infection revealed a downregulation of genes related to Hif-1α and glycolysis pathways in STZ mice compared to normoglycemic mice. Depletion of CD11c cells in CD11c-DTR mice partially restored infection resolution in STZ mice, suggesting that these dysfunctional CD11c$^+$ cells could be detrimental to host defense. During infection, we observed ~80% of MRSA-infected neutrophils (MRSA-iAC) undergoing cell death, and ~40% of those neutrophils were phagocyted by CD11c$^+$Sirp1α$^+$ cells. To determine whether glycolysis is activated during efferocytosis, we co-cultured BMDC with MRSA-iAC and observed increased glycolysis, glycolytic capacity and reserve, as well as upregulation of *Pfkfb3*, *Glut1*, and *Hk2* genes. Conversely, mitochondrial respiration in BMDC was drastically reduced during efferocytosis of MRSA-iAC. We observed a transient decrease in mitochondrial membrane potential accompanied by increased mtROS production and dysfunctional mitochondria within the BMDCs.Treatment with 2-DG decreased IL-1β, IL-6, IL-10, and nitrite production and the inhibition of Hif-1α impaired glycolytic gene upregulation, thus demonstrating that Hif-1α-induced glycolysis occurs during efferocytosis of MRSA-iAC by BMDCs.These results indicate that hyperglycemia negatively impacts glycolysis activation in CD11c$^+$ cells and shed light on additional targets to benefit immunocompromised individuals during skin infections. **keywords:**Dendritic cells;Efferocytosis;Metabolism.

DO-004 - **Persisting Thromboinflammation in COVID-19 survivors**

GONCALVES, R.M.[1]; DE CAMPOS, M.M.[1]; DO AMARAL, L.P.[2]; AZEVEDO-QUINTANILHA, I.G.[2]; MENDES, M.A.[3]; MENDES, J.D.T.[3]; TEMEROZO, J.[2]; ROSADO-DE-CASTRO, P.H.[3]; BOZZA, F.A.[3]; RODRIGUES, R.S.[3]; HOTTZ, E.D.[4]; BOZZA, P.T.[2]. 1. FEDERAL UNIVERSITY OF RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. OSWALDO CRUZ INSTITUTE, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. D'OR INSTITUTE FOR RESEARCH AND EDUCATION, RIO DE JANEIRO - RJ - BRASIL; 4. FEDERAL UNIVERSITY OF JUIZ DE FORA, JUÍZ DE FORA - MG - BRASIL.

Severe COVID-19 patients display platelet activation and hyperreactivity and high rates of venous thromboembolism. However, the long-term consequences of the infection are not clear. Aside persisting symptoms, post-hospitalized patients have higher risk for cardiovascular and thromboembolic events.Here we investigate thromboinflamation and hypercoagulability in COVID-19 pneumonia hospitalization survivors (CS) up to 6 months after symptom onset.This study (CAAE 5523520.3.0000.5249) enrolled 29 healthy donors (HD) and 46 CS from 30 days to 6 months after symptom onset.CS platelets had a higher surface expression of α-granule-CD62p release markers and ex-vivo released of PDGF, RANTES and PF4, which are also elevated in the plasma. CS platelets show higher spread over a fibrinogen coated surface were hyperreactive to thrombin stimuli, indicating elevated platelet activation and hyperreactivity. Incubating HD platelets with CS plasma induced platelet activation, and pre-treatment of platelets with Abciximab (50µg/mL), Brilliant Blue G (5µM) or acetylsalicylic acid (100µM) reduced this effect.CS had elevated D-dimer (median=521.5 ng/mL), CRP (median=1.625 mg/L), and higher circulating levels of Tissue Factor (TF) (median=40.5 pg/mL) than HD (median=10.9 pg/mL). Compared to HD, CS presented higher circulating levels of total Extracellular Vesicles (EVs) and TF$^+$ EVs, and also elevated surface expression of TF in platelet derived EVs.Survivors of COVID-19 pneumonia hospitalization presented persistent platelet activation and hyperreactivity up to 6 months after symptom onset. Additionally, CS had increased levels of pro-coagulant TF bearing EVs, which may contribute to the development of post-discharge thrombotic events. Integrin $\alpha_{IIb}\beta_3$ inside-out activation and subsequent binding to plasma fibrinogen, ATP purinergic signaling and TXA_2 generation are involved in the amplification of platelet activation, and may contribute to the hypercoagulable state found in CS. **Keywords:**Thromboinflammation;Tissue Factor ;Extracellular Vesicles.

PD-001 - **Recombinant BCG expressing SARS-CoV-2 chimeric protein protects k18-hACE2 mice against viral challenge**

SILVA, F.M.[1]; MARINHO, F.A.V.[1]; ANDRADE, J.M.[1]; DE ARAUJO, A.C.V.S.C.[1]; ABUNA, R.P.F.[2]; FABRI, V.M.D.R.[1]; SANTOS, B.D.P.O.[1]; SILVA, J.S.[2]; DE MAGALHÃES, M.T.Q.[1]; HOMAN, E.J.[3]; LEITE, L.C.D.C.[4]; DIAS, G.B.M.[5]; HECK, N.D.B.[5]; MENDES, D.A.G.B.[5]; MANSUR, D.S.[5]; BAFICA, A.L.B.[5]; OLIVEIRA, S.C.[6]. 1. FEDERAL UNIVERSITY OF MINAS GERAIS, FEDERAL UNIVERSITY OF MINAS GERAIS BELO HORIZONTE - MG - BRASIL; 2. OSWALDO CRUZ FOUNDATION-FIOCRUZ, UNIVERSITY OF SÃO PAULO, OSWALDO CRUZ FOUNDATION-FIOCRUZ, UNIVERSITY OF SÃO PAULO RIBEIRÃO PRETO - SP - BRASIL; 3. IOGENETICS LLC, IOGENETICS LLC WISCONSIN - ESTADOS UNIDOS DA AMERICA; 4. BUTANTAN INSTITUTE, BUTANTAN INSTITUTE SÃO PAULO - SP - BRASIL; 5. FEDERAL UNIVERSITY OF SANTA CATARINA, FEDERAL UNIVERSITY OF SANTA CATARINA FLORIANÓPOLIS - SC - BRASIL; 6. UNIVERSITY OF SÃO PAULO, UNIVERSITY OF SÃO PAULO SÃO PAULO - SP - BRASIL.

The COVID-19 has been one of the greatest health crises of recent history, being accounted for more than six million deaths worldwide. Many attempts on finding counter-measures against the ongoing pandemic have been prosed, with the Bacillus Calmette-Guérin (BCG), the existing tuberculosis vaccine, being one of the most appealed options. It has been proposed as potential strategy against SARS-CoV-2 infection mainly due to its ability of inducing trained immunity and heterologous protection against other infections. In this study, we demonstrated the construction of a recombinant BCG expressing a chimeric protein consisting of nucleocapsid- and spike-derived immunogenic epitopes from SARS-CoV-2 (termed rBCG-ChD6). This strategy was meant to provide a powerful combination of the beneficial effects elicited by the BCG upon the innate immune system and also specific cellular and humoral responses against SARS-CoV-2 elicited by the recombinant antigens. We investigated whether rBCG-ChD6 immunization followed by a boost with the recombinant nucleocapsid and spike chimera (rChimera) with alum provided protection against SARS-CoV-2 infection in K18-hACE2 mice. A single dose of rBCG-ChD6 boosted with rChimera/alum elicited the highest anti-Chimera IgG and IgG2c antibody titers with neutralizing activity against SARS-CoV-2 Wuhan strain when compared to control groups. Importantly, following SARS-Cov2 challenge, this vaccination regimen induced IFN-g and IL-6 production in spleen cells and reduced viral load in the lungs. In addition, rBCG-ChD6 immunized mice boosted with rChimera/alum presented no clinical deterioration and no detected viable virus in the lungs when compared to control groups, also being associated with amelioration of overall lung architecture. Altogether, our study demonstrates a protective prime-boost immunization system based on a rBCG expressing a chimeric protein derived from SARS-CoV-2 against viral challenge in K18-hACE2 murine model. **Keywords:**COVID-19;BCG;Vaccine.

DO - 005 - **CORRELATION BETWEEN CIRCULATING IMMUNE MOLECULES AND THEIR CARDIAC GENE EXPRESSION IN CHRONIC CHAGAS CARDIOMYOPATHY**

SILVA, T.G.D.S.E.[1]; NEVES, E.G.A.[1]; NUNES, M.D.C.P.[1]; GOMES, J.D.A.S.[1]; GOLLOB, K.J.[2]; DUTRA, W.[1]. 1. UFMG, UFMG BELO HORIZONTE - MG - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, HOSPITAL ISRAELITA ALBERT EINSTEIN SÃO PAULO - SP - BRASIL.

Characterization of compartmentalized immunological events in target organs is of fundamental importance for unveiling the pathogenesis of several diseases. Nevertheless, accessing affected vital organs is often constrained due to limited safety. Here we aimed to establish a correlation between the levels of immune molecules in the blood and their gene expression profiles in the hearts of patients with Chagas disease cardiomyopathy, a debilitating and lethal cardiomyopathy caused by infection with the protozoan *Trypanosoma cruzi*. Serum levels of immune mediators from individuals with the indeterminate and cardiac (CCC) clinical forms of Chagas disease, as well as healthy donors (CTRL) were measured using the Bio-Plex kit. Heatmap and PCA were employed to assess the discrimination pattern of soluble molecules among the groups.The GEO2R web tool, ExpressAnalyst, and NetworkAnalyst.ca were utilized to evaluate gene expression profiles in the heart from CCC and CTRL, and to construct an interaction network of differentially expressed genes (DEGs) with enriched signaling pathways and biological processes.Our findings revealed significant increase in inflammatory cytokine levels in the serum of CCC, which were associated with markers of worse cardiac function. Notably, 75% of the measured soluble mediators in the plasma of CCC patients showed correspondence with their gene expression profiles in the heart. Interestingly, we demonstrated an association of soluble molecules with the enrichment of inflammatory signaling pathways in both the peripheral blood and heart compartments. Also, the DEGs in the heart tissue from CCC were associated with the enrichment of T-cell receptor pathway and cytotoxicity, showing correlation with CD8+ cells in the heart. This data reveals a strong correspondence between immune molecule profiles in the peripheral blood and within the myocardium of CCC patients, highlighting the blood as a valuable and non-invasive compartment. **Keywords:** Chagas cardiomyopathy;circulating cytokines and chemokines;gene expression profiling.

DO - 006 - **SUCNR1-mediated response in CD11c+ macrophages is essential for the statement of resistance to cutaneous leishmaniasis**

DE ASSUNÇÃO, S.F.V.[1]; ROSA, M.H.[1]; CARVALHO, L.M.[1]; NASCIMENTO, D.C.B.[1]; FUZO, C.[1]; CARVALHO, L.[2]; BENEVIDES, L.[3]; PEREIRA, V.C.[1]; PACHECO, R.[4]; DE CARVALHO FILHO, E.M.[5]; ZAMBONI, D.S.[1]; ALVES FILHO, J.C.F.[1]; SILVA, J.S.[1]. 1. UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. GONÇALO MUNIZ INTITUTE - FIOCRUZ BA, SALVADOR - BA - BRASIL; 3. BI-INSTITUTIONAL PLATFORM FOR RESEARCH IN TRANSLATIONAL MEDICINE, RIBEIRÃO PRETO - SP - BRASIL; 4. FEDERAL UNIVERSITY OF SERGIPE, SERGIPE - PE - BRASIL; 5. FEDERAL UNIVERSITY OF BAHIA, SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis (CL) is a neglected infectious disease prevalent in Brazil, where it is mainly caused by *L. braziliensis* and *L. amazonensis.* The available treatments are chronic and can lead to resistance to the parasites, hence, we investigate the role of succinate receptor 1 (SUCNR1) during the development of CL. SUCNR1 has been described in dendritic cells (DCs) and in macrophages (MOs) promoting inflammation. Therefore, we set to investigate SUCNR1 possible role during the development of CL. Our hypothesis is that SUCNR1 expressed by MOs and/or DCs contributes to the protective immune response to CL. We characterized SUCNR1 expression in the skin lesion of patients infected with *L. brasiliensis*, it is upregulated in the lesion and its expression is correlated with myeloid cell gene markers. To evaluate Sucnr1 role during CL, we infected wild type (WT) and Sucnr1 total knockout ($Sucnr1^{-/-}$) mice with *L. amazonensis* and we observed an increase in the ear thickness, lesion, edema and parasite burden in the absence of Sucnr1. The cytokine profile in the lesion shift in $Sucnr1^{-/-}$ mice with a decrease in TNF, IL-12p70 and IFN-g but an increase in IL-4. Amongst the myeloid cells, we observed an increase in the infected $CD11c^{+}$ MOs, they also presented a decreased intensity of iNOS. To confirm this finding, we differentiated BMDM from WT and $Sucnr1^{-/-}$ mice, and we performed a killing assay. We observed that $Sucnr1^{-/-}$ BMDM did not responded to IFN-g stimulus being unable to kill the parasites. We then constructed a conditional knockout mice to Sucnr1 in $CD11c^{+}$ cells ($CD11c^{cre}Sucnr1^{f/f}$) and we infected them with *L. amazonensis*. The $CD11c^{cre}Sucnr1^{f/f}$ mice showed an increase in the ear thickness, lesion size, edema and parasite burden. Our data suggest that Sucnr1 expressed by CD11c+ macrophages are more responsive to IFN-g signalling which increases their ability of killing the parasite, thereby controlling the infection and the lesion progression during CL. **Keywords:** Cutaneous Leishmaniasis;SUCNR1;Macrophages.

DO - 007 - **Th17 axis activation in the immune response against Cryptococcus gattii and the influence of IL-22 and IL-23 cytokines in the systemic experimental infection**

LIMA, I.D.[1]; FARIA, J.M.D.S.[1]; DE ALMEIDA, G.J.M.[1]; DA SILVA JUNIOR, E.B.[1]; DE OLIVEIRA, J.C.G.[1]; DOS SANTOS, I.M.F.[1]; NASCIMENTO, D.D.O.[2]; DE LIMA, L.F.[1]; DECOTE-RICARDO, D.[2]; GUEDES, H.L.D.M.[1]; FREIRE-DE-LIMA, C.G.[1]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPÉDICA - RJ - BRASIL.

Cryptococcosis is an infection caused by Cryptococcus fungi. Although the infection is most associated with *Cryptococcus neoformans*, *Cryptococcus gattii* has emerged as an important pathogen. Th17 response is crucial in pulmonary cryptococcosis for host defense. The Th17 response, involving cytokines like IL-17, IL-22, and IL-23, plays a crucial role in fighting the infection. However, studies suggest that *Cryptococcus* spp. can manipulate the Th17 response, potentially leading to inadequate immune reactions. Dysregulated IL-22 and IL-23 production may be involved. While IL-22 has protective effects, excessive production can cause inflammation, and IL-23 contributes to sustaining the Th17 response. Both cytokines influence the progression of cryptococcosis. The hypervirulent strain of *C. gattii* R265 in severe cryptococcosis induces higher IL-22 production compared to other species. Therefore, we hypothesize that *C. gattii* modulates the Th17 pathway, leading to lung tissue damage, facilitating fungal colonization and immune evasion. We found that IL-22 deficient mice are more susceptible to *C. gattii* infection, exhibiting uncontrolled dissemination of yeasts in the blood throughout the disease progression, culminating in increased susceptibility to cryptococcal meningitis due to high fungal burden in the brain. The poor containment of fungi in the lungs, as evidenced by reduced pulmonary burden in these animals, seems to be dependent on IL-22 rather than IL-23, as IL-23 deficient mice do not experience uncontrolled fungemia, neither low pulmonary fungal burden. The absence of IL-22 impairs lymphocyte and eosinophil migration to the lungs while increasing the relative percentage of activated macrophages. This suggests a greater role of phagocytes in attempting to control the infection. Therefore, IL-22 production aids in lymphocyte recruitment and regulates the hematopulmonary barrier, limiting systemic fungal dissemination and attenuating the pathological condition. **Keywords:** Cryptococcus gattii;Th17 profile;IL-22.

DO - 008 - **The antigen effect over immune response modulation by IFN-γ-stimulated-FRCs derived from human lymph nodes**

FERREIRA, B.D.O.; DO NASCIMENTO, S.; ALOIA, T.P.A.; ROCHA, F.A.; SILVEIRA, C.R.F.; FLATOW, E.A.; RIZZO, L.V.; SEVERINO, P.; MARTI, L.C.. HOSPITAL ISRAELITA ALBERT EINSTEIN, HOSPITAL ISRAELITA ALBERT EINSTEIN SÃO PAULO - SP - BRASIL.

Background: Fibroblastic reticular cells (FRCs) have been shown to modulate expansion of antigen-specific T cells. However, how IFN-γ-stimulated-FRCs are able to modulate T cells proliferation while not disrupting antigen clearance it is mostly unknown. **Aims:** Investigate the FRCs modulation of T cells proliferation in presence or absence of an antigen. **Methods:** FRCs were isolated from four human lymph nodes (LN), cultured as plastic adherent cells, fully characterized and cell sorted by flow cytometry. FRCs were cultured, treated with IFN-γ (100ng) and microarray analysis was performed. The genes up-regulated by IFN-γ treatment were validated by protein expression and functional tests in presence or absence of antigen (inactivated *E. coli)*. **Results:** IFN-γ-stimulated FRCs up-regulated genes from pathways as endocytosis, peripheral tolerance and pathogen recognition including HLA-DR and CD5L (ligand of CD36). Antigen presence abrogated the modulation exerted by FRCs and IFN-γ treated FRCs over the T cells proliferation. The antigen presence did not alter the expression of PD-L1, PD-L2 or HLA-ABC at protein level. HLA-DR was down regulated in the antigen presence while Rab20, CD5L and CD36 proteins were up regulated. Further, antigen was co-localized in FRCs with CD36 and CD5L. The antigen presence decreased levels of IFN-γ added in cell culture supernatant in lower degree than in their absence, suggesting reduction in FRC-IFN-γ receptors antigen-related. **Conclusions:** Our data suggest that FRC affect T cell proliferation depending on the presence of an antigen. FRCs stimulated by IFN-γ are able to up-regulate genes and proteins related to antigen processing and presentation, which may give them the ability of interfering over T cells proliferation. Instead, the presence of an antigen retracts this ability. We believe that the axis involving CD5L/CD36 is the pathway activated by the antigen that abrogates FRC's effect over T cells proliferation. **Keywords:** Fibroblastic reticular cells;immune modulation;antigen, IFN-γ.

DO - 009 - **ULTRA-FAST PROTOCOL FOR CAR-T CELL GENERATION**

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CAR-T cell therapies are a breakthrough in cancer treatment, which has a great response in B-ALL patients but very expensive. Basically, this therapy occurs with leukapheresis to remove the cells, taken to specialized laboratories, activated to proliferate, genetically modified with viral vectors, expanded for about 12 days, and returned to the hospital – a process that can take up to 30 days. In this work, we propose an ultra-fast protocol to decrease the time, cost, and complexities of CAR-T cell therapy. We use transposon-based non-viral vectors called Sleeping Beauty(SB) or PiggyBac (PB) that allows modifying non-activated cells. We insert CAR into T cells and in less than 24h and further use these cells to treat leukemia bearing mice. PBMC were isolated and electroporated with plasmid encoding SB-19BBz CAR and SB100x transposase. CAR expression on day 1 ranged between 5-15% in all experiment with SB. NSG mice were injected iv. $5x10^6$ RS4;11 or 10^5 Nalm6 and after 3 days were treated with recently electroporated CAR-T cells. Both mice models (RS4;11 and Nalm6) treated with our protocol to produce CAR-T cells (doses of $1x10^5$ and $7x10^5$, respectively) showed improved survival when compared to controls. Decreased tumor burden in several organs was also observed. Head-to-head comparison of 19BBz cells used in ultrafast protocol or expanded for 8 days, showed similar antitumor activity, leading to equivalent improvements of survival. After that, we added an IL15 membrane receptor (mIL15) to the PB-based CAR cassette to improve cell persistence and survival. We noticed that the tumor burden evaluated by bioluminescence was lower and the survival of animals that had 19BBz-mIL15 was better when compared to 19BBz control (dose of $3x10^5$). We can conclude that our proposed ultrafast protocol to CAR cell therapy can be explored as an alternative with less cost, time, and complexities. Furthermore, mIL15 added to CAR appears to bring benefits in fighting tumor in animal model. **Keywords:** Immunotherapy;CAR-T cells;Ultra-fast protocol.

DO - 010 - **Single-cell atlas reveals immunophenotypes associated with clinical outcome and enrichment of CD8 memory cells in adjacent normal tissue of pancreatic cancer**

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and lethal tumor. PDAC also shows poor response to many conventional treatments and is refractory to immunotherapy. We hypothesize that these challenges arise from the highly heterogeneous and immunosuppressive tumor microenvironment (TME) in PDAC. Therefore, to comprehend the players behind this immunosuppressive TME we integrated 10 publicly available datasets into a robust PDAC scRNA atlas covering 201 patients (159 PDAC, 24 non-cancer, and 18 metastatic PDAC). The TME showed distinct composition for each tissue, predominating a fibro-inflammatory phenotype with infiltrating Tregs and dysfunctional CD8 T cells in primary PDACs. In contrast, adjacent normal tissue exhibited enrichment of memory CD8 T cells (FDR<0.1). Metastatic PDAC was characterized by a decrease in stromal cells combined with infiltration of myeloid and naive T cells. Comparison between treated and untreated PDAC revealed enrichment of cancer-associated fibroblasts (CAFs) in treated patients. Besides, T cells from treated patients exhibited higher levels of heat shock genes which has been linked to immunotherapy resistance. Next, we stratified patients based on TME cell composition. Unsupervised clustering revealed 6 clusters: Desert (high tumor cell fraction); Fibrotic (high fraction of CAFs); Normal-like (high fraction of acinar and normal ductal cells); M-enriched (high fraction of myeloid cells), Immune-CD8_high and Immune-CD8_low. The Immune-CD8_high cluster was associated with a good prognosis in a dataset (n=43) present in our atlas. Then, we performed bulk RNA deconvolution of the TCGA cohort (n=145) and were able to recover almost all (except for M-enriched) previously identified clusters. Survival analysis showed better overall survival for the Immune-CD8_high group ($p<0.05$). Collectively, our study identifies distinct features of immune escape in PDAC and unmasks the impact of TME composition on patient prognosis. **Keywords:** Immunotherapy;Adenocarcinoma;scRNA.

DO - 011 - **ROLE OF IKAROS-HDAC COMPLEX IN REGULATING MICRO-RNA AND APOPTOSIS PATHWAY IN MURINE B-1 CELLS**

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Introduction. B-1 lymphocytes are innate B cells capable of self-renewal and recognition of self-antigens, which could facilitate their differentiation into hyperproliferative cells. Interestingly, murine-B-1 cells are very similar, functional and phenotypically to B leukemic cells (B-CLL) in chronic lymphocytic leukemia (CLL). Among the molecules involved in the pathogenesis of CLL, the transcription factors of the Ikaros family (Ikzf1) are deregulated. The main form of Ikaros activity is through association with complexes, which involve the presence of histone deacetylases (HDAC). In the context of CLL, it has been described that HDACs are mostly overexpressed, so our hypothesis is that this may influence the expression of microRNAs (miRNAs) and culminating in the regulation of apoptosis. **Methods and Results.** Murine B-1 cells were obtained from the peritoneum of C57BL/6 mice followed by enrichment by cell sorting. Firstly, interaction of Ikaros and HDAC-1 was confirmed by immunofluorescence and coimmunoprecipitation. Next, Ikaros silence by siRNA increases the cell viability, HDAC1 and c-Myc expression. We also observed an increase in bcl-2, cytochrome-c and caspase-3 expression and no difference in Bax and MCL-1. The array of miRNAs revealed overexpression of 25 miRNAs and low expression of 9 miRNAs. It was interesting observed that HDAC inhibition in Ikaros-silenced cells was able to revert the increase in thecell viability. **Conclusion.** This information confirmed that Ikaros participates in the regulation of molecules involved in B-1 cell survival and further, this involves HDACs and c-Myc molecules. **Keywords:** chronic lymphocytic leukemia;Ikaros;B-1 cells.

DO - 012 - **Pak-2 is involved in the activation of several inflammasomes**

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Inflammasomes are cytosolic multiprotein complexes assembled in the cytosol upon sensing cellular stress and pathogenic microorganisms, and activate inflammatory caspases to produce cytokines and to induce pyroptotic cell death. Inflammasomes play beneficial roles in the host immune response, but dysregulated inflammasome activation is associated with numerous inflammatory diseases. Inflammasome activation is regulated in many levels by posttranslational modifications, but the full mechanism is not fully understood. The aim of this work was to identify new proteins involved in NLRC4 inflammasome activation. For this, we used the BioID technique, followed by mass spectrometry and identified p21-activated kinase 2 (Pak2) as an important player in inflammasome activation. Pak2 is involved in the activation of NLRP3, NLRC4, AIM2 and the non-canonical inflammasome. Pak2 deficiency impairs ASC speck formation, caspase-1 activation and IL-1b secretion. *In vivo*, Pak2 inhibition increases susceptibility to *Legionella pneumophila* infection and improves clinical symptoms of experimental autoimmune encephalomyelitis. Our findings establish Pak2 as an important inflammasome regulator and a new potential target for the treatment of inflammasome-mediated diseases. **Keywords:** inflammasome;Legionella pneumophila;Pak2.

DO - 013 - **Putative role of non-canonical autophagy machinery in the control of HiF-1α function**

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Cell compartmentalization is an intrinsic component of the signaling cascades triggered upon engagement of pattern recognition receptors by innate immune cells to ensure effective responses to a pathogen. For instance, lipopolysaccharide (LPS) of Gram-negative bacterial wall activation of TLR4 on cell surface activates NF-KB through the adaptor MyD88 . LPS also trigger TLR4 endocytosis, leading to TRIF-dependent type I interferon (IFN) production and autocrine signaling [Cell, 147(4): 868–880, 2011]. Of note, non-camonical recruitment of components of the autophagy machinery to compartments of endolysosomal pathway affect macrophage responses [Cell, 178, 536–551, 2019]. Herein, we show that non-canonical autophagy adaptor Rubicon (RUBCN), a molecule recruited to the endocytic compartments, regulates macrophage function in response to TLR4 signaling. Using bone marrow-derived macrophages (BMDM), we show that RUBCN ablation impairs the synthesis of IL-6, pro-IL-1b but not of TNF-a, in response to LPS. Mechanistically, we found that LPS or *Escherichia coli (E.coli)* stimulation required RUBCN to induce synthesis and stabilization of HIF-1a, a hypoxia stress sensor known to regulate macrophage glycolytic metabolism upon TLR4 engagement. Accordingly, the expression of glycolytic enzymes were reduced in *Rubcn*$^{-/-}$ BMDM in response to LPS. In sepsis model induced by *E.coli* infection, we observed that RUBCN knockout mice were protect from death compared to wild type. Our preliminary data indicates that non-canonical autophagy regulates macrophage responses to LPS, suggesting that recognition of compartmentalization participates in macrophage metabolic shift in response to inflammatory stimuli. Besides that, our in vivo data suggest that non-canonical autophagy play an important role for contributing to mice survival in sepsis model induced by *E.coli* infection. Funding: FAPESP (18/25559-4, 19/26311-9). **Keywords:** HIF-1a;Endocytosis;Rubicon.

DO - 014 - **IMPACT OF STING SIGNALING PATHWAY ON THE DIFFERENTIATION AND FUNCTION OF REGULATORY T CELLS**

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The protein STING comprises the intracellular DNA-sensing machinery, promoting the activation of transcription factors such as those involved in the production of type I IFNs. Although STING is widely described in innate immune cells, little is known about its role in lymphocytes. The present study aims to investigate the role of STING on the differentiation and function of Tregs, a T cell subpopulation that has a central role in the immune system's homeostasis and prevention of exacerbated inflammatory responses. Naive T cells were isolated from WT and STING KO mice and cultured under iTreg polarizing conditions in the presence or not of STING agonists. Additionally, peripheral regulatory T cells were isolated from Foxp3-Cre^{YFP} e Foxp3-Cre^{YFP}*Tmem173*$^{fl/fl}$ mice and cultured in vitro with IL-2 or IL-2 + IL-6, with or without STING agonists. The impact of STING activation on the differentiation, stability, and function of Tregs was assessed by flow cytometry and/or RT-qPCR, ELISA, and Western Blot. Our findings so far demonstrate that STING expression increases during the differentiation of Treg cells in vitro. Upon activation, STING triggers the canonical pathway, evidenced by the phosphorylation of STING, TBK1, and IRF3. The activation of STING enhances the differentiation of Treg cells and upregulates key regulatory molecules such as Foxp3 Nt5e, Pd1, Gitr, and Areg. Notably, STING activation also improves Treg cell stability, as demonstrated by sustained FoxP3 expression even in the presence of the Treg-destabilizing cytokine IL-6. Interestingly, the effects of STING activation are independent of SMAD2/3 phosphorylation but rely on AhR activation, as evidenced by the reversal of increased FoxP3 expression upon AhR inhibition. In conclusion, our study suggests a role of STING in Treg cell generation and function, revealing that STING is a potential candidate for Treg-targeted therapies. **Keywords:** Regulatory T Cells;STING;Autoimmunity.

DO - 015 - **IMPACT OF INTESTINAL INFLAMMATION ON THE KIDNEYS OF MICE SUBMITTED TO AN EXPERIMENTAL MODEL OF CHRONIC COLITIS**

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Introduction: Kidney diseases (KD) have an impact on the intestine, such as the production of uremic toxins, which can lead to inflammation and increased intestinal permeability. However, the reverse role remains poorly explored. Therefore, the aim of this study is to evaluate the influence of intestinal inflammation on the kidney. **Materials and Methods:** C57BL/6 (n=5/group) mice were subjected to three cycles of 2% dextran sulfate sodium (DSS) dissolved in water (each cycle consisted of 5 days of DSS treatment interspaced by 4 days of normal water). The Disease Activity Index (DAI) was determined based on weight loss, stool consistency, and rectal bleeding. Colonoscopy was performed before initiating DSS treatment and at the end of each cycle for macroscopic analysis of the intestinal mucosa. Renal function was evaluated by measuring serum creatinine levels and urea levels. The frequency of resident and infiltrating immune cells in the kidneys was assessed by flow cytometry. **Results**: As expected, animals treated with DSS showed a cumulative DAI of 30.6, compared to 0.8 in the control group ($p< 0.0001$). On the last day of each cycle, colonoscopy revealed the intestinal mucosa exhibited translucent appearance with visible blood vessels. The DSS-treated group exhibited reduced levels of serum urea compared to untreated group (32.27 vs 24.98 mg/dL in the control group; $p= 0.0002$), while there was no difference in the serum creatinine levels. Analysis of kidney-immune cells showed that the percentage of resident renal macrophages was decreased in the DSS group (80.28 vs 72.74% in the control group; $p= 0.0285$), while the percentage of monocytes (6.5 vs 9.1%; $p= 0.0268$) and CD8+ T lymphocytes was increased (19.6 vs 28.5%; $p= 0.0103$). **Conclusion**: Chronic gut inflammation changes the profile of immune cells in the kidney. This may indicate increase in susceptibility to develop inflammation-mediated KD. **Funding**: FAPESP (2022/08362-8; 2019/14755-0 **Keywords:** Kidney;Gut;Intestine-kidney axis.

DO - 016 - **Intratumoral delivery of mRNA-TRAIL via Ionizable Lipid Nanoparticles reduces Colon Cancer and Drive Robust Immune Response**

DA SILVA, W.N.[1]; COSTA, P.A.C.[2]; PRAZERES, P.H.D.M.[3]; FERREIRA, H.A.S.[2]; DE CAMPOS, C.L.V.[4]; CARVALHO, K.A.B.[5]; SANTIAGO, H.D.C.[4]; LITWINSKI, V.V.C.[6]; BARCELOS, L.D.S.[2]; TEIXEIRA, M.M.[4]; FRÉZARD, F.J.G.[2]; GUIMARAES, P.P.G.[2]. 1. FEDERAL UNIVERSITY OF MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 2. DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. DEPARTMENT OF PATHOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 4. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 5. GRUPO INTEGRADO DE PESQUISAS EM BIOMARCADORES, INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE - MG - BRASIL; 6. DEPARTMENT OF MORPHOLOGY, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BELO HORIZONTE - MG - BRASIL.

Immunotherapy has revolutionized cancer treatment by harnessing the immune system to enhance antitumor responses while minimizing off-target effects. Among the promising cancer-specific therapies, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has attracted significant attention. Here, we developed an ionizable lipid nanoparticle (LNP) platform to deliver TRAIL mRNA (LNP-TRAIL) directly to the tumor microenvironment (TME) to induce tumor cell death. Our LNP-TRAIL was formulated via microfluidic mixing and the induction of tumor cell death was assessed in vitro. Next, we investigated the ability of LNP-TRAIL to inhibit colon cancer progression in vivo in combination with a TME normalization approach in NOD/SCID mice. Our results demonstrated that LNP-TRAIL induced tumor cell death in vitro and effectively inhibited colon cancer progression in vivo, particularly when combined with TME normalization induced by treatment with losartan. Importantly, TME normalization improved the delivery and efficacy of LNP-TRAIL in the TME and increased the infiltration of cytotoxic CD8+ T cells. In addition, potent tumor cell death as well as enhanced apoptosis and necrosis was found in the tumor tissue of group treated with LNP-TRAIL combined with TME normalization. Together, our data demonstrate the potential of the LNP to deliver TRAIL mRNA to the TME and to induce tumor cell death, especially when combined with TME normalization. Therefore, these findings provide important insights for the development of novel therapeutic strategies for the immunotherapy of solid tumors. **Keywords:** immunotherapy;mRNA-TRAIL;lipid nanoparticle.

DO - 017 - **Sand fly yellow salivary proteins modulate neutrophil and macrophage responses to Leishmania parasites**

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During the sand fly blood meal, *Leishmania* parasites vector, components derived from the insect or parasite, such as saliva and exosomes, are regurgitated in the site of the bite and have the ability to modulate the vertebrate host immune response [1,2]. After the bite, neutrophils are the first cells recruited to the site of infection [3]. Bites of infected or uninfected sand fly leads to the same recruitment of neutrophils, suggesting that factors derived from the insect are responsible for that effect. Our group demonstrated that saliva of *Lutzomyia longipalpis* and *Phlebotomus duboscqi* have a family of proteins (yellow proteins) that act as chemokines, directly recruiting neutrophils. In addition, we observed that yellow proteins exacerbate infection *in vivo* [4]. Here, we evaluated the *in vitro* activation status of human neutrophils infected or not with *L. major* or *L. infantum* and incubated in the presence or absence of yellow proteins of their respective vectors; yellow proteins PduM10 and PduM35, from *P. duboscqi*, and LJM11 and LJM17, from *L. longipalpis*. Our results showed that yellow proteins increased the survival rate of parasites in the presence of treated neutrophils. Higher numbers of parasites were also found in cultures of monocytes and macrophages treated with the proteins. PduM10 and PduM35 or LJM11 and LJM17 partially inhibited neutrophil extracellular traps release. PduM10 and PduM35 increased elastase release. Furthermore, supernatants of neutrophils treated with yellow proteins induced increased survival of *Leishmania* in macrophage cultures. These data indicate that yellow proteins have the ability to modulate neutrophil and macrophages responses, favoring *Leishmania* infection. Data were analyzed using One-way ANOVA test and $p < 0.05$ was considered statistically different. References: 1. Infect Genet Evol. 28:691-703, 2014; 2. Front Immunol. 3:110, 2012; 3. Science. 321:970-974, 2008; 4. Nat Commun. 12(1):3213, 2021. **Keywords:** Leishmania;sand fly saliva;neutrophil.

DO - 018 - **Nasal administration of anti-CD3 mAb (Foralumab) downregulates NKG7 and increases TGFB1 and GIMAP7 expression in T cells in subjects with COVID-19**

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T cells are present in the early stages of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and play a major role in disease outcome and long-lasting immunity. Nasal administration of a fully human anti-CD3 monoclonal antibody (Foralumab) reduced lung inflammation as well as serum IL-6 and C-reactive protein in moderate cases of COVID-19. In a randomized trial, mild to moderate COVID-19 outpatients received nasal Foralumab (100 µg/d) given for 10 consecutive days and were compared to patients that did not receive Foralumab. By employing proteomics and RNA sequencing, we describe an increase in naïve-like T cells in Foralumab-treated subjects and a decrease in $NGK7^{+}$ T cells. Additionally, in Foralumab-treated subjects, a proinflammatory gene module characterized by *CCL5*, *IL32*, *CST7*, *GZMH*, *GZMB*, *GZMA*, *PRF1*, and *CCL4*, was downregulated in T cells, and *CASP1* being downregulated in T cells, B cells, and monocytes. The downregulation of effector functions in known cell types corresponded with an upregulation of *TGFB1* and GTP-binding gene GIMAP7 treated subjects. Conversely, Rho/ROCK1, a downstream pathway of GTPase signaling was downregulated in treated subjects. Finally, Foralumab-treated COVID-19 subjects exhibited similar changes to that of treated MS patients, and mice treated with nasal anti-CD3. Taken together, these findings yield a potential route to treat COVID-19 through which nasal Foralumab can modulate the inflammatory response in the disease. **Keywords:** COVID-19;Immunotherapy;T cell.

DO - 019 - **HIF-1α interaction with HIF-1A antisense long non-coding RNA shift response in macrophages during Leishmania infantum infection**

ZANATTA, J.M.; FERNANDES, J.C.R.; ACUÑA, S.M.; TEIXEIRA, C.A.; POLONIO, C.M.; FRANCISCO, T.; DE OLIVEIRA, L.G.; PERON, J.P.S.; MUXEL, S.M.. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO SÃO PAULO - SP - BRASIL.

Recently, lncRNAs are implicated in regulation of metabolic reprogramming of glycolysis in cancer cells. The activation of HIF-1α contributes to the Warburg effect through a switch from oxidative phosphorylation to glycolysis. In this work, we investigated the correlation of HIF1A and HIF-1α antisense long non-coding RNAs (HIF1A-AS2, HIF1A-AS3) gene expression in macrophages infected with *Leishmania infantum* or stimulated with TLRs agonists. We analyzed the HIF1A, HIF1A-AS2, HIF1A-AS3 gene expression in human THP-1-derived macrophages infected with *L. infantum NCL* (MOI 5:1) or stimulated with LPS (100ng/mL), poly(I:C) (100ng/mL) or gardiquimod (500ng/mL) for 4 and 24 h by RT-qPCR. We evaluated the HIF-1α expression in macrophages infected with L. infantum or TLRs agonists by flow cytometry. Next, we investigated the impact of HIF1A-AS3 knockdown and overexpression to infection and gene modulation using CRISPR-Cas9 strategy. In THP-1 macrophages infected with *L. infantum*, we observed reduced levels of HIF1A, HIF1A-AS2 and HIF1A-AS3 after 4 and 24h. Interestingly, LPS stimulation of macrophages increased the levels of HIF1A, HIF1A-AS2 and HIF1A-AS3 after 4 and 24h, but not with poly(I:C), and gardiquimod stimuli. Also, TLR3, TLR4 and TLR7 stimulation of THP-1 macrophages with poly(I:C), LPS and gardiquimod, respectively, modulated the genes hexokinase II (HKII), pyruvate dehydrogenase kinase 1 (PDK1), pyruvate kinase M2 subtype (PKM2) and lactate dehydrogenase A (LDHA) expression.The *L. infantum* infection modulated these genes in a distinct way.The infection and LPS stimulation increased the frequency of HIF-1α+ macrophages. The knockdown and overexpression of HIF1A-AS3 regulate HIF1A and PDK1 expression. Our initial data suggest that infection of human macrophages with *L.infantum*, reduces HIF1A, HIF1A-AS2 and HIF1A-AS3 expression, working in a distinct way of LPS and in TLR-related stimulation by poly(I:C), LPS and gardiquimod. **Keywords:** leishmania;glycolysis;lncRNAs.

DO - 020 - **Vaccination With Recombinant Listeria Monocytogenes Induces An Effective Anti-Tumor Immune Response In Necroptosis- And Pyroptosis-Deficient Mice**

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Effective induction of CD8+ T cell-mediated anti-tumor immunity is a major goal during the development of novel anti-cancer vaccine strategies. Live recombinant vectors such as Listeria monocytogenes have been tested in preclinical setups and proved to induce a strong in vivo CD8+ T cell response. Importantly, Listeria was shown to induce pyroptosis in immune cells, and inflammatory/immunogenic cell death programs, such as pyroptosis and necroptosis, positively regulate immune responses. Therefore, we hypothesized that a deficiency in regulatory or effector molecules involved in necroptosis or pyroptosis would interfere with the host response to Listeria and the consequent stimulation of CD8+ T cell mediated immunity. To test our hypothesis, we vaccinated Wild-type (WT), caspase-1/11-/-, gsdmd-/-, ripk3-/-, and mlkl-/- C57Bl/6 mice with recombinant L. monocytogenes carrying the ovalbumin gene (LM.OVA). In vivo cytotoxicity assay was carried out to evaluate the efficiency of target cell elimination by OVA-specific CD8+ T lymphocytes. In addition, the in vivo growth of B16 and B16.OVA melanoma cell lines was measured in vaccinated and control mice. Our results showed that although Caspase-1/11 and GSDMD participate in the control of LM.OVA infection, a single deficiency of either Casp-1/11, GSDMD, RIPK3, or MLKL did not impair the LM-OVA-induced antigen-specific anti-tumor response. **Keywords:** Vaccines;CD8+ T Lymphocytes;Cell death.

DO - 021 - **Humoral Responses Among Individuals Infected With Wild-Type Yellow Fever Virus And Vaccinated with 17DD Yellow Fever Vaccine**

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Human yellow fever virus (YFV) infection is a severe disease with a high lethality rate, even though it is vaccine-preventable. Between 2018-2019, an outbreak of YFV infection occurred in the State of São Paulo, Brazil, where the vaccine coverage was low, leading to a 36% case-fatality rate. As a strategy to contain new cases, mass vaccination campaigns with the live-attenuated 17DD vaccine were implemented in areas where the wild-type (wt) virus was already circulating, which resulted in the vaccine being also administered to individuals who were pre-symptomatic or in the early stages of YFV infection. Our study aimed to investigate the impact of pre-symptomatic or early-stage vaccination on humoral responses following wt infection in the disease outcome, as well as to explore the occurrence of coinfection with 17DD/wt-YFV. We analysed 73 YFV cases diagnosed during the outbreak, with 55 unvaccinated patients, and 18 vaccinated with 17DD between six days before symptoms onset and two days after symptoms onset. The unvaccinated group had 45,5% case-fatality rate, while only 11,1% of the vaccinees died. The vaccinated group had higher neutralizing anti-YFV antibody titres than the unvaccinated group ($p = 0.027$). We found no significant differences in viral load between groups. Moreover, the 17DD YFV strain was not detected in plasma samples. Our data suggest that vaccination with 17DD in earlier stages of YFV infection may lead to development of potent neutralizing antibodies and decreased mortality, even in an ongoing outbreak scenario. Although the wt-YFV replication prevailed among vaccinated participants, mass vaccination campaigns can improve disease outcomes, indicating that early vaccination may be a handy strategy to control future YFV outbreaks. **Keywords:** Yellow fever;Humoral immunity;Neutralizing antibodies.

DO - 023 - **CANNABINOID RECEPTOR TYPE 2 AGONIST GP1a ATTENUATES MACROPHAGE ACTIVATION INDUCED BY M. BOVIS-BCG BY INHIBITING NFKB SIGNALING**

VALERIANO, J.D.P.[1]; SILVA, M.A.[2]; BARROS, R.C.M.[3]; PEREIRA-DUTRA, F.[2]; ROSAS, E.C.[2]; VIOLA, P.T.B.[2]; HENRIQUES, M.D.G.M.D.O.[4]; SILVA, M.F.D.S.C.[5]. 1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO NITERÓI - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL FLUMINENSE, UNIVERSIDADE FEDERAL FLUMINENSE RIO DE JANEIRO - RJ - BRASIL; 4. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO RIO DE JANEIRO - RJ - BRASIL; 5. UNIVERSIDADE FEDERAL FLUMINENSE, UNIVERSIDADE FEDERAL FLUMINENSE NITERÓI - RJ - BRASIL.

The cannabinoid receptor type 2 (CB2) is mostly expressed in immune cells. Studies have demonstrated that its activation can influence the course of inflammatory diseases, being a potential target to modulate the immune system. Tuberculosis is one of the leading causes of death worldwide and a major public health problem. Immune evasion mechanisms and antibiotic resistance highlight the need to better understand this disease and explore alternative treatment approaches. *M. tuberculosis* can modulate macrophages response and metabolism of the cell to persist and proliferate inside the cell. Therefore, our study aims to evaluate the effects of the CB2-selective agonist GP1a on *M. bovis* BCG-induced macrophage activation. For that, we pretreated J774A.1 macrophages with GP1a for 30 min, followed by the stimulation with irradiated *M. bovis* BCG (iBCG) at MOI 3 for 1 hour. We observed the increased expression of CB2 in macrophages stimulated with iBCG within 1 and 6 hours. Additionally, GP1a-treatment (10uM) inhibited the iBCG-induced production of inflammatory mediators (TNFa, PGE-2, Il-10, IL-6, nitrite, and COX2) by macrophages. GP1a-treatment also reduced the transcription of pro-inflammatory genes (*inos, il1b, cox2*) and genes related to lipid metabolism (*dgat1, acat1, plin2, atgl, cd36*), measured by qPCR. Indeed, the lipid droplet accumulation and PPARy activity were reduced by GP1a-treatment. Finally, GP1a-treatment reduced the activation of inflammatory signaling pathways (NF-kB, ERK 1/2, and p38), but not JNK. In conclusion, the activation of CB2 by GP1a modulated the macrophage response to iBCG by reducing inflammatory mediators. GP1a also affected lipid metabolism and downregulated inflammatory signaling pathways. These findings highlight the potential of CB2 agonists as therapeutic targets for tuberculosis and drives the need for further investigation. **Keywords:** tuberculosis;cannabinoid;macrophage.

DO - 024 - **Therapeutic effect of roflumilast, a selective PDE4 inhibitor, in a murine model of coronavirus infection**

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In 2019 a new betacoronavirus known as severe acute respiratory syndrome 2 (SARS-CoV-2) emerged. The disease associated with its infection, named coronavirus disease 19 (COVID-19), is characterized by pneumonia, pulmonary infiltrate, acute respiratory failure, and sepsis, which can lead to the patient's death. Several experimental models have been developed to study COVID-19 pathogenesis. Murine hepatitis virus (MHV), a betacoronavirus, naturally infects mice and induces a lung inflammatory disease that mimics some aspects of COVID-19. Since COVID-19 pathogenesis is partially due to the imbalance of the host's inflammatory response to SARS-CoV-2 infection, immunoregulatory therapies represent potential treatments. Inhibition of phosphodiesterase 4 (PDE4), an enzyme responsible for the degradation of the anti-inflammatory second messenger cyclic AMP (cAMP), represents an important inducer of these immunoregulatory pathways. Here, we demonstrated that treatment with roflumilast, a selective PDE4 inhibitor, in a dose of 10 mg/kg, administered 2 days post infection, reduced histopathological score and chemokines levels in the lung of MHV-3 infected C57BL/6 (CEUA 157 / 2021). Meanwhile, roflumilast treatment in a dose of 1 mg/kg roflumilast did not affect these parameters. In contrast, the administration of roflumilast as a prophylactic treatment resulted in an increase in lung histopathological score in animals infected with MHV-3. The combination of 1 mg/kg of roflumilast and 0.1 mg/kg of dexamethasone,which individually do not affect the disease outcome, reduced lung histopathological score in animals infected with MHV-3. In conclusion, we demonstrate that roflumilast treatment has a dose- and time-dependent effect in ameliorating lung inflammation induced by MHV and that roflumilast and dexamethasone combined treatment has a synergistic effect in ameliorating lung injury associated with MHV infection. **Keywords:** MHV;Roflumilast;Anti-inflammatory.

DO - 025 - **Temporal dynamics of murine brain lymphocytes distribution during life**

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The concept of brain immune privilege has been revised considering the findings of the presence of lymphocytes and their possible functions in the brain. The characterization of the lymphopoietic niche in the cerebral meninges has raised interest in the importance of these cells for brain functioning and immunosurveillance. However, little is known about how and when lymphocytes begin to populate the brain and these cells remain in this place, especially in the aging. The aim was to characterize the presence of B/T cells during brain development and identify the location of these cells. Also characterize the molecules that modulate their presence and activity. C57BL/6 mice P3, P21, 3m, 18m and 24m old of both sexes were used. Brains were analyzed by confocal microscopy. Flow cytometry was used to further characterize the lymphocyte populations. Chemokine and cytokine multiplex kit was used.
Antibodies to CD3 and B220 were used to identify T and B cells, respectively, in different regions of the mouse brain. B cells are present from P3 until adulthood when their numbers decrease, followed by a slight increase in aging. In contrast, there are fewer T cells during early postnatal in comparison to the adult brain. Using laminin as a marker of blood vessels, we found T and B cells associated with the vessels, but also some T cells in the brain parenchyma. FACS analysis demonstrated that B-1a lymphocytes have the same profile, while B-1b has the inverse one, decreasing with aging. CD4 T cells are more abundant in aging than young animals. Memory T cells increase at aging. Pro-inflammatory cytokines TNF-a and IL-16 increase while IL-1b and GMCSF decrease. IL-10 is stable. Notably, CXCL13 and CCL5 are upregulated in aging brains.
Lymphocyte distribution is dynamic during brain development, present in all brain regions and modulated by different cytokines and chemokines especially in aged brain. **Keywords:** Neuroimunologia;citocinas;linfócitos.

DO - 026 - **UNDERNUTRITION ALTERS EXPRESSION OF SPLENIC EXTRACELLULAR MATRIX COMPONENTS AND MODIFIES SPLENOCYTES LOCATION IN MICE INFECTED WITH LEISHMANIA INFANTUM**

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Undernutrition is a risk factor for the development of visceral leishmaniasis (VL). Our group demonstrated that protein undernutrition alters the number of splenocytes, the microarchitecture and the immune response in the spleen of infected animals, suggesting that the distribution and localization of T and B lymphocytes in the organ, as well as the expression of molecules involved in cell migration may be compromised. To assess this, we analyzed the distribution, location and activation of subpopulations of lymphocytes in the spleen of undernourished mice infected with *Leishmania infantum*, as well as the expression of extracellular matrix (ECM) molecules in this organ. The animals were fed a control diet (14% protein) or a low protein diet (4% protein). After seven days of diet, each group was divided into two and one of them was infected with *L. infantum*, resulting in four experimental groups: CP, LP, CPi and LPi. After 14 days of infection, the animals were euthanized and the spleen was analyzed. Since tissue organization drives cells location and this in turn determines their correct activation, we evaluated whether undernutrition would alter location, number and proliferation of T and B lymphocytes. We observed significant increase of CD4+ T cells in these cells in the follicular region of LP and LPi animals. Furthermore, we observed a significant increase in CD8+ T cells in the red pulp of LPi animals. While CPi animals show a significant decrease in B220+ cells, LPi animals show a significant increase in the percentage of positive area for this marker in the splenic red pulp. We also observed a significant decrease in the cell proliferation profile in the spleen of LP and LPi mice and a significant and early increase in the splenic parasite load of LPi animals. Together, our results suggest that splenic disorganization induced and aggravated by undernutrition compromises T-cell mediated control of parasites in this organ. **Keywords:** Protein undernutrition;Leishmania infantum;spleen.

DO - 027 - **Reconstruction of pasteurized donor's milk microbiota as an immune interventional strategy for premature newborns: molecular benefits revealed by a multiomic approach of a clinical trial**

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Human milk and its associated microbiota are extremely important especially for premature newborns (PNs). Pasteurized donor's milk (PDM) is the best option if mother's own milk (MOM) is not available, but pasteurization eliminates viable microorganisms reducing PDM benefits. We conducted a clinical study (ReBEC:RBR-729kr8x) to explore reconstructing the microbiome of PDM by adding 10% of MOM followed by incubation at 37◦C for 4 h. Eighty PNs (<32 weeks) and their mothers were randomly assigned either to receive PDM or reconstructed milk (RM). The microbiota of milk (PDM, RM and MOM) and PN's fecal samples were analyzed using 16S rDNA sequencing. Metabolomic profiles of PDM and RM milk were obtained by untargeted liquid chromatography-mass spectrometry, followed by feature extraction and statistical analysis in R and Metaboanalyst®. Interestingly, the microbiota analysis of individualized milk samples showed that the majority of RM samples exhibited a microbiota composition profile more closely to MOM's microbiota rather than to PDM after 4 hour incubation. In addition, the fecal microbiota of PNs that received RM exhibited significantly increased levels of Acinetobacter, Enterobacter, Novosphingobium, and Veillonella genera compared to PDM controls. Moreover, the metabolomic analysis of RM displayed increased area ratios (AR) of oxidized lysophospholipids (means: 0.05696 vs. 0.03980 in PDM), sphingomyelins (0.2436 vs. 0.1455), prostaglandin A1 levels (1.608 vs. 0.9654), tryptophan (0.08042 vs 0.03436) and indoleacrylic acid (0.1965 vs 0.08771) (p: <0.005). Notably, these metabolites play critical roles in signaling, inflammation regulation, and supporting gut microbiota and immune development. Our results highlight the potential use of RM to enhance immunological support for premature and non premature newborns. They also endorse a novel insight that highly improves the quality of PDM in a simple and fast manner that could be easily adopted in neonate care units. **Keywords:** Prematurity;Metabolites;Microbiome.

DO - 028 - **Targeting adrenergic receptors to mitigate iNKT-induced acute liver injury**

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Invariant Natural Killer (iNKT) cells are unconventional T cells responsive to glycolipid antigens presented by CD1d molecules. Upon stimulation with specific ligands the release of TNF-α and IFN-γ makes them targets for cancer immunotherapy. However, acute liver damage following iNKT activation limits therapeutical use. The adrenergic signaling (AS) can modulate immune cell activity through β2 adrenergic receptor (AR), leading to immunosuppression in tumor microenvironment. Considering the lack of knowledge regarding iNKT cell susceptibility to AS regulation and the relevance of this circuit to cancer management, we aimed to investigate the role of AS over iNKT cell responses and related liver injury. iNKT cells activation was analyzed *in vitro* in the presence of noradrenaline and with a model of *in vivo* hyperactivation of the AS (ARα2ac$^{-/-}$ mice), alongside the administration of AR agonists. Cytokine production was measured in mice serum and by intracellular staining. B16F10 melanoma model was used for assessment of anti-tumoral activity. Histological analysis, ALT and AST measurement were performed for evaluation of liver damage. Flow cytometry was used for analysis of cell dynamics in the lung and liver after iNKT activation. We showed that iNKT responses are not inhibited by AS. In fact, adrenergic stimulation was able to prevent acute liver damage while maintaining antitumoral responses, sustaining cytokine production by iNKT cells. Additionally, the AS inhibited the infiltration of myeloid cells into the liver after iNKT activation. Our data indicates that iNKT cells might not be as susceptible as conventional T lymphocytes regarding adrenergic activity attenuation and inhibition of myeloid cell recruitment could be responsible for hepatic protection. The use of adrenergic receptor agonists could be further studied as mean of improving iNKT-based immunotherapies, diminishing their side effects without jeopardizing their therapeutical efficacy. **Keywords:** iNKT cells;Adrenergic signaling;Liver injury.

DO - 029 - **B-1 CELL PROGENITOR AS A NEW CANDIDATE TO EXPLAIN B-1 CELL ACCUMULATION IN AGING AND DISEASE**

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In some experimental murine models, an accumulation of B-1 cells is observed during aging. However, it is not clear if this B cell population enlargement could be related to self-renewal of mature cells or proliferation of progenitor cells. In humans, Chronic Lymphocytic Leukemia (CLL) emerges in aging within accumulation of cells that resemble B-1 lymphocytes. Moreover, it was observed that a small percentage of B-1 cells from healthy mice survive to high doses of irradiation *in vitro* and these cells show CLL characteristics, such as downregulation of miR15a16, correlating B-1 cells and CLL. Due to a lack of knowledge of why B-1 cells accumulate in CLL, here we aim to comparatively investigate molecular characteristics of B-1 cell progenitor (B-1p) cells in young and middle-aged mice, to identify a possible mechanism that results in hyperproliferation of mature cells in aging and disease. We observed that the number of B-1 cell precursors in middle-aged mice (30 weeks) is higher than in young animals (8 weeks). These older cells have downregulation of several microRNAs, including miR15a16, miR181a and let7a, whereas Bcl-2 tends to increase. However, Bcl-2 protein is less phosphorylated in middle-aged precursors. It was also observed that aged B-1p cells survive more and present a higher proliferation index than young cells in vitro. When these cells were submitted to 3.5 Gy gamma-radiation, they showed radioresistance capacity, higher proliferation, downregulation of miR15a16 and higher secretion of IL-6. IL-6 secretion could also be related with downregulation of microRNAs miR181a and let7a found in the middle-aged irradiated group. Our results show that B-1p cells have age-induced molecular changes, augmented with irradiation, which could lead to malignancy. Thus, B-1 cell progenitors have the potential to be a new target to diagnose and treat hyperproliferative diseases in aging. Financial support FAPESP (2019/27009-4); Ethical Committee: 2020/1870040220. **Keywords:** B-1 cell progenitor;Leukemia;microRNAs.

DO - 030 - **Comprehensive characterization of the immune profile in short telomere syndromes**

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Telomere-biology disorders (TBDs) are caused by germline pathogenic variants in genes involved in telomere maintenance, leading to bone marrow failure, pulmonary fibrosis and cirrhosis. The aim of this study was to investigate the immune profile of patients with TBD, by deep-phenotyping their immune cells by CyTOF and assessing the levels of serum cytokines, chemokines and growth factors by Luminex. CyTOF data showed a lower proportion of $CD4^+$ population and an overall predominance of $CD8^+$ cells in patients with TBD. CD4/CD8 ratio is lower than in controls, and B cells count is also decreased. Naïve T and B cells and recent thymic emigrants were reduced in patients, with the accumulation of effector memory cells. T helpers are affected, with T_H1, T_H17 and $T_H17.1$ lymphocytes being decreased, whereas the T_H2/T_H1 ratio was increased in patients. Plasmacytoid DCs were reduced in TBD patients, while a double negative T cell population ($CD3^+CD4^-CD8^-Vd2^-Tbet^+Granzyme\ B^+$) was significantly increased. Frequency of activated effector memory and TEMRA $CD4^+$ cells expressing $CD38^+PD1^+TIGIT^{low}$ and switched memory B cells expressing $Ki67^+$ were higher, suggesting the occurrence of an immunogenic milieu, supported by the prevalence of $CD14^+CD16^+CCR4^+CXCR3^+$ monocytes. Prominent reduction of MAIT cells was observed, which suggests their migration to other tissues in response to inflammation. NK lymphocytes displayed a higher frequency of immature cells as compared to controls. Telomere length was positively correlated to a number of serum analytes, namely angiopoietin-1, IL-1 superfamily, IL-3, IL-4, IL-7, IL-23, IL-27, M-CSF, MCP-1, MIP-3a and PDGF-BB. Our data show that patients with TBD have $CD4^+$ immunodeficiency accompanied by skewing of helper T cells, accumulation of activated subsets expressing inflammatory markers and a dramatic decrease of circulating MAIT cells. Further steps shall unravel the role of the subpopulations shown here in the clinical onset of telomeropathies. **Keywords:** Telomeres;Bone marrow failure;Immunodeficiency.

DO - 031 - **Plasmodium berghei NK65 malaria induces neuroinflammation and promotes cognitive deficits in mice**

SANTOS, F.V.; LOPES, C.D.A.; SILVA, T.L.; SOUZA, J.L.N.; DUVAL, I.D.B.; SILVA, M.C.M.; BRITO, R.; RACHID, M.A.; FUJIWARA, R.T.; MAGALHAES, L.M.D.; BUENO, L.L.. UFMG, UFMG BELO HORIZONTE - MG - BRASIL.

In recent years, cognitive impairment has been documented in individuals from endemic regions affected by both uncomplicated and non-cerebral severe malaria. Nevertheless, investigations employing experimental models have yielded limited insights. Thus, we aim to investigate the impact of non-cerebral malaria infection on the development of behavioral alterations. For that, male C57BL/6j mice were intraperitoneally inoculated with 10^4 *Plasmodium berghei* NK65 (PbNK65)-infected erythrocytes. At 7-, 12-, and 20-days post-infection (dpi), mice were submitted to behavior analysis, to determine possible neurocognitive changes induced by infection. Afterwards, blood and brain samples were harvested to histopathological analysis, cytokine and neurotrophic factor quantification via ELISA, and cell phenotyping using multiparametric flow cytometry. The main findings show increased levels of IFN-γ at 7 dpi followed by increased production of TNF-α and IL-10 at 12 and 20 dpi in the periphery. Despite that inflammation, PbNK65-infected mice do not develop symptoms and typical lesions of cerebral malaria. Even so, the inflammation trigger by the parasite induces changes in the vascular cerebral permeability, which was sufficient to alter the brain microenvironment providing microglia activation, influx of $Ly6C^+$ monocytes, $CD4^+$ and $CD8^+$ lymphocytes, local production of IL-6, IL-12, IL-4 and IL-10, as well as increased N-acetylglucosaminidase activity of macrophages into the brain tissue, specially at 12 dpi. Furthermore, the infection leads to low levels of glial cell-derived neurotrophic factor (GNDF) and fractalkine (CX3CL1) were detected in both brain and peripheral blood. Nevertheless, the impact of PbNK65 infection in the brain homeostasis contributed to the low locomotor activity and memory deficit of infected animals. Taken together, our results provide new perspective on how systemic non-cerebral malaria can trigger neuroinflammation and behavioral changes in the host. **Keywords:** Plasmodium berghei NK65;Neuroinflammation;Neurocognitive deficits.

DO - 032 - **UNRAVELING THE SIGNIFICANCE OF N-GLYCOLYLNEURAMINIC ACID EVOLUTIONARY LOSS IN B CELL DYNAMICS AND ITS CORRELATION WITH THE INTESTINAL MICROBIOTA**

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Every organism presents a dense layer of glycoconjugates on its cell surface that are mostly terminated by two sialic acids isoforms: N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Unlike other mammals, humans lack Neu5Gc due to a mutation in the CMAH gene, which encodes the enzyme responsible for Neu5Gc synthesis, resulting in a different gut microbiome profile, pathogen interaction and immune cell dynamics. To further investigate the influence of Neu5Gc loss on B cell compartments, antibody repertoire and its potential correlation with intestinal microbiota, we first performed flow cytometry analysis of spleen and peritoneal B cells in WT and CMAH-null mice. We observed higher frequencies of B-1b and marginal zone B cells in the peritoneum and spleen of CMAH-null mice, respectively. Both populations comprise an immunoglobulin repertoire that contains self-reactive and pathogen reactive clones. Interestingly, CMAH-null mice presented lower immunoglobulin serum titers across all classes. Furthermore, evaluation of B cell repertoire by blotting culture supernatants (SNs) and mice serum against brain and muscle lysates showed increased binding of IgM from CMAH-null mice serum and SN to both WT and CMAH-null mice lysates, indicating that the absence of Neu5Gc enhances IgM reactivity against self-antigens. Knowing that gut microbiota directly interferes with the natural antibody repertoire, we next evaluated IgA bound-bacteria in fecal samples, revealing a higher number of IgA^+ bacteria in WT mice. Interestingly, the levels of IgA correlated with the levels of Neu5Gc present in the bacteria. Moreover, co-housing WT and CMAH-null mice resulted in increased binding of serum IgM from CMAH-null mice to muscle lysates, correlated to changes in gut microbiota alpha- and beta-diversity. Overall, these findings suggest that the evolutionary loss of Neu5Gc influences the natural antibody repertoire with a direct contribution from the gut microbiota. **Keywords:** Neu5Gc;Natural antibodies;Microbiota.

DO - 033 - **Phenotypic rhythm, integrative networks, and profile of T and B-cell subsets associated with distinct clinical outcomes of severe COVID-19 patients**

DE OLIVEIRA, G.[1]; ROCHA, I.A.D.C.[1]; CARVALHO, N.O.[1]; DOS SANTOS, T.M.A.F.[1]; CAMPI-AZEVEDO, A.C.[1]; PERUHYPE-MAGALHÃES, V.[1]; PRADO, R.O.[1]; MIRANDA, V.H.S.[1]; PEREIRA, A.A.S.[1]; ALVES, C.C.[1]; REIS, L.R.[1]; DA MATA, C.P.S.M.[2]; ANTONELLI, L.R.D.V.[1]; ALMEIDA, G.G.[1]; COELHO-DOS-REIS, J.G.[3]; DE CARVALHO, A.T.[1]; MARTINS-FILHO, O.A.[1]. 1. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS BELO HORIZONTE - MG - BRASIL; 2. HOSPITAL RISOLETA TOLENTINO NEVES, HOSPITAL RISOLETA TOLENTINO NEVES BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS - UFMG, UNIVERSIDADE FEDERAL DE MINAS GERAIS - UFMG BELO HORIZONTE - MG - BRASIL.

The pathogenesis of COVID-19 is associated with pre-existing comorbidities, and the patient's immune status may lead to distinct disease outcomes. The present study aimed to evaluate the kinetics of phenotypic profile, integrative networks, and characterization of T/B-cells in severe COVID-19 patients (n=87), categorized according to disease outcome, and healthy controls (n=13). Peripheral blood was collected at distinct timepoints (at baseline/D0; D7; D14-28, after inclusion) and used for *ex vivo* flow cytometry multiparametric immunophenotyping. Our data demonstrated a decrease in $CD3^+$ and $CD4^+$ T-cell frequencies and an increase in B-cells with mixed activation/exhaustion profiles at D7 and D14-28. The phenotypic rhythms observed along the kinetics timeline were characterized by pulse-like profiles for $CD3^+$ and $CD4^+$ and sigmoidal-like patterns for B-cells and $CD8^+$ T-cells. Higher changes in B-cell and $CD4^+$ T-cells at D7 were associated with discharge or death outcomes, respectively. Regardless of the lower B and T-cell connectivity at D0, distinct profiles from D7 to D14-28 showed that while discharge was related with increasing connectivity for B-cells, $CD4^+$ and $CD8^+$ T-cells, death was related to increased connectivity involving B-cells, but lower connections mediated by $CD4^+$ T-cells. The $CD4^+CD38^+$, $CD8^+CD69^+$, and $CD3^+CD38^+$ cell subsets presented outstanding accuracy in classifying COVID-19 *vs.* HC during the kinetics timeline. The profile of $CD4^+TIM\text{-}3^+$, $CD3^+TIM\text{-}3^+$, and $CD4^+CD45RO^+CD27^+$ cell subsets at D7 were able to classify COVID-19 patients according to disease outcome. Binary logistic regression analysis identified $CD4^+Tbet^+$ and $CD8^+Tbet^+$ at D0 and $CD4^+CD45RO^+CD27^+$ at D7 as cell subsets associated with distinct medical outcomes. Based on these findings, phenotypic rhythmic variations and integrative networks of B and T cells may be used to develop biomarkers for clinical and therapeutic monitoring of COVID-19 outcomes. **Keywords:** COVID-19;Disease Outcome;Activation/Exhaustion.

DO - 034 - **DISTINCT EXHAUSTION MARKER PROFILES REVEAL TIME-DEPENDENT IMMUNE-RELATED ADVERSE EVENTS DURING IMMUNOTHERAPY**

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Immune-related adverse events (irAEs) are consequences of immune checkpoint inhibitors (ICI) therapy, causing extra-tumoral inflammation in about 10%-35% of patients. Majority of irAEs (85%) occur within the first four months, but some patients may experience them later. Here, we propose to clarify immune mechanisms associated with early or late development of irAEs. We enrolled 16 patients with Melanoma or NSCLC eligible for first-line ICI. Severe toxicities (G3-G4), based on CTCAE (v.5.0), were grouped as Early (n=9, ≤4 months) or Late (n=7, >4 mos). Baseline peripheral blood mononuclear cells and plasma were analyzed using flow cytometry and soluble chemokine/cytokines analysis, respectively. Cell frequencies and analytes concentrations (pg/mL) were used for comparison between groups, employing appropriate statistical tests ($p<0.05$). Study median time to onset was 3.35 months (E: 2.4; L: 7.6). Through unsupervised clustering, PD-1, CD161, TCF1, and TOX populations differentiated Early and Late irAEs. The frequencies of PD-1-expressing TCRαβ, CD4eff and CD8eff T cells and NK cells without other exhaustion markers (CTLA-4, TIM-3, LAG-3, CD39) were higher in Early irAEs group, and were associated with elevated CD161 expression. In this group, CD8eff T cells had higher CXCR5 expression, while CD4eff T cells produced more IL-17A. The systemic soluble profile revealed that IL-8 and MIP-1β were increased in Early irAEs. Conversely, polyclonally stimulated cells from Late irAEs showed increased TOX expression, associated or not with TCF1 expression, in TCRαβ, TCRγδ, CD4 T cells and NK cells, suggesting a predominantly exhausted/terminally exhausted profile. Many of these parameters acts as predictors of irAEs onset. In conclusion, early toxicities were associated with a baseline potentially activated immune profile, while Late irAEs were associated with exhausted populations. Results could aid in personalized medicine for early screening and improving treatment regimen. **Keywords:** Immune-checkpoint inhibitors;Immune-related adverse events;Toxicities.

DO - 035 - **Targeting Polycomb Repressive Complex 2 (PRC2) as a strategy to improve the antitumor activity of CAR-T cells**

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Hematologic neoplasies represent challenging clinical conditions, with high prevalence of morbidity and mortality, especially for patients that present with multiple refractory conditions. Recently, the Chimeric Antigen Receptor T (CAR-T) cell therapy was adopted as one of the therapeutic strategies for individuals with specific hematologic malignancies. Although revolutionary, many patients still relapse during the first days after CAR-T infusion. We believe that part of the problem is related to epigenetic mechanisms involved on T cell suppression, which could be reverted trought the use of epigenetic modulators. To investigate this hypothesis , we evaluated the modulatory activity of different inhibitors of Polycomb Repressor Complex 2 (PCR2) using CD8+ T cells from healthy adults, CAR-T cells derived from patients with hematologic malignancies or produced in house from healthy donors. We observed that PCR2 inhibition of CD8+ T cells and anti-CD19 CAR-T cells enhanced the expression activation markers (CD25, CD69), cytoxicity marker (Granzime B) and inflammatory mediators (IFN-γ and TNF-α), with no decrease on cell viability. On the other hand, the treatment led to a discreet increase on cell proliferation and reduced PD-1 expression. In addition, CAR-T cells treated with PCR2 inhibitors presented killing capacity when challenged with Nalm-6 lymphocytes, a B Cell Acute Lymphoblastic Leukemia cell line. Moreover, CAR-T cells derived from hematologic malignancies patients also demonstrated enhanced expression of cell surface markers associated with activation and cytotoxicity. Our results suggest that epigenetic modulation of PCR2 complex may be a promising tool to enhance cell-based oncologic therapies. **Keywords:** CAR-T Therapy;CD8+ T cells;Epigenetic.

DO - 036 - **UBIQUITIN LIGASE SMURF1 REGULATES THE INFLAMMATORY RESPONSE IN MACROPHAGES AND ATTENUATES SYSTEMIC INFLAMMATION DURING MURINE HEPATIC CORONAVIRUS INFECTION**

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Smurf1, an E3 ubiquitin ligase, plays a crucial role in the ubiquitination process of various substrates including proteins involved in immune signaling, inflammation, and viral autophagy. Murine Hepatitis Virus (MHV) is often used as an experimental model that resembles SARS-CoV-2 infection, the causative agent of COVID-19 in humans. MHV belongs to the same genus as SARS-CoV-2 and it induces acute respiratory syndrome in mice, accompanied by elevated production of inflammatory mediators, similar to the inflammatory response in COVID-19. This study aimed to investigate the role of Smurf1 in modulating the inflammatory response during MHV infection. Wild-type (WT) (C57BL/6) or Smurf1-deficient (Smurf1$^{-/-}$) bone marrow-derived macrophages (BMDMs) were infected with 0.01 plaque-forming units (PFU) of MHV-A59 per cell, and lactate dehydrogenase (LDH) production, viral titers, and inflammatory markers production were evaluated at different time-points. Additionally, WT or Smurf1$^{-/-}$ mice were infected with 10^4 PFU of MHV-A59 by the intranasal route, and survival, weight loss, blood cell counts, and inflammatory parameters were evaluated five days post-infection. Our results showed reduced viral titers, LDH production, and increased TNF and CXCL1 levels in Smurf1$^{-/-}$ BMDMs cultures. The expression of mRNA for Smurf1 in the lungs of WT mice was upregulated. The absence of Smurf1 did not affect the weight loss or survival of MHV-A59-infected mice. Notably, the lymphocyte/neutrophil ratio was increased in Smurf1$^{-/-}$ mice 5 days post-infection. In the lungs, MHV-A59-infected Smurf1$^{-/-}$ mice exhibited reduced viral elimination, increased mRNA for IFN-beta, and unchanged inflammatory markers at day 5 post-infection. In the liver, Smurf1$^{-/-}$ mice displayed increased tissue damage, with higher alanine aminotransferase (ALT) levels and unchanged viral titers. These findings suggest that Smurf1 may act as a negative regulator of inflammatory responses during coronavirus infection. **Keywords:** Smurf1;Macrophages;Inflammation.

DO - 037 - **ATP-P2X7 signaling in the pathophysiology of COVID-19**

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Purinergic signaling is involved in the pathophysiology of several pathogen infections. Viral infections induce ATP secretion, and recent reports have shown SARS-CoV-2 infection increases serum ATP levels, suggesting that this danger signal might contribute to disease progression. P2X7 receptor is an ion channel activated by extracellular ATP (eATP) that stimulates pro-inflammatory responses. Therefore, here we sought to investigate the contribution of ATP-P2X7 signaling in the immune response against SARS-CoV-2, infecting mice (K18-Human ACE2) with inactivated-SARS-CoV-2 (iSARS). For the P2X7 receptor inhibition, mice received intraperitoneally 50 mg/kg Brilliant Blue G (BBG) one day before inoculation. Animals were divided into 4 groups: MOCK; SARS; SARS + BBG; MOCK + BBG (n=3-7). Mice were inoculated intratracheally with $2x10^6$ PFU of iSARS or Mock. After 3 days the animals were submitted to euthanasia and lungs were harvested for further analysis. The mRNA levels for P2X7, CD39, IL-1β, and TNF-α were analyzed by RT-qPCR. Plasma was obtained to determine the eATP and ALT/AST circulant levels. We found a significant increase in P2X7, CD39, IL-1β, and TNF-α mRNA levels in the lung of SARS vs MOCK group while a lower increase in P2X7 and CD39 mRNA level in the SARS vs SARS + BBG group. Lung histological analyses showed inflammation and vasculitis in the SARS vs MOCK group and reduced inflammation in SARS + BBG. No significant differences in ATP, AST, and ALT levels were detected in any group. Our results suggest P2X7 receptor is modulated after iSARS-CoV-2 inoculation. In addition, the P2X7 receptor pharmacological inhibition attenuates the iSARS-induced lung inflammation suggesting that purinergic signaling is involved in the pathophysiology of COVID-19. Financial Support: FAPERJ, CNPq. **Keywords:** P2X7;ATP ;SARS-CoV-2 .

DO - 038 - **Evaluation of the role of butyrate in the migration of plasmacytoid dendritic cells during experimental COVID-19.**

PEREIRA, J.A.[1]; RODRIGUES, V.F.[1]; OLIVEIRA, J.E.[1]; ABUNÁ, R.P.F.[2]; PEREIRA, Í.S.[1]; MACHADO, M.S.G.[1]; BARBOSA, S.C.[1]; PACHECO, T.C.F.[1]; SILVA, J.S.[2]; SARTORI, D.C.[1]. 1. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO RIBEIRÃO PRETO - SP - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ RIBEIRÃO PRETO - SP - BRASIL.

Literature data have shown that severe cases of COVID-19, caused by SARS-CoV-2, lead to a decrease and loss of function in plasmacytoid dendritic cells (pDC), which play a crucial role in the antiviral response. Additionally, studies have revealed that the intestinal microbiota of severe patients shows a significant reduction in beneficial short-chain fatty acid (SCFA)-producing bacteria, such as butyrate. Bacterial metabolites may influence hematopoiesis and immune cell function, suggesting that butyrate may play a role in COVID-19. In this context, we infected WT C57BL/6 mice with the gamma variant (P1) of the SARS-CoV-2 virus and observed a mild disease associated with reduced pDCs in the bone marrow and an increase in lung-resident CCR2+ pDCs. Subsequently, we investigated our hypothesis that COVID-19 resistance might be related to an increased abundance of butyrate-producing bacteria in the intestinal microbiota, which could influence pDC production/migration. Interestingly, we observed an increase in the Firmicutes phylum and the species Cluster IV and *Faecalibacterium prausnitzii* in these mice, characterized like butyrogenic bacteria. Therefore, we infected mice with the P1 virus that were Knockouts (KO) for the G protein-coupled receptor 109a (GPR109a-/-), the main receptor for butyrate. Indeed, in the absence of the butyrate receptor GPR109a, the mice became more susceptible to experimental infection, showing an increased pDCs population in the bone marrow and a reduction in the lungs. Furthermore, *in vitro* we demonstrated that butyrate increased the expression of the CCR2 receptor in pDCs isolated from WT mice, but it effect is not observed in pDCs isolated from KO animals. In summary, our data suggest that during a mild COVID-19 course, there is a prevalence of butyrate-producing bacteria, which, through the signaling of the GPR109a receptor, promotes pDC migration to the lungs via CCR2, thereby conferring a protective role against infection. **Keywords:** COVID-19;plasmacytoid dendritic cells ;microbiota intestinal.

DO - 039 - **CD38 increased activity may account for NAD+ metabolism imbalance in the brain of neonate mice during Zika virus infection**

SARAIVA, G.D.N.; SOUZA, N.M.D.S.; DA SILVA, R.C.; CHICHIERCHIO, M.S.; DE LIMA, E.V.; ANTONIO, L.M.D.S.; PEREIRA, J.C.; PASSOS, G.F.; ZEIDLER, J.D.; DA POIAN, A.T.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Here we investigated the causes of NAD^+ metabolism disturbance in the brain of Zika virus (ZIKV)-infected mice. For this, neonate Swiss mice were subcutaneously mock-infected or infected with 10^5 PFU ZIKV on the third day after birth (P3) and euthanized on different days post-infection (3, 6, 9, 12, 15, 18, 21, 24, and 30 dpi). Tissue samples were collected and processed for analysis: NAD^+ levels were measured by enzymatic coupled cycling assay; NADase activity was analyzed using etheno-NAD^+ as a fluorogenic substrate; changes in gene expression were assessed by qPCR; and mitochondrial function was evaluated by high-resolution respirometry. Our findings reveal that NAD^+ levels decrease in the brain of ZIKV-infected mice from 18 dpi, reaching a drop of approximately 30% at 24 dpi. Accordingly, total NAD^+ hydrolase activity increases by 40% during the same period, which was shown to be mainly CD38-dependent, although mRNA expression of NADases such as PARP10 and PARP12 are also induced. By breaking down NAD^+ molecules for signaling purposes, NADases generate nicotinamide as a byproduct, which is recycled by NAMPT for NAD^+ re-synthesis. NAMPT expression increases in the brains of ZIKV-infected mice, suggesting an unsuccessful attempt to compensate for the NAD^+ levels decline through this pathway. Lastly, we found a significant increase in mitochondrial oxygen consumption in response to cytochrome c in mitochondria isolated from ZIKV-infected mouse brains, indicating the presence of damaged mitochondria in this tissue. Our results suggest that the NAD^+ levels decline in the brains of ZIKV-infected mice is likely a consequence of the increased activity of NAD-degrading enzymes, especially CD38, without a sufficient compensation of the NAD^+ salvage pathway through NAMPT. This event may contribute to mitochondrial dysfunction in the brain in ZIKV-infected mice. Financial support: CNPq and FAPERJ. **Keywords:** Zika virus;neuroinfection;NAD metabolism.

DO - 040 - **INTENSITY OF TOLL-LIKE RECEPTORS EXPRESSION PER T CELLS CORRELATED WITH CLINICAL PARAMETERS OF MULTIPLE SCLEROSIS**

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Introduction: Through multiparametric flow cytometry studies, the frequency of different toll-like receptors (TLRs)-expressing IL-17^{+} and IFN-γ^{-} T cell subsets convincingly has been associated with severity of Multiple Sclerosis (MS), an autoimmune demyelinating disease of the central nervous system. Nonetheless, the usefulness of this method in the clinical practice of those patients is unfeasible. **Objectives:** Evaluate the TLRs expression in different RRMS-derived T cell subsets in whole blood and correlated it with plasma cytokines and clinical parameters. **Methods:** According differential expression of CD45RA, CD26L and TLRs, the frequency of different (CD4^{+} and CD8^{+}) T cell subsets in whole peripheral blood from MS and healthy subjects (HS) was determined by cytometry. The plasma cytokines were dosed were quantified via ELISA. Clinical data were obtained through medical records. **Results:** Elevated intensity of TLR3, TLR7 and TLR9 expression per T cells, as well as TLR2, TLR4 and TLR5 density on naïve (N) CD4^{+} T cells, was identified among patient samples. The severity of neurological disabilities and risk of relapsing within 2 years were associated with TLR2^{high} and TLR4^{high} CD4^{+} T_N cells and TLR4^{high} CD8^{+} T_{EMRA} cells. Conversely, MS clinical activity was inversely correlated with TLR3^{high} and TLR9^{high} T_N cells. No relationship was observed with regard to the percentage of TLRs^{+} T cell subsets and clinical parameters. Concerning plasma cytokines, IL-1β and IL-6 concentrations directly associated with clinical parameters and the intensity of TLR2 and TLR4 expression on T cells. While Th17/Tc17-related cytokines inversely correlated with TLR3^{high} and TLR9^{high} T cells, plasma IL-10 levels associated with TLR9^{high} T cells. **Conclusions:** Our results suggest that measuring intensity of expression of different TLRs in T cells, rather the percentage of cytokine-producing TLRs^{+} T cells, can be a valuable tool in the outpatient follow-up of RRMS patients. **Keywords:** Multiple Sclerosis;TLRs;T cell.

DO - 041 - **The role of DPP-IV inhibition in regulating inflammation during acute colitis and colitis-associated colorectal cancer: combining in sílico and experimental approaches**

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Colitis-associated colorectal cancer (CAC) is a high-risk and high-mortality type of colorectal cancer linked to intestinal inflammation. Incretins, such as GLP-1 and GIP, are peptide hormones secreted by specialized intestinal cells called enteroendocrine cells (EECs). These hormones have diverse functions in immune and non-immune cells. The enzyme DPP-IV (also known as CD26) plays a critical role in regulating incretin function. In this study, we aimed to investigate the impact of incretins and DPP-IV on immune cells, intestinal inflammation, and CAC progression. We analyzed gene-expression data from both human and mouse colorectal cancer samples to explore the role of incretins and DPP-IV in CAC that revealed the downregulation of hormone-secretion pathways and reduced expression of EEC-specific gene markers in colorectal cancer tissues compared to healthy tissues. Notably, lower levels of GLP-1 protein (encoded by GCG) and CHGA were associated with poorer survival outcomes in colorectal cancer patients and chemotherapy responders. In a mouse model of CAC using azoxymethane (AOM) followed by 3 cycles of dextran sulfate sodium (DSS), mice treated with a DPP-IV inhibitor (DDP-IVi) by gavage daily during the DSS cycles exhibited delayed tumor formation, less tumor number and reduced disease severity. In addition, the DPP-IVi treatment showed a decrease in CD45+ cells, intestinal macrophages, dendritic cells, monocytes, and CD4 lymphocytes, while enhancing the anti-tumor response. DPP-IVi treatment also alleviated colitis severity, as indicated by lower colitis scores and less colon shortening, as well as reducing CD45+ while increasing T and B lymphocytes in mesenteric lymph nodes. These findings demonstrate that DPP-IV inhibition ameliorates inflammatory bowel diseases and colorectal cancer, suggesting the beneficial role of EECs and incretins in suppressing inflammation and CAC formation and progression. FAPESP 2019/14755-0 and 2020/14388-4. **Keywords:** colorectal cancer;enteroendocrine cell;inflammation.

DO - 042 - **BCG immunotherapy depends on TLR2-MyD88 signaling to control melanoma in mice**

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Bacillus Calmette-Guérin (BCG) has been used in the clinics for over 40 years to treat bladder cancer and has also been proposed for other types of cancer, including unresectable melanoma. BCG modulates the tumor microenvironment (TME), modifying the cellular infiltrate and preventing tumor remission and metastasis. However, the mechanism of antitumoral immune response still poorly understood. In order to investigate how innate immunity is involved in the activation of B16-F10 murine melanoma cells, BCG and other agonists for several pathways were used, such as TLRs, inflammasome, cGAS/STING pathway and type I IFN. BCG was not able to stimulate B16-F10 to produce inflammatory cytokines and chemokine (IL-6, IL-12, TNF-α, IFN-β and CXCL10) related to the different mentioned pathways, and the opposite was observed in bone marrow-derived macrophages (BMDMs) from wild type C57BL/6 mice (WT) used as controls. Despite this, we confirmed that BCG is able to infect B16-F10 cells, which in turn can activate BMDMs and spleen cells from WT mice in co-culture. Furthermore, we established a subcutaneous B16-F10 melanoma model for intratumoral BCG treatment using different deficient mice (KO) for molecules related to innate immunity (TLR2$^{-/-}$, TLR3$^{-/-}$, TLR4$^{-/-}$, TLR7$^{-/-}$, TLR3/7/9$^{-/-}$, Caspase 1$^{-/-}$, Caspase 11$^{-/-}$, IL-1R$^{-/-}$, cGas$^{-/-}$, STING$^{-/-}$, IFNAR$^{-/-}$ and MyD88$^{-/-}$) and adaptive immunity (Rag$^{-/-}$ and IFN-γ$^{-/-}$). BCG was not efficient to control the tumor growth in TLR2$^{-/-}$, MyD88$^{-/-}$, Rag$^{-/-}$ and IFN-γ$^{-/-}$ mice when compared to WT. The importance of MyD88-related pathways was confirmed why we did not detect BCG stimulation in the cell cultures using BMDMs or spleen cells from MyD88$^{-/-}$ mice. MyD88-signaling induced by BCG is also important for the recruitment of CD8^{+} T cells, NKT, NK, neutrophils and macrophages in the TME, which act to control tumor growth. Thus, our work contributes to a better understanding of the immune mechanisms involved in BCG immunotherapy in murine melanoma. **Keywords:** BCG;Immunotherapy;Melanoma.

DO - 043 - **Systemic Immunological Profile from Children with B-Cell Acute Lymphoblastic Leukemia in the Brazilian Amazon**

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Children with B-cell acute lymphoblastic leukemia (B-ALL) have an immune imbalance marked by remodeling of the medullary compartment, with effects on blood circulation. To characterize the systemic immunological profile in children with B-ALL undergoing treatment, we collect peripheral blood samples from 20 B-ALL patients at 2 times of induction therapy (at diagnosis [D0] and after 35 days of follow-up [D35]). In additional, samples from 28 children without leukemia were collected as a control group (CG). The profile of soluble immunological mediators (CXCL8, CCL2, CXCL9, CCL5, CXCL10, IL-6, TNF, IFN-γ, IL-17A, IL-4, IL-10 and IL-2) and cell profile (NK and NKT cells, Treg, CD4^{+} and CD8^{+} T cells) was evaluated using cytometric bead array and immunophenotyping by flow cytometry. B-ALL patients on D0 showed an increase in the percentage of CD4+ and CD8+ T cells and in levels of CXCL9, CXCL10 and IL-10 compared to the CG. At D35, a decrease in CXCL9, CXCL10 and IL-10 levels was observed, with an increase of NKT, TCD4+ and Treg cell counts when compared to diagnosis. Furthermore, correlation analyzes revealed that at D0, B-ALL patients exhibited an integrative network with robust participation of Treg cells and regulatory cytokines. In contrast, on D35, a network like the control group was observed, evolving to a mixed profile, marked by connections between IFN-γ and IL-17A, and a decrease in the participation of Treg cells and regulatory cytokines. Our results show that B-ALL patients have an imbalance in the systemic immunological cells and molecules, which has been shown to be partially corrected at D35, indicating that the immunological profile plays an important role in the progression of childhood leukemia. **Keywords:** childhood leukemia;cellular immunity;cytokines and chemokines.

DO - 044 - **ARYL HIDROCARBON RECEPTOR IMMUNOMETABOLIC ROLE DURING A MURINE BETACORONAVIRUS EXPERIMENTAL INFECTION**

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The COVID-19 pandemic has already caused over 6 million deaths worldwide, making the better understanding of the mechanisms of SARS-CoV-2 infection an imperative. Aryl hydrocarbon Receptor (AhR) is a xenobiotics receptor and its activation by aromatic compounds has been associated with immune suppression. AhR activation on the lungs of COVID-19 patients is responsible for increased mucus release, leading to aggravation of respiratory distress. AhR activation has also been identified as a proviral factor in an experimental murine Betacoronavirus infection. Moreover, AhR activation has been demonstrated to regulate T cell metabolism and differentiation. However, the mechanisms underlying the role of AhR in cell and systemic metabolism are still poorly understood. In this work, we investigated the effect of AhR deletion on the disease induced by murine hepatitis virus (MHV-3) experimental infection. We intranasally innoculated WT and AhR-/- mice with MHV-3 and collected lung and liver samples 3 and 5 days post infection (dpi). Infected WT mice have severe weight loss and die 6 dpi, with a progressive decrease of platelet and leukocyte count, increased liver damage and cytokine release in the plasma by the 5th dpi, together with systemic viral dissemination. Although AhR deletion did not affect weight loss and survival, it prevented platelet and leukocyte count drop while also preventing liver damage. These mice also have reduced cytokine release in the plasma. Yet, AhR-deficient mice presented increased viral burden in the liver at 3 dpi, while presenting lower viral burden in the liver at 5 dpi. These mice also have increased expression of glycolytic genes in the liver at 5dpi. Infected AhR-/- mice have mitigated systemic manifestations of disease, reduced inflammatory cytokines release and increased expression of glycolytic genes. Therefore, our results suggest AhR activation as an important step in the pathogenesis of MHV-3 infection. **Keywords:** Betacoranavirus;Immunemetabolism;AhR.

IC - 001 - **Inflammatory monocyte recruitment and profiling in experimental models susceptible and resistant to cerebral malaria**

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Malaria is a systemic illness that caused 619 million deaths worldwide in 2021 and is still a major public health concern. Cerebral malaria (CM) is an encephalopathy linked to *P. falciparum* infection, responsible for the majority of malaria-related deaths. Despite differences in splenic architecture between humans and mice, both species present splenomegaly as a common sign of CM and experimental cerebral malaria (ECM). Inflammatory monocytes ($CD11b^{+}Ly6G^{-}Ly6C^{hi}$ cells) are considered crucial innate immune cells in infection control through phagocytosis and production of inflammatory mediators. Although the inflammatory response plays a key role in controlling parasite growth, it is also considered one of the possible pathophysiological mechanisms associated with the progression of ECM. The aim of this study is to evaluate the recruitment of inflammatory monocytes to the spleen and to characterize the profile of these cells in BALB/c and C57BL/6 mice infected with *P. berghei* ANKA, experimental models of susceptibility and resistance to ECM, respectively. Our data showed a significant increase in spleen cellularity and a progressive rise in inflammatory monocytes, particularly in BALB/c mice. In contrast, C57BL/6 mice exhibited a comparatively smaller increase of these cells. Analysis of the monocyte profile showed that the percentage of monocytes expressing the mannose receptor, CD206, did not change between the two strains. However, in BALB/c mice, there was a consistent decrease in the percentage of $TNF\text{-}\alpha^{+}$ and $IL\text{-}1\beta^{+}$ inflammatory monocytes throughout the infection, while in C57BL/6 mice, the percentage of these cells appeared to remain relatively stable. Together, these findings suggest that in ECM-resistant mice, inflammatory monocytes may play an important role in regulating the inflammatory response. They appear to down-regulate their inflammatory profile, thereby contributing to a balanced immune response and potentially preventing brain impairment. **Keywords:**Experimental Cerebral Malaria;Inflammatory Monocytes;Plasmodium.

IC - 002 - **Implications of eosinophil deficiency in severe experimental malaria: increased susceptibility associated to uncontrolled parasitemia and imbalanced cytokine profile**

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Plasmodium is a protozoan responsible for malaria, a disease with high morbidity and mortality. The complications are associated with imbalanced immune response and damage to vital organs. Eosinophils are an important player in inflammation contributing to migration and regulation of other cells of the immune system, and can contributes to tissue damage. Herein, we investigate the role of eosinophils in the development of severe experimental malaria. BALB/c (WT) and ΔdblGATA (deficient in eosinophils) knockout male mice were infected with 10^{5} *Plasmodium berghei* ANKA (PbA) parasitized red cells. Parasitemia, weight loss and clinical score (RBMCs) were evaluated daily, starting on the 4th day post-infection, and hemocytometer, ELISA and histology were evaluated at the 12dpi. Despite the deficiency of ΔdblGATA resulted in lower parasitemia at the beginning of infection, at 12 dpi a drastic increased parasitemia was observed in KO mice associated with worst scores clinic, higher weight loss and susceptivity resulting in precocious mortality. Moreover, at the same kinetic the ΔdblGATA KO presented increased platelets and leukocytes, and decreased the granulocytes circulating when compared with WT counterparts. Deficiency of ΔdblGATA also increased in liver, but not in lung and brain, the injury (degeneration) at 12 dpi when compared with WT. Notably, at 12 dpi a reduction of the anti-inflammatory cytokines IL-10 and TGF-b levels, but not of TNF, IL-6, IL-1b, IL-17 and IFN-g, was observed in ΔdblGATA KO. In the lung of KO mice, increased levels of IL-6, TNF and IFN-g were detected at 12 dpi when compared with WT. Of note, in the brain the levels of all cytokines, except IL-10, were similar between WT and KO-infected mice. Our results suggest that eosinophils play an important role in parasitemia control, weight loss, clinical score and immunoregulation during experimental severe malaria. Financial support: CNPq, CAPES and FAPEMIG. **Keywords:**Plasmodium;Eosinophils;Imunoregulation.

IC - 003 - **INFLUENCE OF CD4 T LYMPHOCYTES DURING THE DEVELOPMENT AND MATURATION OF THE CENTRAL NERVOUS SYSTEM**

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The categorization of the central nervous system (CNS) as an immunoprivileged system began in the last century. However, the CNS is responsive to changes in the immune system (IS) and the opposite is also true. A CNS demand by the adaptive IS seems to occur for adequate neurodevelopment, since the absence of lymphocytes in immunodeficient mice leads to impairment of CNS functionality. Proper CNS development depends on the organization of synaptic contacts that result in topographically organized maps and depends on the stimulation of environmental factors and the cellular microenvironment. As lymphocytes seem to be relevant to the development and maturation of the CNS, this work proposes to map the presence, location and profile of T lymphocytes in different brain regions and in the deep cervical lymph node (dcLN) during development and also to observe the influence of T lymphocytes absence through behavioral tests (CEUA/IOC-023/2020). The presence of T lymphocytes in brain parenchyma at all ages was detected through the RT-PCR and immunofluorescence. By flow cytometry, the profile of T lymphocytes seems to oscillate during the neurodevelopment at dcLN and in different brain areas. For investigate the influence of CD4 T lymphocytes on neurodevelopment, pregnant females received anti-CD4 daily from the 15th gestational day until the birth of the offspring. Litters received anti-CD4 every other day until PND7. The absence of CD4 T lymphocytes in early stages of neurodevelopment leads to neurological reflexes impairment in neonates. The results indicated that T lymphocytes with different activation profiles are present at CNS parenchyma during all stages of neurodevelopment and are important for the maturation of the CNS. The elucidation of the mechanisms by which this interconnection occurs is essential to improve the understanding of how cognition and neuronal function are influenced by the peripheral immune system. **Keywords:**neurodevelopment;T lymphocyte;behavior.

IC - 004 - **CORYNEBACTERIUM DIPHTHERIAE EXTRACELLULAR VESICLES CHARACTERIZATION AND ITS EFFECTS ON MOUSE MACROPHAGES**

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Corynebacterium diphtheriae (Cdi) is the etiological agent of diphtheria, a respiratory disease. The main virulence factor of C.dipth is the diphtheria toxin (DT), that is used for vaccine formulation after structural modifications. Despite vaccine success, an increase in case numbers has been reported. Importantly, DT vaccination protects against toxigenic strains, but does not prevent non-toxigenic strains infections, which stimulate the study of other bacterial virulence factors. Besides DT, Cdi has other virulence factors, such as adhesins, neuraminidases, and siderophores. However, little is known about these molecules' interaction with the host. It’s known that bacteria may release extracellular vesicles (VEs) containing different molecules, such as virulence factors, with a role in bacterial physiology, pathogenesis, and immune modulation. Our work aimed to isolate VEs from Cdi and evaluate its content and ability to modulate macrophages. For that, Cdi VEs were concentrated from bacterial supernatant using a VivaFlow system and ultracentrifugation. CdiVEs particle size and shape were analyzed by Particle Matrix and transmission electron microscopy (TEM), respectively. Protein content was evaluated by SDS-PAGE and Western Blot (WB) using horse anti-diphtheritic serum and human serum. The effect of CdiVEs on cell viability was tested on RAW264.7, AMJ2C11, VERO and A549 cells. For macrophage activation, RAW and AMJ2C11 were stimulated with CdiVE for nitric oxide (NO), TNF-a and IL-6 detection. TEM and particle analysis showed particles with mainly 100 nm. CdiVE did not decrease cell viability after 24h, and macrophage stimulation led to NO, TNF-α and IL-6 production. Surprisingly, WB revealed the presence of DT in CdVEs. Here we have shown, for the first time, that Cdi is able to release VEs-containing DT with modulatory activity on macrophages. Ongoing proteomic analysis of VEs will clarify CdiVE content and its ability to interact with host cells. **Keywords:**Vaccine;Extracelular Vesicles;Diphtheria .

IC - 005 - **Omega-3 decreases TNF-α and NO production, alters the glucose usage by macrophages, and leads to better clearance of Pseudomonas aeruginosa.**

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Pseudomonas aeruginosa is an aerobic gram-negative opportunistic bacterium causing nosocomial infections in immunocompromised patients. Polyunsaturated fatty acids are essential to the human body and have already been associated with reduced inflammation. Therefore, to understand the effects of ômega-3 (Ω-3) against virulent *P. aeruginosa* (PA14) infection, bone marrow-derived macrophages (BMDMs) of C57BL/6 mice were stimulated with or without Ω-3 (DHA: 130mg/ EPA: 180mg) for 3 hours when the cells were infected or not with PA14. Six or 8-hours post-infection, the supernatant was collected for TNF-α and IL-10 evaluation by ELISA, and nitric oxide (NO) concentration by the Griess method. Glucose and lactate were assessed by kinetic method and enzyme system respectively. The medium pH was analyzed by test strips. Viable *P. aeruginosa* was evaluated by colony forming units by pour-plating. The results demonstrating that macrophages treated with Ω-3 and infected with PA14 decreases TNF-α production and the concentration of NO when compared to non-treated cells. IL-10 levels were not secreted by cells. Metabolically, glucose levels were significantly higher at 6 hours post-infected cells, while lactate did not show any change during the evaluated times, compared to Ω-3 non-treated group. Furthermore, the pH was not altered in the medium keeping it between neutral to basic. The cells treated with Ω-3 presented less viable extracellular *P. aeruginosa* when compared to non-treated cells, showing a possible influence of this compound to avoid the bacteria replication. In conclusion this results demonstrating that the clearance of extracellular *P. aeruginosa* does not depend on neither the production of pro-inflammatory cytokine TNF-α nor NO secretion when macrophages are treated with Ω-3. Further studies are necessary to better understand the mechanisms in the innate immunity that is influenced by Ω-3 during the acute infection by *P. aeruginosa*. **Keywords:**P; aeruginosa;Omega-3 ;Innate immunity.

IC - 006 - **Sex-related immune responses and gastrointestinal (GI) commitment converge to long COVID-19 sequelae in female patients**

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Introduction: COVID-19 extends concerns beyond acute phase, affecting other organs, including the gut. The post-acute COVID-19 syndrome (PACS or long COVID) involves long-term sequelae and persistent responses, predominantly in women, and we speculate that the gut may play a central role in it. Thus, we investigated the intricate connection between acute GI involvement and PACS sequelae in both male and female patients. **Methods:** Patients presenting at least one COVID-19 sequelae were enrolled, before vaccination, for the AerobiCOVID study. Clinical data and blood samples were collected into the convalescent phase following acute disease. **Results:** Female patients mostly had mild-moderate acute COVID-19, while the frequency of severe disease was higher in men. To avoid interference of ICU procedures, hospitalized patients were excluded. Stress and depression, which are risk factors for PACS and affect immunoendocrine responses, were increased in women. There were augmented circulating lymphocytes and reduced neutrophils, along with elevated plasma IL-6, IL-8, TNF and IL-10. These responses were higher in women with long-lasting sequelae (over 60 days) and, in a sharp contrast, decreased over time in men. The higher inflammatory cytokines in PACS were more evident in female patients who presented diarrhea in the acute stage. Indeed, women had higher frequency and intensity of diarrhea, as well as nausea, vomiting, and abdominal pain. Otherwise, these symptoms were not observed during acute COVID in men who later developed PACS. TNF, IL-6, IL-8 and IL-10 were correlated within each other in female PACS and their acute GI score, which accounts for worse commitment of the digestive tract, was negatively correlated with physical score, while immunological alterations also seemed to be influenced by female mental health status. **Conclusions:** Sexual dimorphic immune responses and GI commitment impact long COVID-19 in female patients. **Funding.** FAPESP, CNPq, CAPES and USP-VIDA. **Keywords:**COVID-19 sequelae;PACS;Gastrointestinal symptoms.

IC - 007 - **Identification of genes associated with immune infiltration and tumor regression in melanoma**

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Introduction: Melanoma represents 90% of skin cancer deaths. Immune response plays an important role in tumor control and regression. In this sense, several signaling pathways have been identified by their ability to modulate immune response and could contribute to oncogenesis or tumor suppression. **Objective:** To perform a screening of potential genes associated with immune infiltration and tumor regression in advanced melanoma. **Methods:** Were included 52 patients with cutaneous or mucosal melanoma with formalin-fixed, paraffin-embedded (FFPE) tissue samples with a minimum of 60% of tumor cells. Tumor-infiltrating lymphocytes (TILs) were categorized in absent, non-brisk and brisk and areas of regression were also evaluated in the hematoxylin and eosin stain. RNA was extracted using RNeasy Mini Kit (Qiagen). The material was submitted to nCounter NanoString technology using the Metabolic Pathways Panel (NanoString Technologies). The results were captured using the nSolver Analysis Software v 3.0 (NanoString Technologies). Statistical analyses were performed using ANOVA and T-student tests through SPSS software v. 23.0, and a *p*-value < 0.05 was considered significant. **Results:** Ninety-eight genes were associated with TILs, and fifteen associated with tumor regression. From those, six genes were highlighted by its association with both, TILs and tumor regression, including *CA12* (*p*=0.015 and *p*=0.007), *SCL2A1* (*p*=0.041 and *p*=0.006), *RPS6KB2* (p = 0.033 and *p*=0.019), *NDUFB7* (*p*=0.045 and *p*=0.044), *CAB39* (*p*=0.003 and *p*=0.037) and *PSMC1* (*p*=0.004 and *p*=0.021). **Conclusion:** This screening highlighted 3 genes associated with metabolic pathways (*CA12*, *SCL2A1* and *NDUFB7*), 2 with mTOR pathway (*RPS6KB2* and *CAB39*) and 1 gene associated with cytokine signaling and hypoxia (*PSMC1*). The study of these pathways could contribute to increase the comprehension of the immune response in melanoma and highlight novel potential biomarkers. **Keywords:**Immune system;Cancer metabolism;Tumor biomarkers.

IC - 008 - **IMMUNOLOGICAL TOUR: A PLAY ABOUT IMMUNITY AGAINST VIRUSES**

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Introduction: Immunological knowledge is extremely important for the prevention and remediation of diseases, as evidenced by the SARS-CoV-2 pandemic. However, this content is taught in schools only in an expository way, making it difficult to assimilate and articulate with reality and, consequently, making it difficult to learn. In view of this problem, the Tutorial Education Program (PET) of Biology at the Federal University of Ceará developed the Immunological Tour activity. Methods and Results: The proposal of this activity is, through alternative methodology, to incorporate science and art in the form of a play to present the content of immunology in a visual and fun way for high school students. The play has 9 characters and stages the entrance of viruses into the body through the respiratory tract, which are fought by the defense cells of the immune system, and at the end there is a parade for each character cell to briefly present itself to the public. The play was staged at the XVIII PET Biology Vacation Course and was attended by 18 students who, at the end of the activity, answered an evaluation form. Based on the results obtained, 55% of the students claimed to have "average" prior knowledge on the subject and 22% "poor" prior knowledge. In addition, 94.4% considered the dynamics adopted "very good" and 44.4% reported increased interest in the content. Conclusion: Therefore, it is observed that the theatrical play as a pedagogical strategy for teaching Immunology, besides drawing more attention of the student and being an alternative and creative methodology, facilitates the contextualization of the content, making the learning process more dynamic and meaningful. **Keywords:**Education;Extension Project;Viruses.

ME - 001 - **FUNCTIONAL CHARACTERIZATION OF ANTI-CD19 HUMANIZED CARs**

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Introduction: The scFv sequences used in clinical trials for anti-CD19 CAR-T cells were based on murine antibody sequences. Different studies confirmed that host immune responses recognized murine scFv epitopes and rendered subsequent infusions ineffective (Beatty et al., 2014). To develop a cell therapy in this context, our group designed two new CARs with humanized scFv and tested their functionality compared with the murine original CAR. Objective: Characterize in vitro and in vivo two new constructions (named H1 and H2) with humanized scFv comparing the new CARs with the murine version based on the FMC63 mAb. Methods: PBMCs from healthy donors were electroporated with CAR-encoding pT4-19BBz Sleeping Beauty transposon vector with two CARs based on the new scFvs (H1 and H2) and the original FMC63-based scFv. Memory, activation, and exhaustion profiles were evaluated in CAR-T cells at day 8 after electroporation, as well as cytotoxicity and in vivo assays. A long-term (up to 96h) cytotoxicity assay was performed using the Nalm6 wt and Nalm6CD19low. CAR-T cells were tested in vivo in NSG mice inoculated with 10e5 Nalm-6 GFP-Luc 48h before being treated. Results: CAR-T cells with the constructs H1 and H2 showed no statistical difference between memory, exhaustion, and activation profiles when compared to FMC63 (n=8). Cytotoxic capacity evaluated showed that all CARs were able to kill the target cell at the lower E:T ratios at all time points, including Nalm6 modified to express intermediate levels of CD19(n=3).Mice grafted with Nalm-6 were treated with 0.7x10e6 CAR+ in total 6.8x10e6 T cells. The FMC63-based scFv group presented better results when compared to the H2 group (p=0.02), however, the H1 construction presented similar results to the FMC63. Conclusion: The H1 construct showed results similar to FMC63. In summary, we demonstrate that we have developed a humanized functional anti CD19 CAR. **Keywords:** CAR-T cells;immunotherapy;acute lymphoblastic leukemia.

ME - 002 - **Genetic screening of autoinflammatory genes in Latin American pediatric patients with Multisystem Inflammatory Syndrome in Children (MIS-C) associated with SARS-CoV-2 infection**

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Background: Multisystem Inflammatory Syndrome in Children (MIS-C) is an inflammatory condition associated with SARS-CoV-2 infection. It is characterized by fever, prominent gastrointestinal symptoms, mucocutaneous manifestations, and patients often present with shock. The occurrence of MIS-C may be associated with congenital defects of immunity. The aim of this study is to identify variants in genes involved in primary autoinflammatory conditions that may be implicated in MIS-C. **Methods**: Cells and clinical and laboratory information were collected from 21patients with MIS-C, recruited from three public hospitals in the Northeast region of Brazil. The cases of MIS-C were classified as severe or moderate, considering, respectively, the need or not for Positive Pressure Ventilation (PPV) and/or vasopressor medication. Then, total exome sequencing (WES) of the individuals was performed and the identified Individual Nucleotide Variants (SNVs) were subjected to an Inborn Errors of Immunity (IEI) prioritization strategy, focusing on 56 genes previously implicated in auto-inflammatory diseases (made available by the Immunity Committee of the International Union of Immunological Societies, 2022). **Results:** 6 SNVs were identified in 5 different genes (*ADAM17, CARD14, IKBKG, PSTPIP1* and *SH3BP12*). All these variants have been found in children/adolescents with the severe form of MIS-C. Notably, two variants (rs1200631089 and rs144458353) were selected in the *ADAM17* gene. This gene encodes a protease implicated in the processing of tumor necrosis factor alpha (TNF-α) and plays a key role in SARS-CoV-2 infection by cleaving the Angiotensin-Converting Enzyme 2 (*ACE*-2), the main human receptor for SARS-CoV-2. **Conclusions:** Our data suggest that rare deleterious variants in genes previously implicated in autoinflammatory conditions, including ADAM17, IKBKG, PSTPIP1, SH3BP2 and CARD14, may explain the occurrence of SIM-P in previously healthy Latin American children and adolescents. **Keywords:** MIS-C;Auto inflammatory;Genetic.

ME - 003 - **Molecular Characterization of Antibodies against the Spike Protein in Hyperimmunized Horses**

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Horse serum has been utilized in serum therapy for over a century due to the presence of neutralizing antibodies against toxins/venoms and infectious diseases, including those associated with COVID-19. However, our understanding of the horse's immune system and the composition of the horse's immune serum remains limited. In this study, an analysis was conducted between B-cell receptor repertoire sequencing (BCR-seq) and serologic repertoire (Ig-Seq) obtained from four horses immunized with SARS-CoV-2's spike protein. High-throughput sequencing was used to sequence antibodies, yielding an average of 57,000 clones for the heavy chain (IGH), 71,000 for the lambda light chain (IGL), and 8,000 for the kappa light chain (IGK). Through serology, we identified 106 specific sequences against the spike for IGH, 49 for IGL, and 19 for IGK. Furthermore, we were able to determine the specific gene segment usage, CDR3 size, and CDR3 composition of amino acid residues for BCR-seq and Ig-Seq. Notably, one of the four horses exhibited the highest titers of neutralizing antibodies, capable of neutralizing 90% of SARS-CoV-2 infection *in vitro* (PRNT) at a dilution of 1:32,000. We found that this horse's antibodies consisted of 19 different IGH variable domains and only 2 IGL variable domains. When compared with the extensive entries of antibodies against SARS-CoV-2 in the Coronavirus Antibody Database (CoV-AbDab), these horse-circulating antibodies represented a maximum of 69% similarity with known antibodies. This observation highlights the exceptional uniqueness of the horse antibodies targeting the spike protein and paves the way for the recombinant production and characterization of these valuable antibodies. **Keywords:** antibodies;SARS-CoV-2;repertoire.

ME - 004 - **Neutrophil extracellular vesicles promote proinflammatory effects of on primary human peripheral blood mononuclear cells**

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Extracellular vesicles (EVs) are small particles limited by lipidic bilayer membrane, secreted by different cell types to the extracellular environment, where they act in the cellular communication and regulate several physiological functions. Studies with EVs from immune cells show a wide range of functions, such as regulation of inflammatory response and antimicrobial activities. Neutrophil-derived EVs act as important mediators between proinflammatory and anti-inflammatory/pro-resolutive responses, such as in endothelial and immune cells. However, it is not completely clear the immunomodulatory effects resulting from the interaction of these EVs with human peripheral blood mononuclear cells (PBMCs). Thus, in this study, we investigated whether neutrophil EVs promote immunomodulatory activity in PBMCs. The EVs were obtained from neutrophil culture supernatant by differential ultracentrifugation. Nanoparticle tracking analysis showed that particle size was <120 nm, indicating that they can be classified as small EVs. PBMCs were stimulated with EVs at the concentrations ranging from 6,25-100 µg/mL for 90 minutes, and 24h later the cytokine levels were measured in the culture supernatant by ELISA, and cellular viability was measured by XTT. The EVs promoted a potent release of the proinflammatory mediators TNF-α, IL-6, IL-8 and IL-1β in a dose-dependent manner, without effects on cellular viability. In contrast, the EVs did not induce IL-10 and IFN-β release by these cells. NF-kB inhibition in PBMCs strongly reduced the proinflammatory cytokine production, suggesting its participation in the EV effect. The endotoxin concentration on EVs was <0,181 EU/mL. Together, these results suggest that neutrophil-derived EVs act as proinflammatory agents on PBMCs, and can be important players in the initiation and maintenance of the inflammatory response. Next, we will evaluate the levels of NF-kB p65 phosphorylation, and the cellular subsets activation in the PBMCs. **Keywords:** Neutrophils;Extracellular vesicles;Proinflammatory.

ME - 005 - **Role of inflammasomes in the immune response to Legionella longbeachae**

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The gram-negative bacteria, *Legionella longbeachae* and *L. pneumophila*, can infect humans and cause Legionnaires' disease. *L. longbeachae* is significantly more virulent and immunologically more silent. The immune response to *L. pneumophila* infection is well characterized, unlike *L. longbeachae* infection. One of the host defense mechanisms to restrict bacterial replication is the activation of inflammasomes, which are multimeric protein complexes formed in the cytosol of cells, including innate immune cells. This activation triggers the release of pro-inflammatory cytokines like IL-1β and IL-18, and pyroptotic death through Gasdermin-D (GSDMD) cleavage. It has already been shown that *L. pneumophila* can activate the Caspase-11, NLRP3, AIM2, and especially NAIP5/NLRC4 inflammasome upon recognition of LPS, dsDNA, and flagellin, respectively. However, *L. longbeachae* does not encode flagellin, and then naturally evade NAIP5/NLRC4 inflammasome activation. Therefore, we aimed to describe the activation of inflammasomes upon *L. longbeachae* infection both in mice and bone marrow-derived macrophages (BMDMs). From that, we observed that *L. longbeachae* activates the inflammasome in C57BL/6 macrophages, by the GSDMD cleavage, but not in Casp11 deficient cells. We also detected the secretion of IL-1β in WT BMDMs, but not in *Nlrp3*$^{-/-}$, indicating that *L. longbeachae* induces the Casp11 inflammasome activation with posterior NLRP3 dependent secretion of cytokines. Interestingly, our results suggest that inflammasome activation is not crucial for bacterial control *in vivo* and *in vitro*. Therefore, we postulate that this bacterium has developed innate defense evasion mechanisms, which remain unknown, and will be the focus of upcoming experiments. The understanding of the effector mechanisms that operate to eliminate the infection will be important for the development of therapeutic interventions aimed at better prognosis of patients affected by *L. longbeachae* infection. **Keywords:** Legionella longbeachae;Inflammasomes;Legionnaires' disease.

ME - 006 - **ANALYSIS OF CHIMERISM OF REGULATORY T CELLS IN PATIENTS RECIPIENTS OF KIDNEY AND LIVER TRANSPLANTS**

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The continuous use of immunosuppressive drugs is one of the factors that prevent transplant patients from achieving operational tolerance. Literature shows the presence of donor cell chimerism in the recipient and the importance of regulatory T cells (Tregs) in operational tolerance. Based on this, we evaluated the presence of donor Treg chimerism in kidney and liver transplant recipients. Thirteen randomly selected kidney or liver transplant recipients at Hospital Felicio Rocho between 2021 and 2023 underwent buccal swab and peripheral blood sample collection. DNA extracted from the buccal swab was used for the evaluation of 20 polymorphic markers using Real-Time Polymerase Chain Reaction (qPCR) to identify markers negative in the recipient that could be informative to identify donor cells in the blood. Subsequently, Tregs were isolated from the peripheral blood, and DNA was extracted for microchimerism analysis using qPCR, focusing on three informative markers.The mean age of the patients was 50.5 years (+/- 14.5), with 53.9% being female and 46.1% male. The average time since the transplant was 10.8 months. Among the patients, 61.6% and 38.4% received an allograft from, respectively, living and deceased donors. Out of the transplants, 77% were renal and 23% were hepatic. A total of 23% experienced an episode of rejection, and 30.7% had an infection. In the chimerism screening, three markers negative in the buccal swab were evaluated on the purified Tregs population of the recipients. All transplanted patients exhibited positive markers in Tregs, indicating chimerism in this T cell population. This study unveils, for the first time, evidence of chimerism in peripheral blood Treg cells obtained from kidney and liver transplant recipients, indicating the presence of circulating donor Tregs in transplant recipients. The implications of these groundbreaking findings are discussed. **Keywords:** Transplant;Chimerism;Regulatory T cells.

ME - 007 - **EVALUATION OF THE IMMUNOMODULATORY ACTIVITY OF A KUNITZ-TYPE PROTEASE INHIBITOR PRESENT IN THE SALIVA OF THE TICK Amblyomma sculptum**

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Amblyomma sculptum are ticks that play a crucial role as *Rickettsia rickettsii* vectors, the causative agent of Brazilian spotted fever. *A. sculptum* saliva contains protease inhibitors that modulate immune and hemostatic responses during blood feeding. Tick-derived protease inhibitors hold promise for therapeutic development. In this study, we intend to further investigate the immunomodulatory activities of AsKunitz, a Kunitz-type inhibitor recently described in *A. sculptum* saliva, using *in vitro* and *in vivo* experimental models. The effects of the recombinant AsKunitz (rAsKunitz) was evaluated on the proliferative capacity and viability of spleen lymphocytes by preincubation for 1 hour followed by stimulation with concanavalin A (Con A) for 72 hours. Carboxyfluorescein succinimidyl ester (CFSE) dilution upon Con A stimulation was significant reduced in cells incubated with rAsKunitz compared to the positive control (cells incubated with ConA only). Furthermore, Live/Dead staining revealed a significant decrease in the viability of cells incubated with rAsKunitz. Thus, the decreased proliferation can be attributed to lower cell viability, suggesting that rAsKunitz can induce lymphocyte death. Interestingly, rAsKunitz also influenced cytokine production by Con A stimulated lymphocytes, leading to increased production of IL-10, IL-17 and IFN-γ. Next, we investigated the effects of rAsKunitz on dendritic cell (DC) biology. Preincubation of immature DCs with the protein for 1 h, followed by stimulation with ultrapure lipopolysaccharide (LPS) for 24 hours revealed no significant effect on CD40 and CD80 expression in these cells, as detected by flow cytometry. On the other hand, in the ELISA analysis, we observed that the supernatant of this culture incubated with rAsKunitz showed a significant increase in IL-12p40 compared to the positive control. Additional experiments are underway to better understand the functional activities of AsKunitz in the vertebrate immune system. **Keywords:** Amblyomma sculptum ;AsKunitz ;protease inhibitors.

ME - 008 - **THE ROLE OF STING IN THE DEVELOP OF KIDNEY INJURY IN LAP-DEFICIENT MICE**

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Introduction: Rubicon (RUBCN) is a protein of a non-canonical pathway that utilizes components of the autophagy machinery to lipidate LC3 onto the phagosome membrane, known as LC3-associated phagocytosis (LAP). RUBCN contributes to cellular debris clearance, important function in tissues to avoid autoimmunity. Accordingly, aged mice deficient for RUBCN develop chronic kidney injury (*Cell Death Dis*.13:236,2022). Deficiency in RUBCN alters the degradation of engulfed apoptotic cells (AC) in macrophages, inducing a shift from anti-inflammatory to a proinflammatory gene expression profile. Additionally, RUBCN deletion causes the activation of STING-dependent type I interferon responses. Herein, we evaluated the role of STING in the development of chronic kidney disease in RUBCN-deficient aged mice. **Methods:** RUBCN-deficient (*Rubcn*$^{-/-}$), RUBCN and STING double-deficient (*Rubcn*$^{-/-}$ *Sting1*$^{-/-}$) and controls aged mice were used to evaluate the development of autoimmune phenotypes and kidney injury. Bone marrow-derived macrophages (BMDM) from those genotypes were used to evaluate macrophages responses to AC in vitro. **Results:** LAP-deficient aged mice showed a mild increase in anti-DNA and ANA autoantibodies serum levels. We also found increased deposition of IgG immune complex in kidney glomeruli in *Rubcn*$^{-/-}$ mice. This phenotype was associated with increased glomeruli size, suggestive of kidney injury. STING is not essential for production of autoantibodies, but STING deletion reduced the deposition of immune complexes in the glomeruli. We confirmed that RUBCN deletion reduced the expression of genes associated with anti-inflammatory function, as determined by qPCR. STING ablation did not affect such responses in *Rubcn*$^{-/-}$ BMDM during efferocytosis. **Conclusion:** STING activation in the absence of RUBCN may contribute to development of chronic kidney injury, possibly without a direct role for STING in deregulating macrophage anti-inflammatory function in response to dying cells. **Keywords:** Non-canonical autophagy;efferocytosis;STING.

ME - 009 - **Development of a mRNA vaccine for Dengue virus serotypes 2 and 3**

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Dengue is the main arbovirus affecting the human population. As pinpoint differential diagnosis and efficient vaccines are still important issues, the disease remains one of the neglected diseases with the greatest impact on Tropical Countries' health systems. Therefore, the development of a prophylactic vaccine is essential to control and reduce the effects of this disease. Nucleic acid vaccines have become excellent options allowing for fast development and adaptability. Therefore, our aim is to develop and evaluate the immunogenicity of a nucleic acid vaccine encoding the envelope (E80) and non-structural protein 1 (NS1) for dengue serotypes 2 and 3. We independently prepared DNA and mRNA molecules coated with lipid nanoparticles and immunized immunocompetent C57BL/6 mice in a two-dose schedule with an interval of 21 days. Blood was drawn between doses for evaluation of the humoral response, and euthanasia 30 days after the boost dose, when spleens were harvested to evaluate the cellular immune responses. Specific humoral responses were observed in primed animals immunized with NS1 D2 and D3, and there was a significant increase in IgG titers after the boosting dose. Such responses were similar for both DNA and LNP mRNA vaccines. As for IFN-gamma production in immunized animals, an increase in titer was generally detected after stimulation. As for immunizations with E80 D2 and D3, was observed specific humoral responses after the booster dose with DNA, including the generation of neutralizing antibodies. Also, for IFN-gamma production in immunized animals, an increase in titer was generally detected after stimulation in immunized animals. These results are encouraging and support further development of a genetic vaccine for Dengue, including immunogens against the other Dengue virus serotypes.
Keywords: mRNA Vaccine;Dengue;DNA Vaccine.

PD - 002 - **COMPARING THE IMPACT OF DIFFERENT DIETS IN THE GUT-LUNG AXIS BY EVALUATING CHANGES IN THE MICROBIOTA AND MACROPHAGES'S FUNCTION**

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Introduction: Gut microbiota composition and function are dependent on several factors, including diet. A high-fat diet has been implicated in the development of several chronic disorders and infections affecting macrophages response that is known to play crucial role in metabolic disease. Conversely, the ketogenic diet has been associated with the improvement of inflammatory disorders and infections due to metabolism modulation. Accumulating evidence indicates that alterations in the gut microbiota due to diet are responsible for chronic disease progression. Macrophage has been indicated as the main immune cell which can sense environmental change and modulate their function on demand. This study aimed to verify if high fat and ketogenic diet can induce microbiota change and consequently macrophage function. **Methods and Results:** Experiments were performed in male and female C57/Bl6 mice (8-12 weeks old; 6 animals per group). Mice were fed with control diet (CT), high fat diet (FAT) and ketogenic diet (KET) for 7 days and cultivable microbiota were analyzed in feces and lung tissue. Systemic parameters and macrophage phagocytosis and ROS production (*ex vivo*) were evaluated. FAT and KET diet increased Enterobacteriaceae family in feces compared with CT. In the lungs, the Enterobacteriaceae family was increased in KET diet compared with CT. Systemically, as expected, KET diet increased levels of ketonic bodies which were accompanied by a reduction of glucose and lymphocytes in the blood compared with CT. There was no difference in macrophage phagocytosis of the enterobacteria pathogen Klebsiella pneumoniae (Kp), for antimicrobial-resistant (AMR) and non-resistant (NR) strains comparing all diets, however, AMR strain lowered ROS production. **Conclusion** These results suggest that diet induces gut and lung microbiota change and consequently macrophage function alteration. **Financial support:** CNPq, FAPEMIG, CAPES, INSTITUTE SERRAPILHEIRA, PRPq-UFMG. **Keywords:** microbiota;diet;macrophages.

PD - 003 - **A potential metabolic and immunoregulatory role of antigen B lipoprotein in Echinococcus granulosus biology: similarities and differences with plasma HDL**

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The larva of the parasite *Echinococcus granulosus* causes cystic echinococcosis, a chronic infection implying a tight control of host immunity. Its location in a medium rich in nutrients shaped parasite's metabolism, losing *de novo* fatty acid and cholesterol synthesis pathways together with the expression of proteins capable of capturing and transporting essential lipids. One of these proteins, antigen B (EgAgB), is a member of the cestode-specific hydrophobic ligand-binding protein family with diagnostic value. In its native form, EgAgB is a 230 kDa lipoprotein containing ~50% lipids in mass. We demonstrated that EgAgB bounds to monocyte and macrophages in a dose-dependent manner using receptors shared with HDL. In addition, we recently found that EgAgB discharges cholesterol from macrophages, mimicking HDL capacity. Since HDL-induced cholesterol efflux on innate cells seems to be linked to modulation, EgAgB effects on the inflammatory activation of macrophages were studied in comparison with HDL. When co-administered with LPS, EgAgB inhibited macrophages activation decreasing: *in vitro* IL-1β, IL-6, IL-12, IFN-β and •NO and *in vivo* IL-6 and IL-12 (together with a potentiation of IL-10) at 4 h post-injection, and MHC-II, CD40 and CD86 in resident macrophages at 24 h post-injection in the peritoneal cavity. Furthermore, EgAgB and LPS exhibited *in vitro* as well as *in vivo* mutual interference in cell recognition and/or effects, indicating the involvement of a common cell receptor and/or the ability of EgAgB to bind LPS. In this scenario, a putative EgAgB-LPS interaction supporting a scavenger activity, as recently described for HDL, is being explored. Contrasting with EgAgB, in the assayed conditions HDL did not modulate *in vitro* LPS-activation of macrophages, suggesting differences between their interactions with macrophages. Overall, our results support a potential metabolic and immunoregulatory role of EgAgB in *E. granulosus* biology, mimicking some HDL properties. **Keywords:** Echinococcus granulosus antigen B;lipoproteins;metabolic and immunoregulatory functions.

PD - 004 - **ROLE OF DEATH-ASSOCIATED PROTEIN 6 IN CHROMATIN REMODELING MECHANISMS IN INTESTINAL CELLS**

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Death-associated protein 6 (DAXX) is a histone chaperone responsible for regulating several biological processes such as repression of repetitive DNA elements and inhibition of replication stress. Unpublished data from our lab suggests that DAXX interacts with crotonylated histones more specifically than with widespread acetylated histones. In the colon epithelium, the presence of histone crotonylation is governed by the microbiota; depletion/absence of gut microbiota leads to decrease in overall crotonylation in the colon of antibiotic treated or germ-free mice. The goal of our study is to determine whether DAXX protein plays a role in intestinal microbiota-dependent gene regulation by mediating or regulating histone modifications (specially crotonylation). To address this hypothesis, we generated a conditional knockout mouse with DAXX deletion in the intestinal epithelial cells (IECs). Western blotting from control and DAXX knockout IECs indicate that pan-crotonylation (KCr) is decreased in the colon and in the small intestine of DAXX knockout mice. Additionally, bulk RNA-Seq data of IECs with and without DAXX show downregulation of genes involved in response to other organisms, response to external stimulus among other biological processes that suggest that DAXX knockout leads to decreased response to microbiota or its elements. Also, interestingly, RNA-Seq data show downregulation of gasdermin C (GSDMC) in DAXX_KO IECs. GSDMCs are upregulated by type 2 cytokines (e.g. IL-4, IL-13) in the gut and are important factors in tumor necrosis factor-mediated pyroptosis. In summary, our data indicates the relevance of DAXX for epigenetic changes and immune regulation in the intestinal epithelium. **Keywords:** histone modification;gene expression;microbiota.

PD - 005 - **MACROPHAGES-MYOBLASTS INTERPLAY: ROLES OF GDF11 SIGNALING IN MYOGENESIS IN CULTURE**

REIS, R.F.; DA SILVA, I.F.; SAVINO, W.; BOU-HABIB, D.C.; COTTA-DE-ALMEIDA, V.; JURBERG, A.D.; RIEDERER, I.. FUNDAÇÃO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ RIO DE JANEIRO - RJ - BRASIL.

Adult skeletal muscle shows the ability to regenerate upon injury from the activation of resident stem cells known as satellite cells. In brief, these cells first proliferate and then differentiate before fusing with the damaged muscle fiber or generating new myofibers. However, stem cell therapies are hampered by impaired cell migration into the injured site and high cell death rates. We seek to improve the outcome of stem cell transfer for muscle injuries by investigating the role of macrophage-derived signals on myoblast behavior in culture. Using macrophage-conditioned media (MØC), we found that soluble factors stimulated myoblast proliferation and inhibited cell differentiation compared to non-conditioned media. In addition, we identified that both macrophages and muscle cells produced growth and differentiation factor 11 (GDF11), a member of the TFG-β superfamily previously implicated in cardiac muscle rejuvenation. Similar to MØC, recombinant GDF11 (rGDF11), increased cell proliferation and impaired cell differentiation in a dose-dependent manner. Since myoblasts exhibited all GDF11-related type I receptors (named ALK4/5/7), we further treated muscle cells with a 1-hour-pulse of the selective inhibitor SB-431542 of ALK4/5/7 receptors. This inhibition stimulated myoblast differentiation in MØC, whereas subsequent incubation with rGDF11 did not induce myotube formation. To dissect the activity of each GDF11- related type I receptor, we generated single-mutant cell lines by CRISPR/Cas9 for ACVR1B (ALK4), TGFBR1 (ALK5) e ACVR1C (ALK7). The inactivation of ALK4 slowed down myoblast proliferation in either non-conditioned or in MØC. Moreover, the loss of ALK4 delayed myotube formation under differentiation conditions. The addition of rGDF11 also did not rescue these phenotypes. In conclusion, manipulating macrophages and GDF11 signaling seem promising for tweaking the muscle niche to develop alternative cell therapy protocols for skeletal muscle regeneration. **Keywords:** Macrophages;Myoblasts;GDF11.

PD - 006 - **Differential immunomodulatory effects of iron in regulatory t (Treg) and Th17 cells**

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Iron (Fe) is a crucial micronutrient that plays a vital role in cellular respiration, DNA synthesis and repair, and various enzymatic reactions essential for cell survival. Disturbances in iron homeostasis have been implicated in the development and progression of numerous inflammatory diseases. In this study, we investigated the impact of iron on the biology of Treg and Th17 cells using an in vitro differentiation protocol for iTreg and Th17 cells. We employed the iron chelators extracellular Deferoxamine (DFO) and intracellular 2,2-bypiridine (BIP), as well as an iron donor (FeSO4). Our initial experiments revealed that DFO enhanced the differentiation of Treg cells while inhibiting both non-pathogenic (npTh17) and pathogenic (pTh17) Th17 cells. Surprisingly, BIP promoted the differentiation of iTreg cells and restrained the differentiation of npTh17 and pTh17 cells. Additionally, BIP induced Foxp3 expression in npTh17 cells. To gain further insights into the mechanism, we investigated the effect of iron on the expression of key genes in lymphocytes. We observed that DFO downregulated *Rorc*, *Il23r*, and *Il21*, while upregulating *Il22*. Furthermore, FeSO4, the iron donor, significantly reduced the differentiation of iTreg cells while increasing the frequency of npTh17 cells. In conclusion, our findings indicate that iron plays a pivotal role in modulating fundamental aspects of Treg and Th17 cell biology. Targeting iron metabolism could be a promising approach for therapeutic interventions aimed at addressing inflammatory disorders. **Keywords:** Iron;Regulatory T cells;Th17 cells.

PD - 007 - **The role of mitochondrial inheritance in the early rise of asymmetric T cell fates**

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T cell immunity is impaired in older adults, which impinges immune responses reliant on diversity. Autophagy and asymmetric cell division (ACD) are both mechanisms that contribute to memory formation but are lost during ageing. Thus, we aimed to decipher whether autophagy regulates the early-rise of asymmetric T cell fates. Proteome characterization of first-daughter-CD8$^+$ T cells enabled us to identify novel cargoes that may play a role in fate-decision and are autophagy-targets. Confocal and electron microscopy imaging of first-daughter-cells validated proteomics results by revealing a correlation between autophagy-sufficiency and asymmetric inheritance of fate determinants, including damaged/aged mitochondrion. To assess the impact of mitochondrial inheritance on T cell fate, we used a pioneer murine model (Mito-SnapTag) that allows tagging of aged vs. young mitochondria. This enabled us to sort cells based on the inheritance of a fate-determinant regulated by mitophagy and to perform functional adoptive transfer experiments. We observed that cells that mostly preserve aged mitochondria are more glycolytic and show poorer memory potential, measured by survival and re-expansion potential upon cognate-antigen challenge. To determine whether autophagy is required for the asymmetric partitioning of aged mitochondria, we created autophagy-deficient Mito-Snaptag CD8$^+$ T cells, which exhibited increased mitochondrial mass in comparison to autophagy-sufficient cells. During mitosis, loss of autophagy led to higher co-localization rates between aged and young mitochondrial structures and resulted in symmetric inheritance of aged mitochondria. We anticipate that these findings will be relevant to better understanding on how ACD is coordinated and on how T cell diversity is early-imprinted. As autophagy can be pharmacologically modulated, these results can potentially lead to the development of more efficient vaccination and advances in the context of regenerative medicine. **Keywords:** Asymmetric T cell fates;autophagy;mitochondrial inheritance.

PD - 008 - **SARS-CoV-2 infection promotes cellular and humoral responses in the human airways**

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sCoronavirus challenge studies in animals demonstrated an essential protective role for immune responses in the airways, induced by mucosal but not parenteral vaccination. However, studies addressing the capacity of peripheral vaccination to generate humoral and cellular immunity in the lung mucosa in humans, and how this is shaped by prior SARS-CoV-2 infection, are very limited. Using human bronchoalveolar lavage (BAL) samples collected before the COVID-19 pandemic, we demonstrated the presence of pre-existing T cells that recognize SARS-CoV-2 in individuals with stronger immunity to seasonal human coronavirus (HCoV), exemplifying how prior immune history shapes effectiveness of airway memory T cells (Nat Immunol. 23:1324–29, 2022). Here we interrogated induction of SARS-CoV-2-specific immunity in the airways of unexposed or infected vaccinated individuals in BAL samples collected during the pandemic. Parenteral SARS-CoV-2 vaccination alone was not sufficient to seed the respiratory mucosa with spike-specific T cells, despite the induction of T cell responses in the circulation. In infected donors, SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells were significantly higher in BAL than blood, showing an enrichment for SARS-CoV-2 T-cells in the airways, particularly in the tissue-resident compartment. Airways SARS-CoV-2-specific T cells were detected for over one-year post-infection, including those targeting the replication transcription complex (RTC), that we have previously shown to abort SARS-CoV-2 infection (Nature, 601:110–17, 2022). Vaccination alone elicited detectable anti-spike-IgG in the respiratory mucosa, but levels were higher and accompanied by IgA post-infection. Spike-specific memory B-cells were only detectable in BAL from donors with history of infection. Our findings underscore the rationale of respiratory mucosal antigen exposure to expand T and B cells in the airways by next-generation mucosal vaccines, aiming to provide frontline immunosurveillance. **Keywords:** Sars-CoV-2;mucosal immunity;T cells.

PD - 009 - **Immune mechanisms involved in the activation of BCG-induced systemic antitumor response**

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Immunotherapy has a prominence impact for its potential to stimulate the immune system to recognize and destroy the cancer cells. Bacillus Calmette-Guérin (BCG) immunotherapy has not been surpassed by any other treatment for its ability to reduce the recurrence and progression of bladder cancer. Due to its systemic effect, immunotherapy with BCG has also been proposed to control melanoma progression. The aim of this work is to evaluate the efficacy of BCG to induce systemic response in mice and the immune mechanism involved in this process. For this purpose, we used wild type (WT) C57BL/6 and deficient ($Rag^{-/-}$, $Batf3^{-/-}$, $IFN\gamma^{-/-}$ and $IFNAR^{-/-}$) mice that received implantation of MB49 bladder tumor cells in both flanks, where the right tumor (local) was intratumorally treated with BCG or PBS and the left tumor (abscopal) was not treated. BCG treatment in WT mice induced the control of both local and abscopal tumors, however lymphocyte deficient mice ($Rag^{-/-}$) did not respond to BCG treatment. Then, we decided to evaluate if Batf3 and type I and II interferons were involved in tumor control. $IFNAR^{-/-}$ mice respond to BCG treatment likewise WT, while BCG was not able to control abscopal tumors in $Batf3^{-/-}$ and $IFN\gamma^{-/-}$, showing that the systemic response depends on adaptive immunity (Rag, Batf3 and IFNγ). BCG treatment promotes the infiltration of inflammatory cells that controls local and abscopal tumors in WT mice. Furthermore, we observed that prior immunization with intravenously BCG (30 days before tumor implantation) potentiated the effect of intratumoral treatment with BCG in local and abscopal tumors and surprisingly decreased tumors in the PBS control group. The effect of prior immunization was also observed in $Batf3^{-/-}$ and $IFN\gamma^{-/-}$ mice, suggesting a possible involvement of the innate immune memory. Our results reinforce the importance of intratumoral treatment and prior immunization with BCG to activate the systemic response, crucial for cancer immunotherapy. **Keywords:** BCG (Bacillus Calmette-Guérin);Cancer;Immunotherapy.

PD - 010 - **Microbial translocation and its association with high neutrophil count and severity in Yellow Fever Virus infection**

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Between 2017 and 2019, a yellow fever (YF) outbreak occurred in São Paulo, Brazil, with a fatality rate of approximately 35%. During this outbreak, a study conducted in our lab identified the neutrophil count in peripheral blood as a prognostic marker for death. Since the neutrophil is a cell type usually associated with bacterial infections and the microbial translocation correlates with the severity of several viral infections, we investigated the occurrence of this event as a factor associated with YF severity. We measured the plasma levels of microbial translocation markers by immunoenzymatic (sCD14, LBP, EndoCAb IgM, I-FABP) and molecular (16S DNA) assays in samples obtained from patients upon hospital admission (n=90) and at the convalescent phase (n=16); samples obtained from individuals vaccinated against YF (YF-17DD 14 days post-vaccine, n=10) and health individuals (n=22) were used as control groups. The 16S DNA was also sequenced to assess the systemic microbiological profile. For neutrophil analysis, we evaluated the Mean Fluorescence Intensity (MFI) of the markers CD66b, CD14, CD16 and CD11b by flow cytometry in 11 samples obtained during the 2019 outbreak as well as 8 samples from health individuals as a control group. The results were analyzed based on laboratory data, MELD score, and clinical outcomes (survivors, n=63; deceased, n=27). In the microbial translocation analysis, deceased patients had higher levels of sCD14 ($p=0.0044$), LBP ($p=0.0098$), and I-FABP ($p<0.0001$) than survivors. A negative correlation was observed between bacterial diversity, neutrophil count, and the MELD score. In the flow cytometry analysis, we observed a higher and lower MFI of CD66b ($p=0.0050$) and CD14 ($p=0.0157$), respectively, in the YF patients. Together, these results suggest that neutrophil were activated in the acute YF infection and that deceased patients had more liver and intestinal damage, favoring the gut-liver axis impairment and microbial translocation. **Keywords:** Yellow fever;Microbial translocation;severity.

PD - 011 - **SREBP induced lipid droplet biogenesis and inflammasome activation during SARS-CoV-2 infection**

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Viruses exploit host lipid metabolism to promote their replicative cycle, and SREBPs play a regulatory role in fatty acid and cholesterol metabolism. Additionally, SREBPs have been linked to lipid droplet (LD) biogenesis. Our group has made the pioneering discovery that SARS-CoV-2 infection induces LD biogenesis in human monocytes. Furthermore, SREBPs are also involved in caspase-1 activation and cell death through pyroptosis. However, the role of SREBPs during SARS-CoV-2 infection remains largely unexplored. Thus, our hypothesis is that SREBPs regulate host metabolism, influencing SARS-CoV-2 replication. In this study, we investigated the impact of pharmacological and molecular inhibition of SREBPs on lipid metabolism during SARS-CoV-2 infection in calu-3 cells. We found that infection leads to the expression and activation of SREBP1 and SREBP2, resulting in the upregulation of genes involved in lipid metabolism and cytokines. This, in turn, leads to the accumulation of triglycerides, cholesterol, and LD biogenesis. Partial inhibition of SARS-CoV-2 replication and cell death was observed when either SREBP1 or SREBP2 was knocked down. Interestingly, combined knockdown of SREBP1 and SREBP2 demonstrated synergistic inhibition, downregulating lipid and cytokine genes. This led to a reduction in LD formation and viral replication. Furthermore, we used the pharmacological inhibitor fatostatin, which effectively inhibited the activation of SREBPs, LD formation, and viral replication. Notably, fatostatin treatment also reduced caspase-1, gasdermin D1, and the release of IL-1β and IL-18. Collectively, our data highlight the crucial role of SREBPs as master regulators during SARS-CoV-2 infection. Inhibiting these factors could prove to be critical in combating viral infection. **Keywords:** Sars-CoV-2;Lipid droplets;Inflammasome.

PD - 012 - **Asymptomatic malaria is associated with an IFN-γ-induced program on adaptive immunity**

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Asymptomatic malaria poses as one of the most challenging obstacles to achieve malaria eradication. Since clinical immunity depends on previous expositions to *P. vivax* infections and is correlated with age, it is reasonable that adaptive immunity has a central role in asymptomatic infection. It is thought that the adaptive immunity against *Plasmodium vivax* is essential to limit parasite growth to low levels preventing the occurrence of symptoms. Here we investigate the adaptive cellular compartment in ASY subjects compared to symptomatic patients (SY) and healthy donors (CTL). PBMC from 47 ASY with positive qPCR for *P. vivax*, 28 SY with both qPCR and thick smear positive and 19 CTL that reported never have had malaria before were phenotyped to assess the B and $CD4^+$ T cell compartments. Immune features were associated with the clinical outcome and parasitological variables. Soluble mediators and antibodies against *P. vivax* antigens were measured in plasma. We found that the soluble mediators IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, and TNF were decreased in ASY compared to CTL. Increased T_H1 related phenotypes, mainly represented by increased frequencies of $CXCR3^+$ T cells, and increased frequencies of replicative atypical B cells were observed in ASY compared to CTL. These frequencies correlated with parasitemia and antibody levels against MSP1 and AMA1 but not DBPII. Frequency of cells expressing activation (ICOS and CD69) or inhibition (TIM3 and PD1) markers were decreased in ASY compared to CTL. Frequencies of T_H1-committed T follicular helper cells were increased in ASY and correlated with higher frequencies of replicative B cells, IgG1, and CCL3, CCL4, CCL5 and PDGF-bb. Our data indicates that ASY bear a pool of $CD4^+$ T and B cells induced by an IFN-γ program contemporaneously with low inflammatory response that maintain an adaptive response able to control parasitemia while preventing symptoms of the disease. **Keywords:** asymptomatic malaria;plasmodium vivax;adaptive immunity.

PD - 013 - **Fast and Efficient Monoclonal Antibody Isolation from Human Memory B cells**

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COVID-reached a pandemic status and became a public health problem due to its high transmissibility and mortality. The etiologic agent SARS-COV2 is an enveloped virus surrounded by Spike (S) protein trimers, which the virus particles interacts with ACE-2 receptor to entry into the host cell. Adaptative immune response plays a important role in the resolution of viral infections, since B cells are able to produce antibodies against distinct epitopes from the viral particles. It's has been vastly described the importance of specific antibodies to S proteins and its subunit RBD due its neutralizing potential. Our group developed a methodology to generate specific monoclonal antibodies (mAbs) in a rapid fashion. For this purpose, samples of peripheral blood mononuclear cells were obtained from vaccinated or convalescent patients and labeled with specific antibodies and a soluble S protein from Wuhan strain conjugated to fluorochromes to access specific memory B cell population by flow cytometry. These S-specific B cells were sorted and subjected to a single cell culture on a monolayer of feeder cells that provides crucial factors to B cell proliferation and antibody secretion. After 7 days of culture, total immunoglobulin secretion and specificity against S protein and its RBD domain were evaluated in supernatant by enzyme-linked immunosorbent assay (ELISA). Cells were removed and placed in lysis buffer to extract and reverse transcribe the mRNA derived from each clone for sequencing. So far, we have isolated 341 S-specific mAbs and we observed that 25.96% of theses mAbs bound to RBD, 10.03% to NTD and 3.83% to RBD and NTD. We performed further characterization to other variants of concern of SARS-CoV2 and neutralization assay for Gamma and Delta pseudovirus on 20 mAbs, which 10 were able to neutralize both. Neutralizing antibodies are promising in treatment of infected patients, so we intent to produce these antibodies to testing for future therapeutic implementation. **Keywords:** SARS-CoV2;Spike;monoclonal antibodies.

PD - 014 - **SCALE-UP PRODUCTION OF A LOW-COST ANTI-CD19 CAR-T CELL FOR LEUKEMIA IMMUNOTHERAPY**

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*Immunotherapy for cancer has been improved by the use of genetically modified T lymphocytes to express Chimeric Antigen Receptors (CARs). Anti-CD19 CAR-T cell therapy has achieved high response rates in patients with B-cell malignancies. However, the currently approved CAR-T therapies for clinical use are based on laborious procedures and high costs, which may limit their widespread use. The strategy developed by our group utilizes the non-viral Sleeping Beauty (SB) transposon-based gene delivery system to generate anti-CD19 CAR-T cells with a short in vitro expansion protocol. Peripheral Blood Mononuclear Cells (PBMCs) were collected from healthy donors and isolated by density gradient centrifugation. Cells were electroporated with plasmids encoding 19BBz CAR and the SB100x transposase, and CAR-T cells were expanded for 8 days. To evaluate the efficacy of these CAR-T cells, we developed a patient-derived xenograft NSG mouse model (PDX) by injecting primary tumor cells from acute lymphoblastic leukemia patient in its tail vein. After 47 days, the tumor burden in PDX mice (inoculated dose of 10^6 cells) was confirmed by detecting human CD19+ and CD45+ positive cells in mice blood by flow cytometry. We next treated the animals with $7x10^5$ anti-CD19 CAR-T cells generated with our expansion protocol. Preliminary results show that after 17 days of CAR-T cell treatment the tumor burden in PDX animals was 16.3% (control group) and 0.5% (treated animals). In addition, the scaled-up expansion protocol of CAR-T cells performed using the G-REX bottle culture starting from $3x10^7$ PBMCs resulted in $1.1x10^8$ total CAR-T cells, showing our capacity for large-scale production of anti-CD19 CAR-T cells with antitumoral function against human B-cell leukemia. This approach generates enough number of potent antitumor CAR-T cells and represents the base for future clinical trials in patients.***Keywords:** Leukemia;Immunotherapy;Transposon.

PD - 015 - **An IgG fusion vaccine strategy increases RBD immunogenicity and protects against SARS-CoV-2**

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The unprecedented global outbreak caused by SARS-CoV-2 has emerged as one of the most significant health and economic challenges in recent history, highlighting the urgent need for safe and effective vaccines. The receptor binding domain (RBD) of SARS-CoV-2, responsible for its entry into the host cell, has been identified as a major target for vaccine development. However, RBD proteins have demonstrated poor immunogenicity, presenting challenges to vaccine efficacy. In response to this limitation, we developed a vaccine by fusing the RBD of the Wuhan Hu-1 strain with a mouse IgG1 antibody, in order to enhance the immunogenicity of the antigen. Based on previous studies indicating that antigens fused to IgG antibodies can improve immune responses, we successfully expressed the RBD-IgG fusion protein in the Expi293 system and formulated it with Poly (I:C) as an adjuvant. Subcutaneous immunization with the RBD-IgG vaccine resulted in the generation of high titers of RBD-specific antibodies compared to non-fused RBD. Furthermore, RBD-IgG induced promising neutralizing antibodies against pseudotyped and live SARS-CoV-2 viruses. The analyzis of the IgG subclass showed that the RBD-IgG vaccine stimulated Th2-type immune responses and induced cross-reactivity against different variants of SARS-CoV-2. To evaluate protective efficacy, K18 mice were challenged with the Wuhan SARS-CoV-2 strain, and their body weight and survival rate were monitored. Additionally, lung and brain samples were collected to analyze viral load. Interestingly, immunization with the RBD-IgG vaccine conferred almost 100% protection against SARS-CoV-2 infection, as evidenced mainly by the absence of detectable viral loads. We believe that this innovative vaccine strategy can make a significant contribution to current and future epidemics, becoming a promising tool to combat other diseases. **Keywords:** SARS-CoV-2;RBD-IgG;vaccine.

DO - 045 - **Mast cell chymase promotes cytoskeleton reorganization and migration in primary human airway smooth muscle cells**

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Background: Increased airway smooth muscle (ASM) mass is a common feature in allergic asthma development, contributing to airway hyperresponsiveness and airway remodeling. Mast cell responses play a significant role in asthmatic conditions, releasing mediators and exceptionally large quantities of MC-restricted proteases upon activation. However, the interactions between these proteases and ASM is only partially understood. **Objective:** Here we investigated whether MC proteases may affect human airway smooth muscle cells (SMCs). **Methods:** Primary SMCs were treated with MC chymase or tryptase, followed by assessment of parameters related to SMC cell viability, proliferation, contraction, migration and gene expression. **Results:** Chymase exhibited major effects on the morphology of SMCs, while tryptase did not elicit a similar response. Treatment with chymase resulted in decreased quantitative metabolic activity and cell proliferation, while tryptase had minimal effects. Both proteases caused a reduction in intracellular calcium levels in SMCs. Chymase induced a partial inhibitory effect on SMC contractility and induced rearrangement of SMCs cytoskeletal components, such as actin filaments and vimentin. Our findings indicate that SMCs express tight junction proteins, including ZO-1, ZO-2, Claudin 2, and CD2AP. Treatment with chymase led to the degradation of ZO-1, ZO-2, Claudin 2, and CD2AP proteins in SMCs. In line with this, chymase was also shown to enhance the migratory capacity of SMCs. **Conclusions:** Our findings suggest that chymase, but not tryptase, has an effect on the migratory capacity of primary SMCs by modifying their cytoskeletal components. **Keywords:** allergic asthma;mast cell;airway smooth muscle.

DO - 046 - **Human immature dental pulp stem cells and its extracellular vesicles possess immunomodulatory activity on monocyte/macrophage populations**

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Mesenchymal stem cells (MSCs) and its derived extracellular vesicles (EVs) have been explored as an attractive tool for the treatment of inflammatory disorders. Once that monocytes and macrophages are main players in orchestrating inflammation, the aim of this study was to evaluate the immunomodulatory potential of human immature dental pulp stem cells (hIDPSCs) and its derived EVs on monocytes and macrophages. For this, we co-cultured monocytes and macrophages derived from two different cell lines (THP-1 and U-937) with hIDPSCs or hIDPSCs-derived EVs. The cell viability, secreted cytokines, and immunophenotype of immune cells were analyzed. Our results showed that the co-culture of hIDPSCs and hIDPSCs-derived EVs with monocytes and macrophages does not affect the cell viability of both immune and stem cells. hIDPSCs produce large quantities of interleukin (IL)-6, and after exposure to monocytes and macrophages, IL-6 secreted by hIDPSCs strongly increases. In contrast, hIDPSCs-derived EVs do not alter IL-6 production in macrophages. Also, the co-culture of macrophages with hIDPSCs decreased the secretion of TNF while increased the secretion of IL-10 by macrophages. Following the same line, when treated with hIDPSCs-derived EVs macrophages showed a decrease of TNF. However, changes in IL-10 secretion were not observed. The monocyte-hIDPSCs co-culture turned monocytes into an IL-10-producing cell. Although changes in secreted cytokines, macrophage immunophenotypic changes of the molecular markers CD64 and CD163 were not detected after co-culture with hIDPSCs. In conclusion, these results demonstrated that hIDPSCs and hIDPSC-derived EVs have immunomodulatory properties in macrophages and monocytes. **Keywords:** Immunomodulation;Monocytes and Macrophages;Human Immature Dental Pulp Stem Cells.

DO - 047 - **The neuroinflammation induced by Aβ oligomers in a preclinical model of Alzheimer's disease is prevented by exerkines**

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposits of soluble amyloid-β oligomers (AβO) and plaques, neuroinflammation, and cognitive decline affecting about 35 million people worldwide. Currently, there is no effective treatment for AD and notable efforts are aimed at developing strategies to counteract the mechanisms leading to the development of the disease. Evidence suggests that physical exercise (PE) can reduce the risk of developing AD or attenuate its progression; however, the molecular mechanisms that underlie its beneficial effects remain poorly understood. An increasing number of exercise-associated signaling molecules with potential roles in improving brain functions have been identified in the last few years. Irisin has been described as an important myokine capable of mediating the beneficial effects of PE on synaptic plasticity and memory in animal models of AD. Additionally, growing evidence points out that irisin promotes an increase in osteocalcin release by osteoblasts. This osteokine plays a pivotal role in the central nervous system and has been recognized as an anti-gerontic hormone that could prevent age-related cognitive decline. Despite recent findings showing the potential neuroprotective effects of irisin and osteocalcin, the role of these exerkines on neuroinflammation involved in the neurodegenerative process of AD remains to be determined. Here, we aim to understand whether irisin and osteocalcin might counteract the neuroinflammation induced by AβOs. Organotypic hippocampal slices culture were previously treated with irisin and osteocalcin and exposed to AβOs. Cytokine levels in culture medium and microglial activation were investigated. We observed a reduction in the levels of TNF-α, IL-1β, IL-6, and IL-10 as well as microglia activation after exerkines treatment. Although preliminary, our findings suggest that irisin and osteocalcin have neuroprotective effects against inflammation induced by ABOs. **Keywords:** ALZHEIMER'S DISEASE;IRISIN;OSTEOCALCIN.

DO - 048 - **A SINGLE SESSION OF STRENGTH TRAINING CAN MODULATE THE IMMUNE PARAMETERS AND THE PLASMA RESISTIN LEVELS IN OBESE MEN**

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Obesity is characterized by excessive accumulation of adipose tissue, which plays several important physiological roles. Adipocytes interact with the immune system by secreting large amounts of inflammatory molecules called adipokines. Strength training is a stressful stimulus to the body physiology, including immune system and it was hypothesized that, this type of protocol, can mobilize different populations of leukocytes into the bloodstream. This study aimed to evaluate the effects of a strength training session on the circulating leukocytes number and on plasma levels of resistin in twelve sedentary obese young men, who performed a single strength training session. The strength training protocol consisted of 4 sets of 12 repetitions in six exercises. Blood samples were collected through venipuncture in the antecubital vein, before (pre), immediately after (post), and 1-hour subsequent after strength training session. The total number of neutrophils increased from baseline (3699 ± 1634.34 mm^3) to post (4883 ± 2368.19 mm^3) and 1-h after (5194 ± 2275.07 mm^3). The number of monocytes changed from pre (535 ± 109.86 mm^3) to post (651 ± 212.42 mm^3). A significant decrease in plasma resistin levels were found 1-h after the strength training session, compared to levels before the training session (2390 ± 1199 pg/mL vs post-1h (1523 ± 798.6 pg/mL). This study showed that, a single strength training session can increase the number of circulating neutrophils and monocytes and also decrease the plasma level of resistin 1 hour after the end of the training session. In this sense, it is possible that, the strength training session could increase the interaction between these cells and the resistin molecule **(Front. Immunol. 12:699807, 2021)**. The mechanism behind this possible interaction are still uncertain, and it was not proposed by this study. These results suggest that a single session of strength training can induce immune and metabolic changes in obese young men. **Keywords:** Strength Training;Immune System;Resistin.

DO - 049 - **T cell exhaustion in experimental malaria: expression of inhibitory molecules during active infection and following parasite clearance**

ORNELLAS-GARCIA, U.; FREIRE-ANTUNES, L.; DE AZEVEDO, V.M.; RANGEL-FERREIRA, M.V.; DE ALMEIDA, M.L.R.; DANIEL-RIBEIRO, C.T.; GOMES, F.L.R.. FIOCRUZ, FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

Cell exhaustion is an immune system mechanism aimed to prevent an exacerbated immune response that could potentially harm the host. This process is characterized by the persistent expression of inhibitory molecules (such as PD-1, PD-L1 and TIM-3) on the surface of T lymphocytes, leading to impaired proliferation and cytokine secretion. It is important to note that this exhaustion can also hinder an effective response to a pathogen. Initially described in chronic viral infections, cellular exhaustion has also been reported in diseases in which acute exposure to the agent is capable of inducing intense inflammation, as in malaria. The aim of this study was to evaluate the expression of inhibitory molecules during experimental infection by Plasmodium berghei ANKA (PbA) and after chloroquine (CQ) treatment (11th day and 40th day post-treatment), by flow cytometry. C57BL/6 mice were infected with PbA and treated with CQ (from the 4th day after infection, for 7 days). This project was approved by the Ethics Committee on the use of Animals (L-022/2021-A3). On the 4th day after PbA infection, before the onset of cerebral malaria and treatment initiation, parasitized animals showed an increase in the percentage of splenic T cells expressing inhibitory molecules associated with T cell exhaustion. The percentage of CD4 PD-1+ and CD4 PD-L1+ T cells, as well as CD8 PD-L1+ T cells, showed an increase in infected animals. Furthermore, an increase in the percentage of CD4 and CD8 T cells co-expressing PD-1+ and PD-L1+ was observed in infected animals compared to uninfected animals. Surprisingly, even after CQ treatment, the percentage of splenic CD4 PD-1+ T cells and CD8 PD-1+ T cells remained high. We also observed a significant increase in the intensity of these molecules on the surface of T lymphocytes. Thus, our data suggest that even after parasite clearance, the expression of inhibitory molecules is maintained, generating a suppressive state in the immune system. **Keywords:** Plasmodium berghei ANKA;Cellular exhaustion;Immune response.

DO - 050 - **Neutrophil Extracellular Traps promote macrophage activation and restrain HIV-1 infection**

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Neutrophils have key roles in innate immune responses and can release extracellular traps (NETs), characterized by chromatin exteriorization associated with granule and cytoplasmic proteins, such as neutrophil elastase, myeloperoxidase and HMGB1. These traps are released upon neutrophils' contact with a number of factors, including infectious agents, which are entrapped and inactivated by NETs. Since neutrophils are recruited to the sites of HIV-1 replication in lymphoid tissues, where they are activated by inflammatory mediators, we aimed to verify whether and how these structures modify HIV-1 production by macrophages. We found that exposure of HIV-1-infected macrophages to NETs released by neutrophils from healthy donors and HIV-1 patients resulted in inhibition of viral replication, through reducing HIV-1 genome integration and infectivity of residual virions produced by NET-treated infected macrophages. We also observed elevated levels of NETs and LPS in the plasma of HIV-1 patients and a positive correlation between them, suggesting that HIV-1 infection and microbial translocation contribute to NET formation. Regarding the mechanisms involved in this phenomenon, we found that, likewise NETs, the NET-associated proteins neutrophil elastase, myeloperoxidase and HMGB1 inhibited HIV-1 replication and were required for NET-mediated HIV-1 inhibition. Concerning macrophage modulation by NETs, we performed RNA sequencing analysis and identified more than thirty genes differentially expressed and sixteen biological processes and two molecular functions enriched in NET-treated uninfected macrophages. Furthermore, NETs induced macrophage production of the anti-HIV-1 molecules β-chemokines, IFN-1β and IL-10, as well as IL-6, IL-8, TNF-α and reactive oxygen species. Our results indicate that NETs and proteins associated to these structures promote macrophage activation and act as an innate mechanism able to control HIV-1 infection. **Keywords:** Macrophages;NETs;HIV-1.

DO - 051 - **MYOGENIC ALTERATIONS AND RESISTANCE FACTORS IN HUMAN MUSCLE CELLS INFECTED BY THE ZIKA VIRUS**

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Muscle cells are targets of several arboviruses, and myalgia and muscular disorders are frequently related to symptoms. In previous work, we observed that Zika virus (ZIKV) infects muscle progenitor cells (myoblasts) while differentiated cells (myotubes) control ZIKV replication. We have evaluated the transcriptional profile induced by ZIKV infection and observed a more robust intrinsic antiviral response in myotubes, with an increase in the expression of genes and pathways that were not modulated in infected myoblasts. Notably, an enrichment of the STING pathway, essential in the innate immune antiviral responses, was observed only in myotubes. This work aims to investigate myogenic alterations caused by infection and to verify whether the STING pathway is involved in the resistance of myotubes to ZIKV infection. Here, we used an *in vitro* model of human skeletal muscle myogenesis, in which proliferating myoblasts differentiate and fuse into myotubes. Muscle cells were incubated with ZIKV for 2 hours (MOI 0,1) and analyzed 72 hours after infection by cytometry, immunofluorescence, viral proliferation, qPCR and WB. ZIKV infection was productive in myoblasts, and viral dsRNA (double-stranded RNA) was detected by immunofluorescence. ZIKV infection reduced the number of cells, but viability reduction was observed only at 120 hours post-infection. However, ZIKV disrupted the cell cycle by reducing cells in mitosis (G2) phase. We also observed impaired myoblasts migration, a decrease in fluorescence intensity of CD56 and MyoD. As expected, there was no detection of viral replication in myotubes, and increase in STING protein levels in myotubes exposed to ZIKV was observed. In summary, our work shows that ZIKV infection disrupts biological processes, including cell cycle, myoblasts migration and may contribute to disrupting muscle development *in vitro*. Next, we will evaluate if STING pathway, a major antiviral innate component, is implicated in myotube resistance to ZIKV. **Keywords:** Muscle cells;STING;ZIKV.

DO - 052 - **Protein malnutrition alters the clinical course of experimental malaria and increases susceptibility to Plasmodium berghei ANKA infection in BALB/c mice**

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Susceptibility to infections is correlated with the nutritional status of the host. Malaria and malnutrition are overlapping public health problems in many countries. However, there are still contradictions regarding the data linking malnutrition and malaria. This study aims to investigate the effects of malnutrition on BALB/c mice infected with *Plasmodium berghei* ANKA (*Pb*A) and their impact on the host susceptibility to infection. Three-week- old BALB/c mice were initially fed a diet containing 20% protein for 3 days or 1 week for acclimatization. After this period, mice were randomly assigned into two groups. One group continued to receive the 20% protein diet (control protein, CP), while the other group received a diet containing only 4% protein (low protein, LP). After 7 days on their respective diets, the CP and LP groups were divided into subgroups. Half of the animals in each group were then infected with 10^6 parasitized erythrocytes, resulting in the CPi and LPi groups, respectively. All experimental protocols were approved by the Animal Ethics Committees of Fiocruz (L-022/2021). The LPi group showed reduction in body temperature, high levels of parasitemia, reduction in hematocrit and a lower survival rate compared to the CPi group. In addition, malnutrition led to reduced serum levels of IGF-1 and leptin. Interestingly, the CPi group showed increased serum leptin levels at day 7 post-infection. Splenomegaly was observed in both groups of infected animals. Additionally, CPi group exhibited a reduced length of the jejunum. In addition, malnutrition itself causes reduction in the size of the colon and cecum. Surprisingly, our study revealed an increase in the blood-brain barrier permeability in the LPi group compared to the CPi group 7 days post infection. Our data suggest that protein malnutrition prior to *Pb*A infection in BALB/c mice contributes to changes in clinical course and disease susceptibility. **Keywords:** Protein-Malnutrion;Plasmodium;Malaria.

DO - 053 - **Increased neutrophil percentage and Neutrophil-T cell ratio precedes clinical onset of experimental cerebral malaria**

FREIRE-ANTUNES, L.; ORNELLAS-GARCIA, U.; RANGEL-FERREIRA, M.V.; DE ALMEIDA, M.L.R.; DE SOUSA, C.H.G.; CARVALHO, L.J.D.M.; DANIEL-RIBEIRO, C.T.; GOMES, F.L.R.. FIOCRUZ, FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

Cerebral malaria (CM) is responsible for thousands of deaths associated with an unbalanced inflammatory response. Neutrophils are key components of inflammation, producing inflammatory mediators that amplify the response. Newly emerging data suggest that several neutrophil defense mechanisms may play a role in both aggravating and protecting against malaria. These exciting findings suggest that the balance of these cells in the host body may have an impact on the pathogenesis of malaria. To fully understand the role of neutrophils in severe forms of malaria, such as CM, it is critical to gain a comprehensive understanding of their behavior and functions. In this way, this study investigated the dynamics of neutrophil and T cell responses in C57BL/6 and BALB/c mice infected with *Plasmodium berghei* ANKA, murine models of experimental cerebral malaria (ECM) and non-cerebral experimental malaria, respectively. All procedures were approved by the Fiocruz Animal Welfare Committee (license number L-041/2016-A2). The results showed a transient increase in neutrophil percentage in the C57BL/6 mice, and a reduction of these cells in the BALB/c mice, in the spleen and blood. Besides, the percentage of CD4 and CD8 T cells decreased in both mice throughout the course of infection. Surprisingly, only the C57BL/6 showed an increased values of the neutrophil-T cell ratio before the development of clinical signs of ECM. Furthermore, despite the development of distinct forms of malaria in the two strains of infected animals, parasitemia levels showed equivalent increases throughout the infection period evaluated. These findings suggest that the neutrophil percentage and neutrophil-T cell ratios may be valuable predictive tools for assessing the dynamics and composition of immune responses involved in the determinism of ECM development, thus contributing to the advancing of our understanding of its pathogenesis **Keywords:** Experimental Cerebral Malaria;Neutrophils;Lymphocytes.

DO - 054 - **Immunological and laboratorial assessment of SARS-CoV-2 infection in people affected by leprosy**

RODRIGUES, T.F.; ILLARRAMENDI, X.; DA FONSECA, M.S.R.; DOS SANTOS, J.B.; TAVARES, I.F.; MATTOS, J.D.S.; HACKER, M.D.A.V.B.; PESSOLANI, M.C.V.; PEREIRA, V.S.. FIOCRUZ, FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

Leprosy, the leading infectious cause of disability worldwide, remains a public health challenge in the endemic countries despite the decline in new cases detection in recent years. The search for biomarkers is essential to a better understanding of the mechanisms of the disease. The COVID-19 pandemic, caused by SARS-CoV-2, brings a layer of challenge for people affected by leprosy given that viral infections may increase the risk of appearance of leprosy reactions, such as Erythema Nodosum Leprosum (ENL). The increased secretion of pro-inflammatory cytokines and blood neutrophil count have been considered essential factors for COVID-19 worsening, and are predominant during ENL. To assess the impact of the SARS-CoV-2 infection on persons affected by leprosy, we developed a longitudinal observational analytical study at the Souza Araújo outpatient clinic (ASA), Fiocruz, Rio de Janeiro. The outcomes evaluated were frequency of ENL, serum levels of C-reactive protein (CRP), IL-6, CD177, myeloperoxidase (MPO), number of circulating leucocytes, and neutrophil lymphocyte ratio (NLR). Patients at any stage of multidrug therapy (MDT) for leprosy, with borderline leprosy (BL) and lepromatous leprosy (LL), in surveillance at ASA between mid 2021-2023 are selected for SARS-CoV-2 antigen rapid test and blood sampling in three scheduled visits: on inclusion, at 3-month, and 12-month follow-up. Out of the 16 patients that completed follow-up, none developed ENL. We identified 2 cases of SARS-CoV-2 and *Mycobacterium leprae* co-infection. NLR, CRP, IL-6, CD177 and MPO analysis showed no significant difference between the three visits. Similar wise, at ASA there has been no evidence of increase of reactional episodes in the cohort of patients under surveillance. These preliminary results point to an absence of effect of the COVID-19 pandemic on immune parameters and leprosy reactions. However, in ongoing analysis we evaluate the effect on mental health and other clinical parameters. **Keywords:** Leprosy;Covid-19;ENL.

DO - 055 - **Periodontal pathogen, Aggregatibacter actinomycetemcomitans JP2, causes colonic leucokytes decrease and gut microbiome impairment in mice**

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Dysbiosis is a quantitative and functional alteration of intestinal microbiome species and is related to the pathogenesis of several multifactorial diseases, such as inflammatory bowel diseases. Due the presence of oral bacteria in extra-oral sites in disease conditions, the plausibility of an oro-intestinal axis raised, where the intestinal community loses homeostasis and dysbiosis induces the breakdown of the intestinal barrier, allowing bacteria to spread through the bloodstream, reaching other organs and participating in the etiology of several systemic diseases. *Aggregatibacter actinomycetemcomitans* (Aa) is a gram-negative, periodontal pathogen that produces a Leukotoxin (Ltx) lethal to leukocytes. This work shows that oral administration of *Aa* genotype JP2 is capable of disturb the intestinal microbiome and imune *status*, inducing local and systemic inflammation. For this, we used 8-week-old male C57BL6 mice, divided into 2 groups: control, which received PBS and JP2 group, which received 10^9 CFU/mL of Aa suspension, 2x a week, for 4 weeks. After euthanasia, colon lamina propria cells were collected to study leukocyte populations. The proportion of colonic macrophages (cMPs), neutrophils and monocytes in the animals in the JP2-infected group was significantly lower than in the control group. There was a reduction in the concentration of IL-1β in the colonic supernatant, as well as an increase in Myeloperoxidase activity in colon and spleen. Regarding the microbiome, it we sought that the alpha diversity of the microbiome was severely decreased in JP2 group. In addition, the presence of several phyla was strongly correlated with the increase in the concentration of TNF-α, MPO and alveolar bone loss, suggesting a pro-inflammatory and pro-dysbiosis role of this periodontal pathogen, however, with the dose and frequency used in the treatment. study, without a sudden break in intestinal homeostasis. **Keywords:** IBD;Periodontitis;Oral-Gut Axis.

DO - 056 - **Monensin induces secretory granule-mediated cell death in eosinophils**

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Eosinophils contribute to the pathology of several types of disorders, in particular of allergic nature, and strategies to limit their actions are therefore warranted [1]. In this work we evaluated the possibility of targeting the acidic, lysosome-like eosinophil granules as a potential means of inducing eosinophil cell death. To this end we used monensin, an ionophoric drug that has previously been shown to permeabilize the secretory granules of mast cells, thereby inducing cell death [2,3]. Our findings reveal that monensin induces cell death in human eosinophils, whereas neutrophils were less affected. Blockade of granule acidification reduced the effect of monensin on the eosinophils, demonstrating that granule acidity is an important factor in the mechanism of cell death. Further, monensin caused an elevation of the granule pH, which was accompanied by a decrease of the cytosolic pH, hence indicating that monensin caused leakage of acidic contents from the granules into the cytosol. In agreement with a granule-targeting mechanism, transmission electron microscopy analysis revealed that monensin caused extensive morphological alterations of the eosinophil granules, as manifested by a marked loss of electron density. Eosinophil cell death in response to monensin was caspase-independent, but strongly dependent on granzyme B, a pro-apoptotic serine protease known to be expressed by eosinophils. We conclude that monensin causes cell death of human eosinophils through a granule-mediated mechanism dependent on granzyme B. Our results suggest that granule-targeted drug therapy by monensin or other lysosomotropic agents may have potential in the management of eosinophil-associated diseases. **References**
[1] Clin Exp Allergy. 38:709-50, 2008. [2] Allergy.77:1025-8, 2022. [3] J Innate Immun .13:131-47,2021. **Keywords:** Eosinophils;monensin;apoptosis.

DO - 057 - **Therapeutic drug monitoring and analysis of genotypes associated with the use of adalimumab in patients with Crohn's disease.**

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Crohn's disease (CD) is a chronic inflammatory condition that can affect the intestine, causing damage and functional limitations. The monoclonal antibody adalimumab (ADA) is one of the biological treatments used in CD, and it can modify the disease course and reduce the need for surgery. However, up to 40% of patients relapse, even under this medication. Our study aims to evaluate ADA serum levels by lateral flow assay, and 3 genetic polymorphisms associated with ADA response. Sixty-three patients were enrolled and classified into active disease (CDA) or remission (CDR) according to radiological, endoscopic findings, or calprotectin levels. Results: Serum ADA concentrations showed no significant differences between the CDA and CDR groups. Among the active patients, 8 had subtherapeutic, 10 had ideal, and 21 had supratherapeutic levels. As for the patients in remission, 2 had subtherapeutic, 6 had ideal, and 16 had supratherapeutic levels. For genotypic evaluation, ATG16L1, CD155, and CD96 genes were analyzed. No patients carried the AA variant genotype for CD155; therefore, we did not proceed with the analysis. The CC genotype for CD96 was associated with colonic CD, and higher CRP values compared to heterozygotes or variants. Individuals with the CC genotype for ATG16L1 exhibit higher levels of fecal calprotectin (2-fold more), and elevated CDEIS values compared to both the variant genotype alone and when present in heterozygous form. Based on our results, we characterize a population sample of CD patients treated with ADA in a tertiary hospital. Insufficient drug levels could not explain disease activity in ADA-treated patients, as most received high doses. Therefore, other immunological pathways probably contribute to disease activity perpetuation. We observed a potential association between the CC genotypes of ATG16L1 and CD96 with the increase of CD activity biomarkers. This suggests that the wild-type genotype may be implicated in the lack of response to ADA. **Keywords:** Crohn's disease;Adalimumab;Therapeutic drug monitoring.

DO - 058 - **Essential role of SOCS-2 mediating immune response in betacoronavirus MHV-3 and Trypanosoma cruzi coinfected mice.**

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The balance of immune response (IR) is essential for the survival of both *Trypanosoma cruzi* and host during the acute phase of Chagas Disease (CD). Sars-cov2 infection can causes unbalanced IR associated to pulmonary and systemic damage in its host. Suppressor Cytokine Signaling (SOCS) 2 is an important regulator of the innate and adaptive response to various infections, such as viral and parasitic, but its role during chronic CD and MHV-3 infection are unknown. Our aim was to evaluate the role of SOCS2 during the chronic CD, MHV3, and coinfection chronic CD/MHV3 in mice. C57BL/6 (WT) and SOCS-2 (KO) animals were infected with trypomastigotes forms (Y strain), and after reaching the chronic CD (100 days), was co-infected or not with MHV-3. Deficiency of SOCS2 increased the gut and pulmonary tissue damage at chronic CD and acute MHV3 infection, respectively. During chronic CD, the coinfection with MHV3 increased the gut and cardiac tissue damage in SOCS2 KO mice when compared with WT counterparts. Moreover, a greater parasitism in the colon and higher viral load in the lung during co-infection was observed in SOCS-2 KO mice. The profile of infiltrating cells analyzed by flow cytometry demonstrated that the absence of SOCS2 in chronic CD increased in lung and gut the TNF production by macrophages and IFN-g by CD8 T cells and reduces IL-10 by Tregs. Absence of SOCS2 in MHV-3 infection increased TNF and reduced IL-10 production by all innate cells analyzed, and increased CD4- and CD8 T cells-producing IFN-g in both organs; in lung was observed an increased production of IL-17 by CD8 T cells. Deficiency of SOCS2 during coinfection was marked by a reduction in IL-10 production by all innate cells and lymphocytes analyzed, and by increased CD4- and CD8 T cells-producing IFN-g in lung and gut. In summary, SOCS-2 is essential to modulate the immune response and progression of pathogenesis, especially in co-infection by beta coronavirus MHV-3 in the chronic phase of CD. **Keywords:** SOCS2;Chagas Disease;Co-infection.

DO - 059 - **PEPTIDE SCREENING FOR DEVELOPMENT OF A RAPID DIFFERENTIAL TEST FOR HTLV-1 AND HTLV-2**

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The *Human T-lymphotropic virus* (HTLV-1 and HTLV-2) is the first primate retrovirus isolated in humans. HTLV-1 is associated with neoplastic and demyelinating diseases[1]. HTLV-2 causes more marked and slowly progressive neurological changes, as well as secondary infections that impact the respiratory and urinary tracts[2]. There are no effective treatments or vaccines. An efficient and widely available diagnosis is the best way to control the dissemination of both viruses, highlighting that Brazil has the largest absolute number of HTLV-1 infected individuals in the world, also with a high number infected by HTLV-2[3,4]. Therefore, this work aims to develop diagnostic tests using differential peptides for HTLV-1 and HTLV-2, aiming the production of a point of care (POC) test. Sequences of structural and nonstructural HTLV-1/2 proteins were analyzed in silico using BepiPred 1.0. Linear peptides (n=173) were synthesized on membranes and their reactivity evaluated by immunoblotting with pools of sera pre-characterized in commercial assays. From those, twelve peptides synthesized in soluble form were evaluated by indirect ELISA in house, with sera from HTLV-1/2 positive individuals from Minas Gerais, São Paulo and Pará cohorts. The results showed four peptides that differentially detect HTLV-2 and three peptides that detect both viruses. From these, eight different pools ranging two to five peptides were evaluated by indirect ELISA and three chimeric multiepitope proteins were constructed, produced in eukaryotic system, and purified by affinity chromatography. In lateral flow assay (LFA), the HTLV-1L2C protein was able to detect both HTLV-1 and HTLV-2 positive sera, while a peptide pool was able to detect HTLV-2 positive sera. Further tests using the other proteins constructs will be carried out in LFA platform. 1.Rev Esp Quimioter. 32(6):485-496, 2019; 2.Emerg Infect Dis., v. 10,n. 1, p. 109–116, 2004; 3.Rev Soc Bras Med, v. 54, 2, 2021.4.Front. Microbiol., v. 3, p. 388, 2012. **Keywords:** HTLV-1/2;Diagnostics;Peptides.

DO - 060 - **The neutrophil migration on Liver-Kidney axis on a diet-induced NASH zebrafish model**

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. NAFLD is the hepatic manifestation of metabolic syndrome that is associated with prevalence of obesity, type 2 diabetes, and chronic kidney disease (CKD). Recent studies demonstrated an increased risk of CKD in patients with NAFLD progression. However, little is known about the mechanisms that contribute to NAFLD-associated CKD. We have recently demonstrated that neutrophils primed at the NASH liver migrate through the body and respond toward secondary inflammatory triggers in an exacerbated and dysfunctional manner. In addition, our data suggest that steatosis and neutrophil infiltration to the liver occurs prior to SCI. In that sense, the systemic inflammatory process associated with NAFLD has the capacity to overflow to other tissues and disseminate inflammation. One of the tissues that might be affected by neutrophil migration is the kidney. Here we aim to better understand the liver-kidney axis and to evaluate if neutrophils primed at the liver migrate to the kidney and contribute to CKD under NAFLD conditions. Using different diet-induced NAFLD/NASH models combined we tested the hypothesis that neutrophils from the NASH liver disseminate inflammation to the kidney and contribute to CKD. For such, we are feeding transgenic zebrafish larvae with fluorescently tagged neutrophils with western-type diets to induce NASH and CKD. Time-lapse microscopy of the liver and kidney areas shows a dynamic movement of neutrophils migrating between the two organs. We also used a transgenic line with neutrophils tagged with Dendra, a photoconvertible protein, that allowed us to track cells that migrate from the liver toward the kidney. Using this line, we evaluated the neutrophil infiltration rate from liver to the kidney at different stages of NAFLD and assess if correlates with CKD progression. Our studies help to clarify regulatory mechanisms that contribute to NAFLD-associated CKD. **Keywords:** Liver-kidney crosstalk;Neutrophil;Zebrafish.

DO - 061 - **Targeting different portions of trans-sialidase for the development of Chagas disease vaccine**

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Trans-sialidases (TS) are proteins present on the surface of ***Trypanosoma cruzi***, which only a subgroup of TS has catalytic activity, responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism that is related to the parasite capacity to evade the host immune system. Some TS have a C-terminal domain containing 12 amino acids repeats known as SAPA (shed acute parasite antigen). In addition, the SAPA domain increases the stability of the enzyme in the bloodstream, which is considered a parasite virulence factor. To evaluated an active TS as vaccine candidate, three recombinant versions of the protein were produced in ***E. coli***: full-length protein, TS without repeats, and only SAPA repeats. BALB/c mice were immunized with each protein and then challenged with a virulent strain of ***T. cruzi***. Analyzes of the cellular immune response showed that immunization with TS without SAPA resulted in higher levels of IFN-γ and lower levels of IL-10 produced by splenocytes from animals, compared to splenocytes from animals immunized with the other two antigens. Furthermore, after challenge, mice immunized with protein containing only SAPA repeats resulted in higher parasitemia and mortality compared to immunization with TS without SAPA. It is important to emphasize that tissues of animals immunized with TS without SAPA did not show inflammatory infiltrate or detectable levels of parasite DNA in the heart. Taken together these results indicated that immunization with TS antigen without SAPA induces the development of a protective Th1 response, essential for intracellular pathogen infection control, and that the presence of SAPA repeats results in the negative modulation of this protective response. Since RNA vaccines have several advantages and TS without SAPA is a promising antigen for a vaccine model against Chagas disease, the mRNA of this protein was produced and tested in a lipid formulation for animal immunization. **Keywords:** Trans-sialidase (TS);SAPA repeats;T; cruzi.

DO - 062 - **Functional Diversity in Circulating Neutrophils in Leprosy Clinical Forms**

DOS SANTOS, J.B.; DE OLIVEIRA, R.G.F.; RODRIGUES, T.F.; TAVARES, I.F.; CABRAL, N.; ROSA, T.L.S.A.; DOMINGUES, C.C.; FAPERJ, R.O.P.-.; PEREIRA, V.S.. FIOCRUZ, FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

Lymphocytes and macrophages polarization is a well establishment phenomenon observed in leprosy clinical forms which is characterized by a spectral disease caused by *Mycobacterium leprae*. Paucibacillary (PB) patients are considered resistant to infection because they present *M. leprae*-specific cellular response and a Th1 cytokine profile, contributing to the control of the bacillary load. On the other hand, multibacillary (MB) patients have a specific humoral response and a predominance of Th2-type cytokines, which contribute to the dissemination of the bacillus. In this study, we studied phenotypic, functional and morphological differences in neutrophil compartment of PB and MB leprosy patients. To assess the impact of *M. leprae* infection in neutrophils, we evaluated the phagocytic capacity, ROS generation, cytokine secretion profile, production of CD177 and MPO and nuclear morphology of highly purified neutrophils from PB, MB and endemic healthy donors (HD). Serum levels of CD177, a marker of neutrophil activation, were quantified by ELISA. No differences were observed in nuclear morphology among neutrophils of HD, PB and MB patients. The groups had similar levels of MPO, TNF, IFN-g, CD177 and ROS production *in vitro* under stimulus with LPS or dead *M. leprae*. Although there was no difference in the phagocytic capacity of PB x MB neutrophils, HD neutrophils showed greater phagocytic capacity when compared to PB after 1h of stimulation. IL-8 secretion was higher in HD and MB cultures when compared to PB cultures, and stimulation of MB neutrophils with LPS or *M. leprae* increased IL-8 secretion. Based on these data, we conclude that HD neutrophils have greater phagocytic capacity when compared to PB neutrophils and HD and MB neutrophils respond to the stimulus of LPS and *M. leprae* with secretion of the cytokine IL-8, but not other markers. Furthermore, our preliminary findings provide new insights surrounding neutrophil function in leprosy. **Keywords:** neutrophils;neutrophil function;leprosy.

DO - 063 - **Porphyromonas gingivalis oral administration triggers intestinal Inflammation: The role of P2X7 receptor**

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Porphyromonas gingivalis (P.g) is a key gram-negative bacteria associated with periodontitis. *P.g* can transmigrate to other body tissues, inducing gut dysbiosis, dissemination of enterobacteria to the liver, and hepatic and inflammatory bowel diseases (IBD). P2X7 receptor is important in exacerbating inflammation during colitis. Therefore, we hypothesize that oral administration of *P.g* may aggravate 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, affecting the liver in a P2X7 receptor-dependent manner. Wild type (WT) and P2X7$^{-/-}$ (n=5) mice were orally inoculated with 10^9 CFU of *P.g,* and colitis was induced by intrarectal TNBS (2,5%) injection, resulting in the following groups in both mice strains: VEH/VEH; *P.g*/VEH; VEH/TNBS; *P.g*/TNBS. Macroscopic and histological analyses of the colon and liver were performed. Zonula occludens-1 (ZO-1) protein expression in colon samples was assessed by immunofluorescence. Bacteremia in the liver was also evaluated. We found that *P.g* upregulated P2X7 receptor mRNA levels in the colon WT mice. *P.g* oral administration also triggered intestinal inflammation and reduced the colon length in WT mice but not in P2X7$^{-/-}$ mice. *P.g* decreased the expression of ZO-1 in the colon of WT, but not in the colon of P2X7$^{-/-}$ mice. Histological analysis showed oral administration of *P.g* also induces liver injury in WT mice. The *P.g*-induced liver alterations were reduced in P2X7$^{-/-}$ mice compared to WT/*P.g* group. Furthermore, the liver of mice from the WT/*P.g*/TNBS group showed more bacteria colonies than WT/VEH/TNBS and P2X7$^{-/-}$/*P.g*/TNBS. Our results suggest that the P2X7 receptor contributes to the deleterious effects of oral *P.g* administration, promoting gut inflammation and translocation of bacteria to the liver. Financial Support: FAPERJ, CNPq. **Keywords:** Porphyrmonas gingivalis;P2X7 receptor;Colitis.

DO - 064 - **Ivermectin-induced gut bacteria imbalance does not enhance suscetibility to Pseudomonas aeruginosa lung infection but intensified liver damage**

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Excessive usage of drugs, including antiparasitics such as ivermectin, may lead to bacterial gut microbiota dysbiosis. Bacterial gut dysbiosis situations can lead the organism to a greater susceptibility to infectious processes states. To better understand the continuous ivermectin usage over bacterial gut community and susceptibility to *Pseudomonas aeruginosa* PA14 pulmonary infection, C57BL/6 isogenic mice were treated for 7 consecutive days with ivermectin or phosphate buffer saline (PBS) by gavage. It was found that ivermectin-induced bacterial gut dysbiosis is characterized by a decrease in Bacteroidetes, Firmicutes, Proteobacteria, and Tenericutes and an increase in the phylum Verrucomicrobia, especially *Akkermansia* sp. Furthermore, a pro-inflammatory immunostimulatory caecal content, inducing an increase in CD86 expression and interleukin 6 (IL-6) secretion in bone marrow-derived macrophages (BMDM) was observed. Moreover, an alteration in the caecal tissue arrangement has been verified. However, ivermectin-induced bacterial gut dysbiosis did not lead to acute susceptibility to intratracheal *P. aeruginosa* infection, showing similarity between the gut-dysbiotic and non-dysbiotic groups infected in the recovery of viable bacteria in organs, histopathological analysis, and cytokine expression in the lung or secretion of pro- or anti-inflammatory cytokines from heat-killed *P. aeruginosa* (HKPa) stimulated mice splenocytes. Thus, an extension in liver damage and up-regulation in the pro- or anti-inflammatory cytokines expression were observed in groups ivermectin-treated non-infected and infected with *P. aeruginosa*, evidencing that the ivermectin treatment generated liver tissue damage in mice, which was exacerbated in infectious conditions. **Keywords:** Gut dysbiosis;Ivermectin;Pseudomonas aeruginosa.

DO - 065 - **Acute infection with T. gondii EGS strain and the role of P2X7 receptor during the inflammatory process**

MARCELO, T.P.R.; MOREIRA-SOUZA, A.C.A.; DA SILVA, S.R.B.; ARAUJO, T.B.; VOMMARO, R.C.; COUTINHO-SILVA, R.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO RIO DE JANEIRO - RJ - BRASIL.

Extracellular nucleotides activate the P2X7 receptor and trigger the innate immune response against different intracellular pathogens, including *T. gondii* (Savio LEB, Coutinho-Silva R. *Curr Opin Pharmacol*. 47:53-58, 2019). The protozoan *T. gondii* is an obligate intracellular parasite known to establish a long-latent infection. P2X7 receptor activation during *T. gondii* infection triggers several intracellular pathways involved in chemokines production, ROS, lysosomal fusion, and cytokines such as IL-1β (Moreira-Souza and Coutinho-Silva R., *Curr Top Med Chem*. 21,3: 205-212, 2021). The EGS strain was isolated in 1998 from the amniotic fluid of a patient in Minas Gerais – Brazil, and presents a recombinant genotype (I/III). We investigated the P2X7 receptor contribution during acute infection induced by *T. gondii* EGS strain. C57Black/6 wild-type mice (WT) and P2X7 receptor knockout ($P2X7^{-/-}$) mice were analyzed 8 days post-infection. The infection increased morbidity in all infected animals and presented a decrease in the small intestine length and loss of intestinal villus, indicative of inflammation. Plasma aspartate transferase (AST) was evaluated to assess liver damage and dysfunction. EGS strain promoted liver damage independent of the presence of P2X7 receptor, despite the pronounced increase in liver weight of $P2X7^{-/-}$ mice. The RT-qPCR assay showed increased parasite load in $P2X7^{-/-}$ mice compared to WT. Besides, we observed up-regulation of IL-12 and TNF-α expression in WT-infected mice and an increase in IFN-γ expression in $P2X7^{-/-}$ mice compared with WT-infected mice. The results indicate that, although the infection caused by the EGS strain was severe, the presence of P2X7 receptor was important in controlling the parasite load, contributing to the classic immune response against *T. gondii* during infection. **Keywords:** Purinergic Signaling;Bowel disease;Parasitology.

DO - 066 - **Modulation of the inflammatory response and pain relief in collagen-induced rheumatoid arthritis through phosphatidylserine-containing liposomes.**

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by damage to the joints, cartilage, and bone tissue due to synovial cell infiltration. In this study, we investigated the effect of phosphatidylserine (PS)-containing liposomes on the inflammatory response and clinical manifestations in the collagen-induced arthritis (CIA) model, which resembles RA in humans. We observed that treatment with PS-containing liposomes resulted in significant improvements in the treated animals. There was an increase in motor capacity and a decrease in weight loss, suggesting functional recovery. Additionally, the treated animals exhibited reduced pain sensitivity, as indicated by a decrease in mechanical nociception. Further analysis revealed that treatment with PS-containing liposomes led to a reduction in synovial inflammatory infiltration. This resulted in joint protection, with a decrease in pannus formation, a fibrous inflammatory tissue that replaces cartilage and bone tissue. Moreover, we observed a decrease in serum levels of TNF-α in the treated animals, indicating a reduction in the inflammatory response associated with the disease. However, we did not observe a decrease in IL-17 levels, another inflammatory cytokine. These results suggest that PS-containing liposomes may be a promising therapeutic approach for autoimmune inflammatory conditions such as RA. The modulation of the inflammatory response and the improvements in clinical manifestations observed in this study indicate potential for alleviating some of the symptoms associated with this debilitating disease. **Keywords:** Autoimmunity;Collagen-induced;Phosphatidylserine.

DO - 067 - **DNA Extracellular Traps (DETs) induced by SARS-CoV-2 in monocytes: characterization and procoagulant activity.**

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Foi demonstrado que o SARS-CoV-2 induz a liberação de armadilhas extracelulares de neutrófilos (NETs), que foram encontradas em autópsias pulmonares, foram prejudiciais às células epiteliais do pulmão e correlacionadas com a gravidade do COVID-19. Os monócitos também liberam armadilhas extracelulares de DNA (DETs) análogas às NETs em resposta a vários estímulos. Aqui, mostramos que monócitos do sangue periférico humano estimulados por SARS-CoV-2 inativado por betapropiolactona e sua proteína Spike liberam DETs, com a morfologia característica da teia. Mostramos que o SARS-CoV-2 induz a produção de ROS em monócitos, cuja inibição diminuiu a extrusão de DET. Usando inibidores específicos, demonstramos que os DETs induzidos por SARS-CoV-2 em monócitos dependem de mieloperoxidase, peptidil arginina deiminase, cálcio, elastase e gasdermina-D. Os DETs foram tóxicos para as linhagens de células epiteliais pulmonares (A549 e Calu-3) e endoteliais (hBMEC), conforme medido pela redução da liberação de resazurina, lactato desidrogenase (LDH) e coloração SYTOX Green. DETs pré-tratados com anticorpo anti-histona citrulinada inibiram a toxicidade das células A549 medida tanto pela liberação de LDH quanto pelo SYTOX Green. Tratamentos de DETs com inibidores de DNAse, elastase ou mieloperoxidase diminuíram a toxicidade para células A549 conforme testado para liberação de LDH. Dados os fenômenos trombóticos no COVID-19 grave, comparamos a atividade pró-coagulante de DETs e NETs induzidos por SARS-CoV-2 em neutrófilos e monócitos dos mesmos doadores. De acordo com nossos achados, os DETs têm uma atividade pró-coagulante significativamente maior quando comparados aos NETs. Também demonstramos que, quando expostos ao SARS-CoV-2, os monócitos expressam níveis mais elevados de fator tecidual do que os neutrófilos, conforme determinado por qPCR. O fator tecidual também foi identificado associado aos DETs. **Keywords:** Monocytes;SARS-CoV-2;DETs.

DO - 068 - **IL-17 profile of γδ T cells is associated with the pathogenesis of clinical severity of COVID-19**

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Background: COVID-19 includes a broad spectrum of clinical manifestations, with viral damage and uncontrolled inflammation contributing to disease severity. Understanding the nature of the immune response to COVID-19 pathogenesis is crucial for developing effective treatments. We aim to assess the role of gamma delta (γδ) T cells in this process, observing the potential response of these cells *in vitro* to correlate to the severity and outcomes of COVID-19 cases. **Methods:** Twenty-seven COVID-19 inpatients admitted at the Hospital Center for COVID-19 Pandemic from the National Institute of Infectious Disease-FIOCRUZ, Brazil, were included in this study. Based on the WHO Clinical Progression Scale, patients were classified as moderate without oxygen support (MWO_2; n=13), moderate with oxygen support (MO_2; n=8), or severe disease (SD; n=6), requiring mechanical ventilation support. γδ T cells were isolated from peripheral blood mononuclear cells by magnetic beads and cultured under OKT3 antibody and K562 lineage cells for 16 hours. Cytokines production (TNF-α, IFN-γ, IL-17) and expression of TRAIL, CD107a, IL-23R, and IL-1R were evaluated in the context of total γδ T cells and Vδ1 and Vδ2 subsets, by flow cytometry. **Results:** SD patients had significantly higher frequencies of IL-17$^+$ γδ T cells than MWO_2 and MO_2 patients (p<0.05), and also significantly higher frequencies of IL-1R$^+$ γδ T cells than MWO_2 (p<0.001), regardless γδ T subsets. Moreover, for all patients, higher frequencies of TNF-α$^+$ cells were observed among Vδ2 than Vδ1 subsets (p<0.05). No differences in other cell receptors investigated were observed among the clinical groups. **Discussion:** Although preliminary, our findings suggest that *in vitro* stimulated γδ T cells from COVID-19 patients exhibit different potential responses based on disease severity. Severe COVID-19 patients present γδ T cells with more inflammatory IL-17/IL-1R profile, suggesting their participation in disease pathogenesis. **Keywords:** COVID-19;γδ T cells;innate immunity.

DO - 069 - **Circulating and lesional immunological biomarkers associated with localized cutaneous leishmaniasis**

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Localized Cutaneous Leishmaniasis (LCL) is a neglected chronic inflammatory disease that causes ulcers and can lead to deformities, which significantly impacts the quality of life of patients. Ulcer formation is result of exacerbated activation and recruitment of defense cells. Thus, the immune response profile plays a central role in disease control, requiring a balance between type 1 and type 2 immune responses. Here, we evaluated the profile of immunological mediators circulating and presents in the microenvironment of lesion of patients with LCL. For this study, patients with skin lesions suggestive of leishmaniasis and that presented a positive parasitological or molecular diagnosis for leishmaniasis [LCL (n=47)], as well as patients with skin lesions of non-leishmaniasis [NL (n=14)] with compatible histological diagnosis of chronic dermatitis or vasculitis, were included. The control group consisted of healthy volunteers, without skin lesions [SL (n=8)]. Levels of chemokines, cytokines and growth factors were quantified using the Bio-Plex Pro Human Cytokine 27 kit. It is known that LCL generates a local response, however an overproduction of circulating biomarkers was observed as compared to control group. Interestingly, our data demonstrated an elevated levels of CXCL8, CCL3, CCL4, IL-1β, IL-6, TNF-α, IL-12, IL-15, IFN-γ, IL-17A, IL-1Ra, IL-4, IL-5, IL- 9, FGF, PDGF, VEGF, G-CSF, GM-CSF and IL-2 in the microenvironment of lesion in the LCL group as compared to NL. The correlation analysis indicates presence of strong correlations between growth factors and pro-inflammatory/modulatory cytokines in the microenvironment of lesion in LCL group. Taken together, these findings may contribute to better understanding of the immunopathology of leishmaniasis, and immune response profile, necessary for parasitism control. The use of biomarkers can be a useful tool in future applications to predict the clinical outcome in LCL. **Keywords:** Localized Cutaneous Leishmaniasis;Immune response;Biomarkers.

DO - 070 - **Involvement of the mTOR pathway in experimental Trypanosoma cruzi infection in macrophages**

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Introduction: Chagas disease (CD) is a chronic systemic infection caused by the protozoan *Trypanosoma cruzi* (*T*. *cruzi*). During *T. cruzi* infection, changes in lipid metabolism occur as formation of lipid droplets (LDs) in infected cells. LDs are central organelles in the production of eicosanoids regulating the immune response against intracellular pathogens. Recent work demonstrates that the mammalian rapamycin target protein (mTOR) pathway participates in the macrophage response against infection by modulating the synthesis of pro- and anti-inflammatory cytokines. Objective: This study aimed to evaluate the involvement of mTOR signaling pathway in the modulation of macrophage response by analyzing lipid body biogenesis, cytokine secretion and *T. cruzi* replication, using the treatment with rapamycin, an mTOR activity inhibitor. Methodology: Murine macrophages of the RAW 264.7 strain were infected with *T. cruzi* and treated with rapamycin. The number of LDs was quantified by fluorescence microscopy after Bodipy® stainning. The number of parasites in the supernatant were quantified on day 10 post infection. The concentration of the cytokines TNF-α (tumor necrosis factor alpha) and IL-10 (interleukin 10) in the supernatant was determined by ELISA. Results: The results obtained indicate that the inhibition of the mTOR pathway by rapamycin is able to reduce parasite replication and partially reduce LDs biogenesis, and suggest an involvement of the signaling pathway in the secretion of TNF-α and IL-10 cytokines. Conclusion: The results presented indicate an involvement of mTOR in both *T. cruzi* replication and in the mechanisms of biogenesis of LDs, while providing data to subsidize new studies for the use of rapamycin as an adjunct therapy for the treatment of CD. Support: CNPq, FAPEMIG. **Keywords:** Trypanosoma cruzi;Lipid droplets;mTOR pathway.

DO - 071 - **Anti-metalloproteases: Production and characterization of polyclonal antibodies IgG anti-F2 fraction antibodies purified from the venom of the snake Bitis arietans**

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Bitis arietans is a snake found in sub-Saharan Africa responsible for a large number of serious accidents. Metalloproteases represent the major protein part of *Bitis arietans* venom (BaV). Our work aimed to identify toxins and develop antitoxins to analysis their immunotherapeutic effect on the local lesion in mice. Through affinity chromatography, an enriched fraction of metalloproteases was obtained, and titration assays carried out with the immunization of mice demonstrated the development of anti-F2 fraction antibodies by the animals. Determining the affinity of antibodies against different venoms revealed that only BaV could be recognized by anti-F2 fraction antibodies. In vivo analyzes demonstrated the hemorrhagic capacity of the venom and the effectiveness of the antibodies in inhibiting activity of the hemorrhage and lethality caused by BaV. The hemorrhage inhibition kinetic effects were monitored, *in situ,* through Fc immunostaining. Histopathological analysis of the subcutaneously areas injected with BaV expressed signals of typical acute inflammation as edema and hemorrhagic focus. Inhibition of the inflammation was attempting by using different dilutions of specific antibodies. Significantly inhibition was observed with 1:5 antibody dilution. Furthermore, the detection of protective mechanisms at the inflammatory site in the cutaneous tissue provided the bioavailability and local action of the anti-F2 fraction mechanisms. Together, the data indicate the effectiveness of therapeutic effects in neutralizing and partially controlling tissue damage during the local acute process, including the appearance of hemorrhagic and leukocyte infiltrate, a fact that helps to understand the mechanism of envenomation and the emergence of studies of new complementary therapies. **Keywords:** Antibodies ;Antivenoms;Bitis arietans.

DO - 072 - **NEUTRALIZATION PROFILE AGAINST VARIANTS OF CONCERN AFTER IMMUNIZATION WITH RECEPTOR BINDING DOMAIN (RBD) RECOMBINANT PROTEINS**

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Introduction: Although the COVID-19 pandemic is under control, the constant mutations of the virus still pose a significant challenge. The Delta Variant of Concern (VOC) presented elevated morbidity and severe symptomatology, whereas, most recently, the Omicron VOC was responsible for a global wave, mainly due to its increased transmissibility. The VOCs acquired mutations with the majority occurring in the Receptor Binding Domain (RBD) sequence. The RBD is also the most immunogenic sequence in the Spike protein and the target of potent neutralizing antibodies against SARS-CoV-2. Here, we aimed to assess the immunogenic capacity of different RBD recombinant proteins. Methods and results: We immunized mice with equimolar amounts of monomeric (Wuhan), dimeric (Wuhan:Wuhan) or trimeric (Beta:Gamma:Delta) RBD recombinant proteins in the presence of the adjuvant AS03. A two-dose regimen (15-day-apart) was implemented, and blood was collected 15 days after each dose. All three proteins induced high titers of anti-RBD IgG antibodies after the second dose, with mice immunized with the dimeric and trimeric RBD displaying the highest titers. In a pseudovirus neutralization assay using the Wuhan strain, all three proteins induced high NT_{50} titers, although mice immunized with the monomeric RBD displayed significantly lower NT_{50} titers. Using the Omicron variant, the trimeric RBD induced a higher neutralizing capacity, while groups immunized with the monomeric and dimeric proteins presented a more than 10-fold decrease in NT_{50}. These findings are consistent with the Virus Neutralization Test (VNT), where the same pattern was observed. Conclusions: Our findings demonstrate that all recombinant proteins were immunogenic and induced neutralizing antibodies against the Wuhan strain. The trimeric construct, however, was able to neutralize all tested VOCs (Beta, Gamma, Delta, and Omicron). **Keywords:** Vaccine;SARS-CoV-2;Variants of Concern.

DO - 073 - **Impact of MR1-blockage On Soluble Mediators Present in Visceral Leishmaniasis Disease**

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In Brazil, Visceral Leishmaniasis (VL) is a significant public health challenge, impacting marginalized communities. It is caused by *Leishmania infantum* parasite, with approximately 30,000 new global cases reported annually. *Leishmania* exhibits tropism for mononuclear phagocytic cell-rich organs, which serve as the parasite's primary host cells. Factors that contribute to disease progression include loss of immune homeostasis. Asymptomatic infection is characterized by a balanced immune response, whereas classic VL presents a modulated immune response. Recent studies focus on enhancing our understanding of immunity in the context of VL. There is an increasing interest in exploring the role of “innate-like” T cells, particularly MAIT cells. However, knowledge about the involvement of MR1, a receptor associated with MAIT cell activation, in parasitic diseases remains limited. Additionally, the consequences of blocking MR1 on the production and secretion of biomarkers by cells in the infection microenvironment remain largely unexplored. Thus, this study evaluates immunological biomarkers profile in the supernatants of peripheral blood cultures in the context of VL. Supernatants from uninfected (NI) and asymptomatic (AS) individuals, and patients with classical VL, both before (VL-BT) and after treatment (VL-AT) were assessed using luminex technology. Our data revealed that MR1-blockade significantly reduces the expression of pro-inflammatory, Th17/Th22 axis mediators and growth factors, inducing a substantial impact on biomarker production across the evaluated groups. Additionally, we observed that the blockade influenced the interactions mediated by these biomarkers. Notably, there was significant decrease in the connections involving regulatory cytokines in the VL-BT and VL-AT. Conversely, increase in the number of connections mediated by growth factors was observed in AS, VL-BT, and VL-AT groups. **Keywords:** Visceral Leishmaniasis;Soluble mediators;MR1.

DO - 074 - **NLRP3 inflammasome is required to control SARS-CoV-2 replication in astrocytes.**

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Inflammasomes are multi-protein platforms found in the cytosol that activate the protease caspase-1, resulting in the release of IL-1β and IL-18 and causing cell death through pyroptosis. In the Central Nervous System (CNS), inflammasomes have been linked to neurodegenerative diseases, neuroinflammation, and responses to infections. Astrocytes, as the most abundant cells in the CNS, play a critical role in maintaining CNS function and balance. However, the role of inflammasomes in astrocyte functions during infections remains unclear. Hence, this study aims to investigate the impact of inflammasome activation on astrocytes in response to SARS-CoV-2, a virus known to cause various neurological manifestations. Our findings demonstrate that SARS-CoV-2 induces the secretion of IL-1β, activates caspase-1, and leads to ASC speck formation in astrocytes derived from C57BL/6 wild-type (WT) mice. Notably, these indicators of inflammasome activation were not observed in astrocytes lacking NLRP3 or caspase-1, indicating that the NLRP3 inflammasome and its effector molecule, caspase-1, are crucial for this activation. Interestingly, SARS-CoV-2's structural proteins, specifically Spike (S) and Nucleocapsid (N), appear to activate the NLRP3 inflammasome. Transfection of these recombinant proteins induced time-dependent ASC speck formation and IL-1β secretion in astrocytes from WT mice, but not in astrocytes from NLRP3 and caspase-1-null mice. Importantly, the absence of NLRP3 and caspase-1, but not NLRC4, resulted in a higher viral load in astrocytes, indicating that NLRP3, but not NLRC4, plays a role in controlling SARS-CoV-2 replication in these cells. Furthermore, NLRP3 does not seem to be involved in astrocyte pyroptosis, as the absence of the NLRP3 inflammasome did not impact LDH release in response to SARS-CoV-2. These findings demonstrate that astrocytes can control SARS-CoV-2 infection through a NLRP3 and caspase-1-dependent mechanism. **Keywords:** Inflammasomes;Astrocytes;SARS-CoV-2.

DO - 075 - **Impact of exogenous CD40L on the proliferation and M(IL-4) polarization of peritoneal cavity macrophages.**

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The interaction between CD40 ligand (CD40L) and CD40 plays a key role in diverse processes in the immune system. CD40L is most highly expressed by activated CD4+ T cells and constitutes an important signal for antigen presenting cells. However, CD40L can also signal in the absence of an antigen-specific interaction. Macrophages express CD40, the main receptor for CD40L. The role of CD40L, in macrophage responses has been well characterized in the context of classical activation induced by Th1 cells. However, little is known on the role of CD40L in type 2 contexts. In such contexts, macrophages can accumulate by proliferation in response to the cytokines IL-4 and IL-13, which also induce polarization to M(IL-4) phenotypes. In type 2 contexts, macrophages can also proliferate in response to increased availability of M-CSF, the growth factor that maintains the homeostatic levels of these cells. In contrast to M-CSF, IL-4 does not induce macrophage proliferation *in vitro*, for unknown reasons. Since IL-4 acts in synergy with CD40L for the proliferation of B cells, we explored whether exogenous CD40L would allow IL-4 to induce macrophage proliferation *in vitro* and/or potentiate proliferation induced by M-CSF.
Stimulation with recombinant soluble CD40L (sCD40L) did not allow IL-4 to induce the proliferation of resident or recruited macrophages in total peritoneal cell cultures (PCCs), nor did it enhance proliferation induced by M-CSF. The expression of M(IL-4) markers Relm-α and ChiL-3 induced by IL-4 in resident and recruited macrophages in PCCs was inhibited by sCD40L at high doses. In addition, the *in vivo* proliferation of resident macrophages and their expression of Relm-α induced by injection of IL-4 was blunted by co-injection of sCD40L, according to our initial results.
In sum, sCD40L does not promote peritoneal cavity macrophage proliferation *in vitro*, and it instead appears to negatively regulate IL-4 driven proliferation and M(IL-4) polarization in these cells. **Keywords:** Macrophages;Polarization;CD40L.

DO - 076 - **GLOBOTRIAOSYLCERAMIDE ACCUMULATION INDUCES INFLAMMASOMES ACTIVATION IN A CATHEPSIN- AND CASPASE- DEPENDENT MANNER**

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Fabry disease (FD, OMIM 301500) is an X-linked lysosomal storage disease (LSD) caused by the deficiency or absence of the α-galactosidase A enzyme due to mutations in the *GLA* gene, leading to accumulation of globotriaosylceramide (Gb3) in the lysosomes of various cells. However, Gb3 storage alone does not fully explain the different phenotypes observed among patients, and it is believed that pathophysiological processes are triggered by Gb3. In this context, several studies indicate a pro-inflammatory profile in FD patients, suggesting the activation of innate immune pathways, as inflammasomes. Inflammasomes are intracellular multiprotein complexes responsible for caspase-1 activation and maturation and secretion of IL-1β and IL-18. The role of inflammasomes in LSDs is still not fully understood and may represent an important therapeutic target since inflammasomes impact the progression of various inflammatory diseases. Thus, this study aimed to evaluate inflammasomes activation in response to Gb3 in a cellular model of Fabry disease. Gb3 accumulation leads to inflammasomes assembly (ASC specks) and IL-1β secretion in PMA-differentiated THP-1 cells (IL-1β: $p=0.013$) and in human monocyte-derived macrophages ($p=0.04$). To understand the mechanisms involved in this process, pharmacological inhibitors of inflammasome activation were used: Ca-074Me, MCC950, Ac-YVAD-cmk and Z-VAD-FMK, which inhibit cathepsin B, NLRP3, caspase-1 and pan-caspases, respectively. Treatment with Ca-074Me ($p=0.0010$), Ac-YVAD-cmk ($p=0.01$), and Z-VAD-FMK ($p=0.0002$) but not MCC950 ($p=0.85$) resulted in reduced IL-1β secretion in response to Gb3. These findings indicate that Gb3 accumulation induces inflammasome activation in a cathepsin B- and caspase-dependent manner but independent on NLRP3, suggesting the involvement of other inflammasomes. Further studies are needed to clarify which inflammasomes and molecular mechanisms are activated upon Gb3 storage and how they act in Fabry disease scenario. **Keywords:** fabry disease;inflammasomes;sterile inflammation.

DO - 077 - **Spleen-liver communication in systemic inflammation**

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A recent study has revealed that TNF production is regulated by a spleen-liver axis in a rat model of systemic inflammation. In this axis, blood-borne signals derived from the spleen prime the liver to produce and secrete more TNF in response to LPS. To investigate this mechanism in more detail, the present study had two goals: (1) to verify if this mechanism is also present in mice; and (2) to determine, *in vivo*, whether liver macrophages are the cells in which TNF production is subject to modulation by the spleen**.** Five days before an experiment, adult male C57/BL mice were subjected to splenectomy or sham surgery. On the day of the experiment, mice were fitted with infusion jackets and injected with LPS (1 mg/kg) via extensions of pre-implanted peritoneal catheters. Blood, liver and lung were harvested 90 min after the injection. TNF levels and expression were determined. For comparison, the levels of the anti-inflammatory cytokine, IL10, were also evaluated. The results showed that, in the absence of the spleen, the LPS-induced production of TNF by the liver was significantly attenuated, while the production of IL10 was not altered. In the lung, splenectomy did not affect the production of either TNF or IL10. Leukocytes obtained from liver samples were subdivided into two subpopulations: CD45+ F4/80+ Ly6G- (macrophages) and CD45+ F4/80- Ly6G+ (neutrophils). In the splenectomized group, there was a reduction in the percentage of TNF-positive macrophages in the liver, with no change in the percentage of these cells in the lungs. In addition to being organ-specific, this effect was macrophage-specific, as no intergroup difference was noticed in the percentage of TNF-positive neutrophils in liver or lungs. These findings extend the spleen-liver axis that drives TNF production to mice and provides *in-vivo* evidence supporting that it is the hepatic macrophages (Kupffer cells) that are the target for the regulation by spleen-derived signals. **Keywords:** Spleen;liver;TNF.

DO - 078 - **ROLE OF THE SPLANCHNIC NERVES IN HOST DEFENSE**

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It is known that autonomic nerves regulate the immune system, but there is limited information about the roles played by sympathetic nerves. In this study, we evaluated whether and how sympathetic splanchnic nerves regulate host defense in a rat model of peritonitis. Bilateral splanchnic denervation or sham surgery was performed one week prior to the challenge with *E. coli*. The challenge involved surgically implanting E. coli-contaminated gauze in the peritoneum. Bacterial burden, leukocyte infiltration, and injury markers were evaluated. Bacterial burden was more pronounced at the peritoneum, followed by the spleen and lungs. Compared to controls, SplancX rats were more efficient in eliminating bacteria in the spleen and the peritoneum when *E. coli* was administered at a dose of $1x10^4$/rat. However, at a higher dose ($1x10^6$/rat), denervation only reduced bacterial load in the spleen. Heightened immunity in SplancX rats was not promoted by increased leukocyte migration, as the groups did not differ in terms of neutrophils or macrophages in the peritoneum, lungs, or spleen. We then raised the hypothesis that increased microbicidal activity in SplancX rats could come at the expense of increased collateral damage to organs. This hypothesis did not find support in the finding that, compared to sham surgery, SplancX did not increase the levels of established organ injury markers. It should be noted, however, that the levels of such markers were not well above reference values within the experimental time period (24 hours). In conclusion, the study reveals a suppressive effect of splanchnic nerves on bacterial clearance, which was not related to changes in tissue infiltration by phagocytes. Presumably, changes in the microbicidal activity of these leukocytes are involved. Improvement in bacterial elimination in the absence of splanchnic nerves did not result in increased collateral damage, but such costs may become evident in other contexts, such as prolonged infection. **Keywords:** bacterial load;splanchnic nerve;inflammatory reflex.

DO - 079 - **Differential regulatory T cell signature after recovery from mild COVID-19**

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COVID-19 is characterized by a range of symptoms with immune response being associated with disease progression. However, the role of regulatory T cells (Tregs) in COVID-19 outcomes has not been thoroughly investigated. Here, we compared Tregs between volunteers not previously infected with SARS-CoV-2 (healthy control - HC) and volunteers who recovered from mild (MR) and severe (SR) COVID-19. Peripheral blood mononuclear cells (PBMC) were stimulated with SARS-CoV-2 synthetic peptides (Pool Spike CoV-2 [PSC] and Pool CoV-2 [PC]) or staphylococcal enterotoxin B. Results of a flow cytometric assay showed higher Treg frequency and expression of IL-10, IL-17, perforin, granzyme B, PD-1, and CD39/CD73 co-expression in Treg among the PBMC from the MR group than in the SR or HC groups for certain SARS-CoV-2 related stimulus. Moreover, MR unstimulated samples presented a higher Tregs frequency and expression of IL-10 and granzyme B than did that of HC. Compared with PC stimuli, PSC reduced IL-10 and improved PD-1 expression in Tregs in the MR group. Interestingly, PSC elicited a decrease in Treg IL-17$^+$ frequency in the SR group. In HC, expression of LAP and cytotoxic granule co-expression by Tregs was higher in PC stimulated samples. While PSC stimulation reduced the frequency of IL-10$^+$ and CTLA-4$^+$ Tregs in the MR group who had not experienced certain symptoms, higher levels of perforin and perforin$^+$granzyme B$^+$ co-expression by Tregs were found in the MR group in volunteers who had experienced dyspnea. Finally, we found differential expression of CD39 and CD73 among volunteers in the MR group between those who had and had not experienced musculoskeletal pain. Collectively, our study suggests that changes in the immunosuppressive repertoire of Tregs can influence the development of a distinct COVID-19 clinical profile, revealing a possible modulation of Tregs among volunteers of the MR group between those who did and did not develop certain symptoms, leading to mild disease. **Keywords:** Regulatory T cells;Mild symptoms;Recovered COVID-19.

DO - 080 - **Protective role of STING in Idiopathic Pulmonary Fibrosis (IPF)**

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Idiopathic Pulmonary Fibrosis (IPF) is the most common chronic and progressive Interstitial Lung Diseases (ILD) with fatal ending [N. Engl. J. Med. 378:1811-1823, 2018]. Immunological processes involved remain poorly understood. STING (STimulator of INterferon Genes), encoded by *Tmem173* gene, is an innate immune sensor activated by cyclic dinucleotides upon sensing of cytoplasmic host or foreign DNA leading to type I/III interferons (IFN) production as well as cell death and autophagy regulations [Nat. Rev. Mol. Cell Biol. 21, 501–521, 2020]. We recently showed that STING displays a protective role using the mouse model of bleomycin (BLM)-induced lung fibrosis, independently of type I IFN signaling [Front Immunol, 8;11:588799. 2021]. After oropharyngeal BLM administration, we showed that STING pathway is upregulated in lung tissue and now aim to identify lung cell subsets that increase STING expression. Using *Tmem173*^{OST} mouse strain enabling specific STING staining by targeting its associated One Strep Tag (OST) by flow cytometry, we observe little STING expression in non-hematopoietic cells and neutrophils in saline or BLM-treated mice. In contrast, while STING appears strongly expressed in both lymphocytes and macrophages at baseline, its expression significanlty increases upon BLM treatment in macrophage subsets but not in lymphocytes. Furthermore, we ambition to characterize STING-mediated mechanisms that trigger protection with a special emphasis on autophagy. Our data indicate that STING induces the conversion of the common autophagosome marker LC3BI to LC3BII associated with an increase of P62 level. In addition, BLM-treated C57BL/6 wild-type (WT) mice that received the autophagy-enhancer carbamazepin display a significant decrease of recruited CD45$^+$ immune cells in the lungs together with reduced collagen deposition and fibrosis. Together, our data support an immunoregulatory function of STING in myeloid subsets through the regulation of autophagy. **Keywords:** STING;Fibrosis;lung.

DO - 081 - **The DNA Sensor AIM2 mediates psoriasiform inflammation by inducing type 3 immunity**

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Psoriasis is a chronic and recurrent inflammatory skin disease characterised by abnormal proliferation and differentiation of keratinocytes and activation of immune cells. However, the molecular driver that triggers this immune response in psoriatic skin remains unclear. The inflammation-related gene absent in melanoma 2 (AIM2) was identified as a susceptibility gene/locus associated with psoriasis. In this study, we elucidated the role of AIM2 in the pathophysiology of psoriasis. We found that mitochondrial DNA levels are elevated in patients with psoriasis and that AIM2 is highly expressed in the psoriatic epidermis of humans and in a mouse model of psoriasis induced by topical imiquimod (IMQ) application. Genetic ablation of AIM2 suppressed the development of IMQ-induced psoriasis by reducing the production of pro-inflammatory mediators such as IL-1β; IL-6; IL-17, and IL-23 and decreasing migration of neutrophils, dendritic cells, and macrophages to the inflammatory site. Furthermore, we demonstrate that AIM2 is involved in the activation/proliferation of keratinocytes and that IL-17A modulates its expression. Finally, the genetic absence of ASC and caspase-1 alleviated IMQ-induced skin inflammation. Collectively, our data show that AIM2 is involved in developing psoriasis through its canonical activation. **Keywords:** DNA sensor;AIM2;skin inflammation.

DO - 082 - **HYDROGEL-BASED SULFORAPHANE DELIVERY SYSTEM ALLEVIATES TRANSPLANT REJECTION IN FULLY MISMATCH SKIN MODEL**

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Introduction: Transplantation (Tx) is an alternative therapy for hematological and end-organ diseases and despite immunosuppression, acute rejection (AR) is a common setback among patients and is still a risk factor for graft survival. We aim to use the developed thermosensitive hydrogels, loaded with sulforaphane (SFN), a phytochemical with anti-inflammatory and immunoregulatory properties, and evaluate the action of treatment with this hydrogel in the prevention of AR in a mouse model of skin Tx. **Methods and Results:** A thermosensitive hydrogel containing SFN (0.1%) and hyaluronic acid (0.5%) dispersed in poloxamer PL407 (at 20% w/v) matrix was developed (GEL-SFN) and evaluated their biocompatibility and efficacy for preventing AR in Tx. Formulation was physicochemically characterized, favoring the structure of a liquid-viscous hydrogel adequate for *in vitro* and *in vivo* subcutaneous (s.c) application. Fully MHC-mismatch skin tx was performed using donor skin balb/c mice transplanted into C57BL/6 recipients mice. *In vitro,* treatment with GEL-SFN proved no cytotoxicity on bone marrow dendritic cell (BM-DC) viability and no difference in their maturation molecules (CD80/86 and MHCII) after LPS stimulation for 24 h. At the 9th day post-tx, 100% of untreated mice rejected their Tx. Interestingly, treatment with s.c injections of GEL-SFN every two days increased allograft survival (80%) for 14 days ($p<0.001$), showing the efficiency of the GEL-SFN treatment. Analyzing the possible effects that GEL-SFN promotes graft survival, cells from draining lymph nodes were analyzed by flow cytometry 5 days post-tx. The treated group presented a modulation of the immune system. Gel-SFN treatment reduced the frequency of DC, CD4+-naïve, -effector memory, -central memory T cells, and there was a trend of decreasing lFNγ-producing CD4+ T cells. **Conclusion:** The GEL-SFN treatment alleviates immune cell activation *in vivo*, preventing AR and contributing to increasing allograft survival. **Keywords:** transplant;acute rejection;sullforaphane.

DO - 083 - **MTOR gene variant rs1057079 is associated with TNF levels and severe COVID-19 outcomes in a Brazilian population**

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Introduction: The worst outcomes linked to SARS-CoV-2 infection have been attributed to the cytokine storm, which appears to be a key player in the immunopathogenesis of the disease. The poor prognosis is also associated with advanced age and pre-existing comorbidities, like diabetes and cardiovascular diseases. The mTORC1 pathway has been proposed as a typical molecular pathway that may be overactivated in people with a genetic predisposition. In this study, we aimed to investigate the association between *MTOR* gene variant rs1057079 and COVID-19 outcomes. **Methods:** This case-control study recruited mild and severe COVID-19 individuals from Brazilian states. *MTOR* variant rs1057079 was genotyped. Logistic regression analysis and Kaplan-Meier survival curves were performed to explore the clinical significance of the rs1057079 genotypes. We applied a genotyping risk score to estimate the cumulative contribution of the risk alleles. TNF and IL-6 plasma levels were measured. **Results and Conclusions:** The T allele of the *MTOR* rs1057079 variant was associated with a higher chance of developing the most severe form of COVID-19, and a higher likelihood of need the Intensive Care Unit. TNF levels were significantly higher in individuals with the TT risk genotype. *MTOR* gene variants showed a cumulative risk when inherited collectively and may be useful in predicting a severe outcome of COVID-19, resulting in a more effective allocation of health resources. **Keywords:** mTOR;genetic variant;COVID-19 severity.

DO - 084 - **Development of Optimized SARS-CoV-2 anti-spike Monoclonal Antibodies**

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2020 arrived, bringing with it the biggest pandemic in recent memory. A virus of the *Coronaviridae* family, SARS-CoV-2, hit the world population spreading rapidly within a few months. Although most people recover from it very well or are even asymptomatic, the circulation of the virus allows the daily identification of mutations that generate new strains, some of which are able to escape the immune system and generate more severe cases such as Alpha, Beta, Gamma, Delta, Omicron, among others. This fact increases the importance of epidemiological and genomic monitoring, as well as the constant development of therapies capable of protecting against infection and/or treating those already affected by the disease. In this context, the use of plasma from convalescent patients for the isolation of neutralizing antibodies, the improvement and development of optimized monoclonal antibodies, combined with vaccination, is an interesting strategy. To this end, neutralizing antibodies were selected from the convalescent plasma of COVID-19 patients, sequenced at the Federal University of Rio de Janeiro and kindly provided by Professor Dr. André Vale. The primary structures were obtained, and the antibodies were modeled using the tool ABodyBuilder2. Target structures of the SARS-CoV-2 spike protein were selected from the Protein Data Bank and the antibody-antigen complex was docked in the ClusPro 2.0 program (antibody mode). The free energy of the formed complexes was assessed with FoldX. Subsequently, the amino acid residues involved in the interaction were analyzed for possible mutations. The changes that proved to be energetically advantageous were maintained for antibody optimization. Finally, via computational methods, we proposed possible anti-spike monoclonal antibodies that will be tested *in vitro* in the future. In addition, this study serves as a pipeline for further therapeutic planning. **Keywords:** Immunoglobulins;COVID-19;Biopharmaceutical.

DO - 085 - **CHARACTERIZATION OF DTL8, A CONSERVED PROTEIN OF Leishmania, AND EVALUATION OF ITS POTENTIAL AS VACCINE CANDIDATE.**

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Human visceral leishmaniasis (HVL) is the second most lethal tropical parasitic disease. There is no HVL prophylactic vaccine available, therefore, antigen discovery and development of an efficacious vaccine formulation is badly needed. We performed an immunoproteomics analysis to identify immunodominant antigens in mice immunized with parasite extracts and protected against *Leishmania amazonensis*. Among the identified antigens, a conserved repetitive hypothetical protein, the DTL8, was selected for further tests, as a potential vaccine candidate. This protein is distributed in different trypanosomatids and conserved in several *Leishmania* species. After differential centrifugation of *Leishmania* extract, DTL8 was found in the same fraction as mitochondrial proteins. Endogenous labeling of the protein by Crispr-Cas9 and immunofluorescence confirmed that DTL8 is a mitochondrial protein. We also generated Crisp-Cas9 DTL8 knockout parasites and performed phenotypic analyzes. We still do not know the function of DTL8 gene. Our results indicate that DTL8 is not required for differentiation of promastigotes to amastigotes, or infectivity and amastigote multiplication in macrophages. Nevertheless, we found that mice immunized with a recombinant version of a truncated DTL8 (rDTL8) associated with Poly (I:C) showed a strong cellular and humoral response and significant decrease in tissue parasitism, when challenged with *L. infantum*. We also generated a A2 and DTL8 chimeric recombinant protein that induced a superior protective immunity than rDTL8 or rA2 alone. In conclusion, the discovery and characterization of DTL8 as well as the vaccination with the DTL8/A2 chimeric protein reveal a promising vaccine candidate for HVL. **Keywords:** Leishmaniasis;Recombinant vaccine;CRISPR-Cas9.

DO - 086 - **The role of SARM1 in the activation, differentiation, effector function, and memory formation of CD4 T cells.**

OLIVEIRA, S.D.S.[1]; DA SILVA, J.V.H.[2]; SILVA, L.M.[2]; LEITE, J.A.[2]; DA SILVA, E.M.[3]; BOS, N.N.[2]; .CAMARA, N.O.[2]. 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

The activation of CD4 T Cells is licensed by the reprogramming of cellular metabolism to a predominantly glycolytic profile, which promotes rapid production of high levels of ATP and conservation of macromolecules, both important phenomena in lymphocytes that will initiate their proliferative activity. The objective of this study is to evaluate the role of SARM1, a protein capable of degrading NAD, the important enzymatic cofactor in glycolysis, in the activation, differentiation, effector functions, and memory formation in T cells. Initially, we conducted a general characterization of CD4 and CD8 T lymphocytes and B cells in the thymus and spleen of wild-type and SARM1 KO animals. We did not find any difference in the quantity of these cells in these organs. However, we found a considerable increase in the spleen weight of SARM1 KO animals associated with an increase in CD69 expression in Tγδ and Tαβ cells in these animals under homeostatic conditions. In vitro, we observed that T lymphocytes isolated from SARM1 KO animals and activated with anti-CD3 and anti-CD28 produce higher levels of pro-inflammatory cytokines IL-4 and IL-17. When analyzing microarray data from CD4 T lymphocytes isolated from healthy donors, we observed a negative correlation between SARM1 expression and IL-4. Our preliminary data suggest a possible involvement of SARM1 in T cell activation and their cytokine production profile. **CEUA:** 4660110123 aprovado em 04/04/2023. **Keywords:** SARM1;Inflammation;CD4 T cells.

DO - 087 - **THE EFFECTS OF OBESE ADIPOSE TISSUE INTERACTION ON LUNG FIBROBLASTS**

DA COSTA, A.C.[1]; GUALBERTO, A.[2]; VENERANDO, L.A.F.[1]; GONÇALVES, D.D.A.[1]; CANGUSSU, V.[3]; GAMEIRO, J.[1]. 1. UFJF, UFJF JUIZ DE FORA - MG - BRASIL; 2. UNB, UNB BRASILIA - DF - BRASIL; 3. HOSPITAL E MATERNIDADE THEREZINHA DE JESUS, HOSPITAL E MATERNIDADE THEREZINHA DE JESUS JUIZ DE FORA - MG - BRASIL.

Introduction Obesity, which is characterized by the exacerbated accumulation of adipose tissue in the body, as well as by conditioning the individual to chronic low-grade inflammation, is an important public health problem in Brazil and worldwide. This condition is mainly associated with cardiometabolic diseases, although it is also involved in the development of cancer, such as breast cancer. The mechanisms by which obese adipose tissue may favor the generation of metastatic disease still need to be better elucidated. Moreover, cells from pre-metastatic sites, such as resident fibroblasts, may be previously modulated to generate a permissive environment for the reception and development of tumor cells. In this context, we hypothesized that chronic systemic inflammation in obese individuals, as well as the small extracellular vesicles (EVs) secreted by this tissue, may activate cells in sites secondary to breast cancer, such as lung fibroblasts, Methods: Visceral adipose tissues (VAT) from eutrophic (VAT-EU) and obese (VAT-OB) subjects were collected and from them, extracellular vesicle-free conditioned medium and small EVs were obtained. Subsequently, lung fibroblasts of the Wi-38 strain were cultured in culture medium containing the conditioned media or the EVs to perform viability, proliferation, migration and clonogenic assays. Results and Conclusions TAV-EU and TAV-OB conditioned media did not significantly impact cell viability and proliferation. However, we observed that fibroblasts treated with TAV-EU formed more colonies and exhibited more prominent migratory activity compared to the untreated (NT) and TAV-OB group. The data suggest that adipose tissue-derived soluble factors may affect lung fibroblasts in a systemic manner. However, further investigations will be performed to verify whether these changes directly impact breast cancer progression. **Keywords:** Fibroblasts;Obese;adipose tissue.

DO - 088 - **Immunomodulatory activities of capsular polysaccharides (GXM and GXMGal) on murine macrophages infected by Trypanosoma cruzi**

DE OLIVEIRA, J.C.G.[1]; GONÇALVES, M.S.[1]; DE SOUSA, B.A.[1]; LIMA, I.D.[1]; DA SILVA JUNIOR, E.B.[1]; DOS SANTOS, I.M.F.[1]; RODRIGUES, A.G.[1]; DE LIMA, M.F.[1]; DE LIMA, L.F.[1]; NASCIMENTO, D.D.O.[2]; DECOTE-RICARDO, D.[2]; FREIRE-DE-LIMA, C.G.[1]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO SEROPÉDICA - RJ - BRASIL.

Cryptococcus neoformans is an opportunistic fungus with global distribution and causes a disease called cryptococcosis, which starts in the lung and is prevalent in immunosuppressed individuals, which, in severe cases, can lead to meningoencephalitis and even death. Biochemical studies have shown that the capsule of this fungus is mainly composed of glucuronoxylomamann (GXM), which represents approximately 88% of the total. In addition, there is the presence of glucuronoxylomannogalactan (GXMGal), which corresponds to approximately 10% of the total, and mannoproteins, representing 2% of its total composition. It has already been seen that purified capsular components show different immunomodulatory effects. While GXM has mostly immunosuppressive activity, leading to inhibition of the immune response, for example, GXMGal has mostly immunoprotective activity. To evaluate this difference in the immunomodulatory effects of the two major capsular polysaccharides in another infection model, we used the in vitro infection model of murine macrophages infected with the DM28c strain of *Trypanosoma cruzi* (*T. cruzi*) and, later, we performed the treatment with GXM or GXMGal. The results obtained showed that at 7 and 10 days after infection, the number of trypomastigotes released under conditions with GXM was higher than under conditions with GXMGal, even in the control where interferon γ was added. Furthermore, the proportion of infected cells and the number of amastigotes count is higher in cells infected in the presence of GXM. On the other hand, conditions with GXMGal showed higher production of nitric oxide. We also performed inflammatory mediators measurements by ELISA, which showed an increase in TGFβ in cells with GXM, TNFα in GXMGal and PGE2 in both conditions, regardless of the presence of *T. cruzi.* These results reinforce the immunomodulatory activities of the capsular components already demonstrated in previous studies. **Keywords:** Capsular polysaccharides;Trypanosoma cruzi;Cryptococcus neoformans.

DO - 089 - **From Complexity to Clarity: Decoding the Molecular Mechanisms of Gal-3BP and SP-2 Interaction**

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The Galectin-3 Binding Protein (Gal-3BP) is a highly glycosylated protein involved in the modulation of cell-cell and cell-matrix interactions. Its interactome is complex and involves over 200 proteins as partners. In this way, Gal-3BP has been predominantly explored through innate immunity and neoplastic progression contexts associated with immune evasion, proliferative signaling, angiogenesis, and metastasis. Gal-3BP has emerged as a promising therapeutic target, particularly in the context of Antibody-Drug Conjugates. The utilization of the anti-Gal3BP antibody (SP-2) as a carrier has shown successful results. However, the molecular basis of this interaction is not clearly understood. The knowledge of the epitope-paratope pair can contribute to the development of new immunotherapeutics for the treatment of oncological diseases. Thus, this work aims to propose a validated binding pose of SP-2 and Gal-3BP through in silico methods. The Gal-3BP and the SP-2 Fv region were modeled to obtain the respective tridimensional structure. In the following steps, the molecular docking approach found candidate poses for the complex. Then, the complex stability and flexibility were evaluated with the heated molecular dynamics simulation. The preliminary results show that the SP-2 binds to BTB/POZ or BACK domains. Both regions are involved with the complexation of Gal-3BP into the multimeric form. Also, this region is essential for interaction with its partners, especially lectins. Heated simulation indicated the most favorable epitopes in Gal-3BP, highlighting the importance of the hydrogen bond mediated by protonated glutamic acids in the CDRH3. In conclusion, we propose the molecular basis of the binding pose of SP-2 and Gal-3BP, with a comprehensive understanding of the stability and flexibility of the complex. These findings pave the way for further advancements in biopharmaceuticals and targeted cancer treatments. **Keywords:** Molecular dynamics simulation;Protein-protein interaction;Antibody design.

DO - 090 - **THE UBIQUITIN LIGASE SMURF1 REGULATES BACTERIAL INVASION IN MACROPHAGES AND HOST RESISTANCE TO SALMONELLA TYPHIMURIUM INFECTION**

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Smurf1, an E3 ubiquitin ligase, ubiquitinates a number of substrates related to innate immune signaling, inflammation, and antimicrobial autophagy. However, how Smurf1 works on immune responses against bacterial infections is not fully understood. Here we aim to study the role of Smurf1 in modulating host-bacteria interaction in macrophages and *in vivo* in the context of infection with *Salmonella typhimurium* (S.tm). To assess how Smurf1 can modulate bacterial invasion and replication in macrophages, WT or Smurf1$^{-/-}$ macrophages were infected with S.tm, and bacteria invasion under different conditions was evaluated by the gentamicin protection assay. Our results show that invasion of S.tm opsonized by the complement system was significantly higher in Smurf1$^{-/-}$ macrophages, while S.tm active invasion mediated by the T3SS secretion system (invasive bacteria) or internalization by phagocytosis receptor was unchanged in Smurf1$^{-/-}$ macrophages. Moreover, replication of invasive S.tm was reduced in Smurf1$^{-/-}$ macrophages at 18h post-infection. To evaluate the role of Smurf1 in host resistance to infection, WT or Smurf1$^{-/-}$ mice were infected intraperitoneally with S.tm and bacterial burn was evaluated in the liver and spleen at 48h post-infection. Smurf1$^{-/-}$ mice showed less bacterial load in the liver and spleen, and histopathological analysis of the liver showed less tissue damage compared to WT animals. A survival assay performed with WT or Smurf1$^{-/-}$ mice infected with S.tm by the oral route showed that Smurf1$^{-/-}$ mice survived significantly longer compared to WT mice. Overall, these data suggest that Smurf1 modulates bacterial invasion in macrophages and host resistance against S.tm infection. This study opens new perspectives for the development of therapeutics for treating infectious and inflammatory diseases. **Keywords:** Smurf1;innate immunity;macrophages.

DO - 091 - **MODULATORY EFFECT OF CTX FROM Crotalus durissus terrificus VENOM ON ACUTE INTESTINAL INFLAMMATION INDUCED BY TNBS IN MICE AND ON HUMAN CELL MIGRATION IN VITRO.**

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Introduction: Inflammatory bowel diseases (IBD) are characterized by loss of the tolerance for enteric bacteria and uncontrolled inflammatory process with intense immune cell infiltration. The mechanisms involved in the exacerbated inflammatory process of IBD are not yet clearly established. Crotoxin (CTX) is the main component of the *C.d.terrificus* snake venom and, among its biological activities, the immunomodulatory effect can be highlighted. **Objective:** Evaluate the modulatory effect of CTX in IBD induced by TNBS *in vivo* model and *in vitro* cultures. **Methods and Results:** To evaluate the effect of CTX in the intestinal mucosa, Caco-2 cells were stimulated with IFN-γ in the presence or absence of CTX for 18 h and analyzed the ICAM-1 expression and IL-8 production. The ability of caco-2 supernatants to induce monocyte and neutrophil migration in *transwell* model were evaluated. The results showed that the CTX inhibited the ICAM-1 expression and IL-8 production in Caco-2 cells, as well as human monocyte and neutrophil migration. The effect of CTX on the IBD process was studied in mice group that received the intrarectal instillation of TNBS and after 12 h, the toxin was injected (ip). After 24, 48 and 72 h of TNBS-instillation, the CTX administration attenuated the weight loss, clinical score and presence of necrotic area in intestinal tissue of TNBS-group. The involvement of LOX-derived mediators in the modulatory effect of CTX was also evaluated in TNBS-mice groups treated with NDGA. The data showed that the effect of CTX on IBD in TNBS-group was partially abolished. The role of FPRs in the CTX effect in TNBS-mice was analyzed using Boc-2. The results demonstrated the dependence of FPRs for the modulatory effect of CTX in TNBS-mice group. Therefore, the data indicate the role of FPRs, partial involvement of LOX-derived mediators in the modulatory effect of CTX *in vivo* and also the direct effect of the CTX on intestinal epithelial cells. **Keywords:** Crotoxin;Intestinal Bowel Disease;Immunomodulation.

DO - 092 - **Aspergillus fumigatus extract induces severe lung inflammation and the formation of multinucleated giant cells**

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Macrophages are one of the most abundant immune cells in the lung and key players in allergic response, but their role remains poorly understood (Inflamm Res.71(12):1417-1432.2022). Here, we showed that intranasal administration *of Aspergillus fumigatus* extract (ASP) in mice induces lung inflammation with the recruitment of neutrophils and eosinophils, a mixed Th2 and Th17 response but also the formation of multinucleated giant macrophages (MGCs) in lung. In addition to the production of extracellular dsDNA in bronchoalveolar lavage fluid (BALF), ASP induces cell death by PANoptosis. Analysis of metabolites in BALF revealed an increase in the polyamine spermidine and a decrease in glycolysis metabolites L-glutamine promoting the polarization of M2 macrophages (Cell Mol Immunol.19(3):384-408,2022). Transcriptomic analysis by RNA sequencing showed an upregulation of specific M2 macrophages genes signature with increased *Arg1*, *Mrc1, Retnla*, *Chil3*, *ccl17*, *ccl24*, *IL-4ra*, *Csf1*, *IL-10* and *IL-13* (Nat Commun.10(1):4353,2019), but a downregulation of efferocytosis genes *MerTK, Tyro3, Axl, Cx3cr1, Gas6, Lrp1, Pros1, Tulp1 and Abca1* (Nat Rev Immunol.20(4):254-267,2020). Thus, our study suggests that the formation of MGCs is initiated by high Th2 immune response environment and cell death by PANoptosis (Eur J Immunol.37(1):33-42,2007). However, the mechanism of MGCs formation and severe lung inflammation need to be further explored. **Keywords:** Allergic lung inflammation;Aspergillus fumigatus;multinucleated giant macrophages.

DO - 093 - **THE ROLE OF MELATONIN ON THE MODULATION OF MITOCHONDRIAL FUNCTION, INFLAMMATION, AND CARCINOGENIC PARAMETERS OF HUMAN GASTRIC CANCER CELLS**

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Introduction: Melatonin is a pleiotropic molecule with numerous biological activities. It is mainly produced by the pineal gland in response to darkness. There is an increasing focus on melatonin in the field of oncology since this molecule can modulate cell growth. However, the role of melatonin in human gastric cancer is poorly understood. Therefore, this work aimed to analyze the role of melatonin in the modulation of carcinogenic parameters, inflammation, mitochondrial function, and oxidative stress in the gastric cancer cell line (AGS). **Methods:** AGS cells were stimulated with melatonin at concentrations of 0.625, 2.5, and 5 mM at different times. Mitochondrial viability and function were assessed by MTT assay, and high-resolution respirometry, respectively. The cell death profile was assessed by annexin-V/propidium iodide (PI). The enzyme lactate dehydrogenase (LDH) release was evaluated by the CyQUANTTM kit. Cell proliferation was assessed by the CFSE probe staining. The cell cycle and membrane pore formation were assessed by the PI probe staining. Oxidative stress was assessed by DCF-DA. Cytokine levels were evaluated by ELISA. **Results:** Both melatonin at 2.5 and 5 mM promoted a reduction in mitochondrial viability, cell proliferation, oxidative respiration, and ROS production. In addition, these concentrations significantly increased apoptotic death compared to unstimulated cells. Moreover, melatonin was able to reduce pyroptosis-related membrane pore formation and LDH release. **Conclusion**: Taken together our data showed that melatonin at the higher concentration was able to promote an antitumor effect by reducing mitochondrial viability, increasing cell death, and reducing oxidative phosphorylation in AGS gastric cancer cells. Importantly, our data highlight that melatonin could promote the inhibition of pyroptosis in gastric cancer cells which could be crucial in the development of melatonin in therapeutic approaches. **Keywords:** Cancer;Melatonin;Pyroptosis.

DO - 094 - **Bothrops jararaca Snake Venom Inflammation Induced in Human Whole Blood: Role of the Complement System**

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The clinical signs of bothrops species envenomation are marked by notable local consequences that may lead to tissue loss, physical impairment, or amputation. Systemic symptoms like bleeding, coagulopathy and acute renal failure may manifest. The presence of mediators of the inflammatory process arising from tissues injured by the venom coincides with the fast onset of local clinical symptoms. Given the crucial role the complement system plays in the inflammatory response, in this study we used an ex vivo human whole blood model to examine the effects of Bothrops jararaca snake venom on the complement system and cell surface receptors involved in innate immunity. In the human whole blood model, B. jararaca venom was able to activate the complement system and significantly boost the production of the anaphylatoxins C3a/C3a-desArg, C4a/C4a-desArg, C5a/C5a-desArg, and sTCC. The venom of B. jararaca decreased the expression of CD11b, CD14, and C5aR1 in leukocytes. Cp40, a C3 inhibitor, inhibited the C3 component, which caused the levels of C3a/C3a-desArg, C5a/C5a-desArg, and sTCC in samples stimulated with the venom to drop to baseline levels. In the human whole blood model, exposure to B. jararaca venom increased the production of inflammatory cytokines and chemokines such TNF-, IL-8/CXCL8, MCP-1/CCL2, and MIG/CXCL9. TNF-, IL-8/CXCL8, and MCP-1/CCL2 production were all markedly reduced after treatment with Cp40. In the samples triggered by venom, C5aR1 inhibition with PMX205 also facilitated a decrease in TNF- and IL-8/CXCL8 to baseline levels. In conclusion, the data provided here indicate that the inflammatory process is considerably aided by the complement system activation induced by the venom of the snake B. jararaca in the human whole blood model. The ability of Cp40, a C3 component inhibitor, and PMX205, a C5aR1 antagonist, to reduce a number of inflammatory markers suggests that complement inhibition may hold promise as a treatment for B. jararaca envenoming. **Keywords:** Bothrops jararaca;complement system and inhibitors;human whole blood.

DO - 095 - **Impact of diet-induced obesity on the small intestine immune system of OT-II mice**

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Studies demonstrate that obesity is associated with the loss of peripheral tolerance (PT) and suggest it can be linked to allergy and autoimmunity. The small intestine (SI) immune system is crucial to the development of PT since it responds to antigens from diet, microbiota and pathogens while maintaining immune homeostasis. It is known that obesity alters SI immunity, but it is not known whether it impairs the mechanisms of PT in an antigen-specific system. Thus, here we investigated whether diet-induced obesity (DIO) promotes loss of PT focusing on the immune processes in the SI of OT-II mice. OT-II mice were submitted to high-fat diet (HFD) for 12 weeks to induce obesity (HF) or not (CT). During DIO, glucose tolerance test (GTT) was performed. In parallel, oral tolerance protocol to ovalbumin (OVA) were induced by giving egg white (20%) in drinking water for 3 days to HF and CT, followed by immunization and challenge with OVA. Epididymal adipose tissue (epiWAT) was weighed and collected for histology, and spleen and SI were collected for flow cytometry analysis of Foxp3+ regulatory T cells (Tregs). OT-II mice submitted to HFD developed obesity (higher body weight), showing increased area under curve on GTT (p=0,027) and higher epiWAT weight (p<0,001). Analysis of SI lamina propria (SILP) showed no difference between HF and CT in the frequency of total Tregs. However, when OT-II mice were submitted to oral tolerance protocol, HF showed increased frequency of total Tregs (p=0,002) and OVA-specific Tregs (p=0,001) in SILP. In spleen, there was no difference between HF and CT in frequency of total Tregs, but HF showed reduced frequency of OVA-specific Tregs (p=0,006). Our preliminary data show that HFD promotes obesity in OT-II mice. DIO seems to interfere with OVA-specific Tregs in OT-II mice, suggesting alteration of antigen-specific PT mechanisms. Further refined experiments will be performed to elucidate these findings. Funding: FAPESP (2021/03913-3; 2019/14755-0). **Keywords:** obesity;small intestine;peripheral tolerance.

DO - 096 - **Atazanavir Upregulate Viability and Ameliorate NO Production of Spike S1-stimulated Microglia**

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COVID-19 patients can develop various neurological symptoms probably due to their exacerbated immune response against the SARS-CoV-2 infection. Microglia takes an important place in central nervous system (CNS) physiology, as well, it responds against local infections by producing inflammatory mediators such as nitric oxide, and oxygen species (EROs). Atazanavir (ATZ) can modulate the immune response and it appears to have an antiviral effect against SARS-CoV-2. Here, the ability of atazanavir to modulate reactive species production by microglia stimulated with the spike S1 protein of SARS-CoV-2 was evaluated. For this, C20 cells (human microglia cell line) cultured in DMEN-F12 supplemented with 2,5 mmol/L L-glutamin, 10% fetal bovine serum and 1% penicillin/streptomycin were stimulated with 0.1, 0.5 and 1 µg/mL of spike S1 protein for 24 hours at 37°C in a humid chamber with 5% CO2. After stimulation, cells were treated or not with ATZ 1µM and 3µM for more 24 hours. Cell viability was performed by MMT assay, nitric oxide (NO) production was evaluated by Griess assay and EROs by flow cytometry. The microglia stimulation with spike S1 decrease the cells viability, but ATZ was not cytotoxic and improves de viability of stimulated cells. The spike S1 decreased NO production, but it did not alter EROS production. In general, ATZ did not modify EROS production by stimulated cells, but It influenced NO production depending on the treatment and spike S1 concentration. Low levels of reactive species are necessary for proper functioning of the CNS. Probably, the maintenance of reactive species low levels by spike S1-stimulated cells might contribute to alter the physiology, neurotransmission, and plasticity of CNS during infection. ATZ repositioning as adjuvant treatment might be an interesting issue, as it can prevent spike S1-mediated cytotoxicity and increase a little the NO production, without hyper stimulate EROS. However, more studies should be carried out. **Keywords:** Microglia;SARS-CoV-2;Neuroinflammation.

DO - 097 - **ABSET: A STANDARDIZED ANTIBODY DATASET FOR MACHINE LEARNING APPLICATIONS**

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Antibodies are immune system proteins produced by B lymphocytes and are regarded as potent therapeutic candidates due to their low toxicity to the human body and high affinity for their molecular targets. Structural and Functional Biology on Biopharmaceuticals Group at FIOCRUZ-CE is developing an integrated computational platform with deep learning aimed at the identification and optimization of potential biotherapeutics against diseases of public health interest. For this purpose, the construction of a dataset of antibody structures, with a focus on the variable regions, is crucial. We thus proposed to implement a structural antibody dataset containing descriptors based on their biochemical properties, such as molecular surface, atomic positions, and hydrophobicity. Firstly, the antibodies were identified from PDB database through sequence analysis, followed by filtering the structures whose antigen is not a protein and have resolution above 4 Å. Subsequently, the structures were numbered and standardized using an internally developed Python script, followed by the implementation of molecular descriptors aimed at extracting the structural characteristics of the antibody-antigen complexes and representing them in graph format. To increase the quantity and diversity of the dataset, antibody-antigen complexes that interact incorrectly were generated using the molecular docking approach. Finally, the effects of mutagenesis on the antibody-antigen interaction were retrieved from the SKEMPI database. As a result, a dataset with 4600 standardized and curated structures was constructed. A total of 1859 mutation points distributed across the antibody and antigen were retrieved, along with their effects on the interaction affinity between these biomolecules. Finally, the molecular docking calculations produced 123,491 incorrect models. **Keywords:** Computational Biology;Biopharmaceuticals;Structural characterization.

DO - 098 - **pDNA delivery mediated by ionizable lipid nanoparticles generates functional CAR T cells from primary human lymphocytes**

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Introduction: CAR T cell immunotherapy represents a major breakthrough in treatment of hematological tumors. Its manufacture is based on the *ex vivo* manipulation of human T cells. New methodologies for the generation of CAR T cells are needed, with a focus on reducing the *ex vivo* manipulation period as well as preventing adverse effects. Here, we developed lipid nanoparticles (LNPs) encapsulating pDNA to produce CAR T cells *in vitro*. **Methods:** To assess the transfection efficiency of LNPs, Jurkat cells were transfected with an LNP encapsulating a pDNA for the expression of CAR (LNP-CAR). Activation of transfected Jurkat cells was evaluated by coplating CAR expressing cells with target cells for 24 hours and measuring the expression levels of CD69 in CAR^+ cells. Primary human T cells isolated from healthy donors were transfected with the optimized LNP and coplated with target cells to evaluate specific killing by flow cytometry. **Results:** Jurkat cells transfected with LNP-CAR expressed the CAR receptor in a transient manner. Transfection with our optimized LNP induced higher levels of CD69 on effector cells when coplated at a 1:1 ratio with target cells. This activation was also dependent on the effector-to-target ratio. Finally, this optimized LNP was capable of transfecting primary human T cells, which were also effective against target cells in a ratio-dependent manner. **Conclusion:** We developed an LNP platform for the delivery of pDNA to immune cells, namely T cells, as a novel approach for the production of CAR T cells. Our optimized LNP showed low toxicity when compared to standard methods, and generated CAR T cells that were able to induce specific killing of target tumor cells. As a non-viral delivery method, LNPs open an alternative to produce CAR T cells in less time, reducing the associated costs and improving the effectiveness of the adoptive cells. LNPs can also be used for other CAR constructs with a fast optimization protocol. **Keywords:** CAR T cells;Ionizable lipid nanoparticles;Immunotherapy.

DO - 099 - **ANTIBODIES AGAINST EFFLUX PUMP PROTEIN TOLC PROTECTS AGAINST ESCHERICHIA COLI INFECTION**

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The development of alternatives to antimicrobials is of utmost importance, due to the rapid spread resistant bacteria. Among the mechanisms of antimicrobial resistance is the overexpression of efflux pumps. We aim to evaluate the immune response against the efflux pump outer membrane protein TolC of Gram-negative bacteria of clinical importance, such as *Escherichia coli* (*E. coli*) for the discovery of antibodies capable of inhibiting their infection. *In silico* analysis predicted epitope regions in the extracellular regions of TolC. Anti-TolC IgM, IgG and IgA were detected in human serum, especially in patients with Gram-negative infections, through ELISA. TolC *in vitro* stimulation of lymph node cells obtained from TolC-immunized mice, led to increased percentage of T cell proliferation, and IFN-γ secretion, evaluated by flow cytometry and ELISA, respectively. TolC immunization induced increased percentage of TolC-specific memory B cells in spleens and lymph nodes, assayed by flow cytometry, and stimulated anti-TolC IgM and IgG production, detected by ELISA. IgG from TolC immune serum binds to TolC in bacterial cell extract and to live *E. coli,* assayed by western blotting and flow cytometry, respectively. Serum from TolC-immunized mice increased *E. coli* macrophage uptake *in vitro* when compared to non-immune serum. Immunization with TolC protected mice from infection with *E. coli*, by reducing their mortality. Our results showed that TolC is immunogenic, activating T and B cells, culminating in the production of antibodies in mice and humans. Anti-TolC antibodies present protective activity, since they were able to bind live *E. coli*, increase *E. coli* macrophage uptake and protect mice from a lethal infection. Further experiments are needed to evaluate the activity of anti-TolC antibodies against antimicrobial resistant Gram-negative bacteria. **Keywords:** Tolc;Antibodies;Efflux Pump.

DO - 100 - **Cellular and serological response to a heterologous vaccination against SARS-CoV-2**

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Introduction: With the COVID-19 pandemic, the search for vaccines against SARS-CoV-2 due to the important cellular and humoral stimulation, increased. This way, different combinations of heterologous doses were used in different countries. However, the knowledge about the mechanisms driving immune responses post-vaccination in this scenario is still scarce. In this study, we recruited a cohort of vaccinees and analyzed their cellular and serological response in four phases during the vaccination. **Methods:** We selected 6 vaccinees who received two doses of CoronaVac and a third dose of BNT162b2. Blood samples were collected before any vaccination and after each dose (pre-vaccine, after 1st dose, after 2nd dose and after 3rd dose). Serum was used for serological analyzes and PBMCs' proteins were extracted, reduced, alkylated, digested with trypsin, and labeled with TMT tagging. LC-MS/MS analysis were performed in a Q-Exactive HF-X hybrid quadrupole-orbitrap mass spectrometer. Raw data were processed using Proteome Discoverer Suite and analyzed in R software. Differentiated abundant proteins (DAPs) were subjected to a co-expression, protein-protein interaction, and pathway enrichment analysis. **Results:** After identifying and applying our filters, we ended up with 1,022 proteins, of which 157 were differently abundant when comparing pre vaccine and each vaccine dose. In addition, we observed an important protein-protein interaction network in metabolic pathways and a big co-expression cluster with an increase of protein abundance throughout the vaccination regarding the neutrophil degranulation, innate immune system and metabolism of RNA. Serological results also had a positive correlation with proteins associated with these pathways. **Conclusions:** In conclusion, we observed a significant alteration in the protein profile in PBMC samples and a positive correlation between specific antibodies against SARS-CoV-2 and important immune pathways due to vaccination. **Keywords:** Vaccine;Proteomics;SARS-CoV-2.

DO - 101 - **TARGETING ACYL-CoA:CHOLESTEROL ACYLTRANSFERASE DURING SARS-CoV-2 INFECTION**

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Lipid droplets (LD) play vital roles in storing and metabolizing neutral lipids such as triacylglycerol and cholesterol esters. They also contribute to cell signaling and have multiple functions in infections and inflammation. The intracellular cholesterol is esterified by the acyl-CoA:cholesterol acyltransferase (ACAT) enzyme and stored within LD. Maintaining cholesterol homeostasis is crucial for various stages of viral replication, including entry, replication, assembly, and egress. Notably, metabolic syndrome and hyperlipidemia have been associated with worse outcomes in SARS-CoV-2 infection, while cholesterol-lowering inhibitors like statins have shown potential in improving COVID-19 survival. This highlights the possibility of targeting cholesterol metabolism as a treatment strategy. In this study, we investigated the significance of the final step of cholesterol ester synthesis by pharmacologically inhibiting the ACAT enzyme in a SARS-CoV-2-infected lung epithelial cell line (Calu-3). Following SARS-CoV-2 infection, we observed an increase in LD and cholesterol levels, which could be prevented by treatment with the ACAT inhibitor (CI-976). Moreover, CI-976 treatment suppressed viral replication, the release of CXCL10, and cell death through pyroptosis and necroptosis, leading to the reversion of caspase 1 and MLKL activation. Our results demonstrate that SARS-CoV-2 infection induces the activation of lipid metabolism, accumulation of LD and cholesterol, and subsequent cell death. Inhibiting ACAT prevents the biogenesis of LD, accumulation of cholesterol, viral replication, release of chemokines, and cell death through pyroptosis and necroptosis. These findings highlight the crucial role of ACAT, cholesterol metabolism, and LD in viral replication and cell death, suggesting their potential as promising targets for antiviral interventions. Funding: CAPES, FAPERJ, and INOVA/FIOCRUZ. **Keywords:** SARS-CoV-2;Lipid Droplets;ACAT.

DO - 102 - **Association of Monocarboxylate Transporter Family Genes with Immunotherapy Outcomes in Melanoma Patients**

SORROCHE, B.P.[1]; DE SOUZA, V.G.[1]; TEIXEIRA, R.D.J.[1]; TOSTES, K.[1]; KINKER, G.S.[2]; LAUS, A.C.[1]; PEREIRA, C.A.D.[3]; MEDINA, T.D.S.[2]; SANTANA, I.V.V.[4]; VAZQUEZ, V.D.L.[5]; ARANTES, L.M.R.B.[1]. 1. MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL, MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 2. INTERNATIONAL RESEARCH CENTER, A.C. CAMARGO CANCER CENTER, INTERNATIONAL RESEARCH CENTER, A.C. CAMARGO CANCER CENTER SÃO PAULO - SP - BRASIL; 3. DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL, DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 4. DEPARTMENT OF PATHOLOGY, BARRETOS CANCER HOSPITAL, DEPARTMENT OF PATHOLOGY, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 5. MELANOMA, SARCOMA AND MESENCHYMAL TUMORS SURGERY DEPARTMENT, BARRETOS CANCER HOSPITAL, MELANOMA, SARCOMA AND MESENCHYMAL TUMORS SURGERY DEPARTMENT, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL.

Background: Several studies have shown the correlation between tumor metabolism, immune response, and disease progression. Glycolytic activity and lactate uptake lead to immune exhaustion in melanoma, but the associated pathways and their implications in clinical practice are yet to be elucidated. This study aimed to investigate the prognostic potential of two genes from the solute carrier (SLC) 16 gene family, namely *SLC16A11* and *SLC16A13*, in a cohort of melanoma patients treated with immune checkpoint blockade (ICB). **Methods**: FFPE tumor samples from 30 patients with advanced melanoma who underwent anti-PD-1 immunotherapy were collected. Patients exhibiting tumor progression were categorized as non-responders, while patients demonstrating partial and complete response or with stable disease for more than six months were classified as responders. The expression levels of *SLC16A11* and *SLC16A13* were analyzed using the NanoString nCounter methodology. The release of immune mediators into plasma was evaluated by Cytometric Bead Array. Statistical analyses were performed using SPSS 23.0 software. **Results**: Sixteen (53.3%) patients presented disease progression after ICB treatment. Lack of therapeutic response was associated with increased expression of both *SLC16A11* and *SLC16A13* genes (p=0.024 and p=0.011, respectively). Moreover, higher *SLC16A11* expression was associated with higher IL-4 plasmatic concentration (r=0.363; p=0.048) and inversely correlated with PD-L1 primary tumor expression (r=-0.371; p=0.044). Finally, elevated expression of these two genes led to reduced overall survival following immunotherapy treatment (p=0.017 and p=0.003, respectively). **Conclusion**: *SLC16A11* and *SLC16A13* emerged as predictive markers of poor response and lower overall survival in advanced melanoma patients undergoing anti-PD-1 therapies. Future prospective studies are crucial to validate their clinical potential in a broader setting. **Keywords:** Melanoma;Biomarkers;Immunotherapy.

DO - 103 - **Context-dependent impacts of endogenous CD40L on peritoneal cavity macrophage responses associated with type 2 settings.**

MARTINS, M.S.[1]; ALAYÓN, I.D.G.[1]; FINLAY, C.[2]; PAPOTTO, P.[3]; PARKINSON, J.[3]; CHAN, B.[3]; ZHANG, L.[3]; BALDWIN, G.[3]; CASARAVILLA, C.[1]; ALLEN, J.E.[3]; DÍAZ, Á.[4]. 1. FACULTAD DE CIENCIAS, UNIVERSIDAD DE LA REPÚBLICA, FACULTAD DE CIENCIAS, UNIVERSIDAD DE LA REPÚBLICA MONTEVIDEO - URUGUAI; 2. TRINITY COLLEGE DUBLIN, TRINITY COLLEGE DUBLIN DUBLIN - IRLANDA; 3. THE UNIVERSITY OF MANCHESTER, THE UNIVERSITY OF MANCHESTER MANCHESTER - REINO UNIDO; 4. FACULTAD DE QUÍMICA, UNIVERSIDAD DE LA REPÚBLICA, FACULTAD DE QUÍMICA, UNIVERSIDAD DE LA REPÚBLICA MONTEVIDEO - URUGUAI.

The accumulation of macrophages during inflammation results from the recruitment of monocyte precursors and/or the proliferation of pre-existing macrophages. The second mechanism was initially described in type 2 contexts, in which it is mainly driven by the cytokines IL-4 and IL-13. These cytokines additionally polarize macrophages towards M(IL-4) phenotypes, functionally opposed to classical activation. The CD40-CD40L interaction has central roles in immunology, including being critical for classical macrophage activation. However, scarce data exist on its impacts on macrophage responses in type 2 contexts. We studied the impact of endogenous CD40L on macrophage proliferation and M(IL-4) polarization ***in vivo*** both after injection of IL-4 and during ***Heligmosomoides polygyrus*** infection, which induces IL-4 production by $CD4^+$ T cells systemically. CD40L was detectable in peritoneal cavity $CD4^+$ T cells under basal conditions on the cell surface (~ 1% of the cells) and was increased by ***H. polygyrus*** infection (~ 2.5% of the cells). In the context of IL-4 injection, blockade of CD40L blunted macrophage proliferation without affecting M(IL-4) polarization. In contrast, during ***H. polygyrus*** infection, CD40L blockade enhanced peritoneal macrophage proliferation as well as their expression of the M(IL-4) marker Ym1 (Chil-3). Our interpretation is that under conditions of low CD40L availability, CD40L contributes to IL-4-driven macrophage proliferation whereas under conditions of high CD40L availability and/or an ongoing specific adaptive response, CD40L acts as a negative regulator of type 2-associated macrophage responses. The second part of our preliminary conclusion is backed by results using high-dose soluble CD40L (presented in an accompanying poster). This scenario has parallels with pre-existing data about the induction of Th2 responses, which requires endogenous CD40L but is deviated towards Th1 in the presence of high-dose exogenous CD40 agonists. **Keywords:** CD40L;Macrophages;Th2.

DO - 104 - **PD-L1 expression is associated with reactive oxygen response, metabolic and proliferative genes in advanced melanoma**

TEIXEIRA, R.D.J.[1]; SORROCHE, B.P.[1]; DE SOUZA, V.G.[1]; TOSTES, K.[1]; KINKER, G.S.[2]; LAUS, A.C.[1]; PEREIRA, C.A.D.[3]; MEDINA, T.D.S.[2]; SANTANA, I.V.V.[4]; VAZQUEZ, V.D.L.[5]; ARANTES, L.M.R.B.[1]. 1. MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL, MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 2. INTERNATIONAL RESEARCH CENTER, A.C. CAMARGO CANCER CENTER, INTERNATIONAL RESEARCH CENTER, A.C. CAMARGO CANCER CENTER SÃO PAULO - SP - BRASIL; 3. DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL, DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 4. DEPARTMENT OF PATHOLOGY, BARRETOS CANCER HOSPITAL, DEPARTMENT OF PATHOLOGY, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL; 5. MELANOMA, SARCOMA AND MESENCHYMAL TUMORS SURGERY DEPARTMENT, BARRETOS CANCER HOSPITAL, MELANOMA, SARCOMA AND MESENCHYMAL TUMORS SURGERY DEPARTMENT, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL.

Background: Immune checkpoints, such as Programmed Death Ligand-1 (PD-L1), have been highlighted by their importance as prognostic biomarkers and/or used as a predictor of immune checkpoint therapy response. The expression of PD-L1 may be induced by extrinsic pathways, like inflammatory cytokines, or intrinsic pathways, including oncogenic signaling. The comprehension of intrinsic pathways and genes associated with PD-L1 expression could contribute to the understanding of tumor evasion mechanisms. **Objective:** To evaluate genes associated with PD-L1 expression in advanced melanoma. **Methods:** Fifty-two patients with advanced melanoma (stages III or IV) with a minimum of 60% of tumor cells in formalin-fixed, paraffin-embedded (FFPE) tissue were included. PD-L1 expression was assessed by immunohistochemistry using the Benchmark® ULTRA platform and the anti-PD-L1 (Cell Signaling Technology, clone E1L3N and dilution 1:200). The tumor proportion score was evaluated in a minimum of 100 viable tumor cells. Gene count (GC) was evaluated in RNA samples submitted to Metabolic Pathways Panel using the nCounter NanoString (NanoString Technologies). Results were compensated by housekeeping genes in nSolver Analysis Sofware v. 4.0. Statistical analyses were performed in SPSS 23.0 software using Pearson Correlation and Multiple Linear Regression tests. **Results:** PD-L1 expression was detected in 6% of cases, with expression varying between 10 to 15%. In univariate analysis, 21 genes were correlated with PD-L1 expression. Multiple Linear Regression indicated four genes with a higher prediction of PD-L1 expression: *INSR*, *NOX4*, *FOLH1,* and *PEBP1*. The model was statistically significant [$F(4, 47) = 34.127$; $p = 0.006$; $R^2 = 0.722$]. **Conclusion:** This screening highlights that reactive oxygen response (*NOX4* and *PEBP*), metabolic pathway (*FOLH1*), and proliferative pathway (*INSR*) genes could induce PD-L1 expression and may be associated with tumor evasion. **Keywords:** Melanoma;PD-L1 expression;Immune checkpoints.

DO - 105 - **Comprehensive analysis of immunophenotyping, microbiome and clinical outcome in high-grade cervical intraepithelial lesion patients treated with imiquimod**

DIAS, T.C.; TOSTES, K.; SORROCHE, B.P.; RODRIGUES, N.D.C.; LIMA, J.A.; DE OLIVEIRA, B.F.; ROBERTO, F.A.Z.; POSSATI-RESENDE, J.C.; DOS REIS, R.; PERES, A.F.. BARRETOS CANCER HOSPITAL, BARRETOS CANCER HOSPITAL BARRETOS - SP - BRASIL.

Introduction: High-grade intraepithelial lesions (NIC 2/3) precede cervical cancer. Current treatment involves removing the distal cervix, impacting female fertility. (Obstet Gynecol. 122(6):1154-9, 2013). As an alternative, the use of Imiquimod, an immunomodulator with antiviral and antitumor activity, has been explored as a non-ablative option. (Obstet Gynecol, 51(4):533-8, 2012) Similarly, in the search for less aggressive methods, recent discussions have focused on the influence of the microbiota on tumor development, as well as the potential effects of alterations in its composition, which may play a significant role in modulating immune responses (Arch Microbiol. 202(2):323-7, 2020).
Objectives: To characterize the immunophenotypic profile of the local immune response, the cervicovaginal microenvironment, and the microbiological profile in women with CIN 3 undergoing imiquimod treatment. **Materials and Methods:** This randomized clinical trial includes three groups: 1) HPV-positive CIN 3 patients receiving 16 doses of imiquimod, applied twice a week for 8 weeks, followed by Excision of the Transformation Zone (ETZ); 2) HPV-positive CIN 3 patients undergoing standard ETZ; 3) Healthy patients with negative high-risk cytology and HPV. Cervicovaginal lavage samples will be collected at different time points to compare cellular response profiles in imiquimod treated and ETZ patients, as well as baseline levels in healthy patients. Samples will be used for immunophenotypic analysis using flow cytometry, immune mediator quantification using *Cytometric Bead Array*, and microbiome evaluation using MiniION. **Expected results:** We expect to generate valuable insights into the molecular signatures of the cervicovaginal microenvironment, with the ultimate goal of facilitating personalized treatment approaches. By leveraging this knowledge, we aspire to achieve improved clinical outcomes, enhance patient selection accuracy, and minimize the need for ablative procedures. **Keywords:** Microbiome;HPV;Imiquimod.

DO - 106 - **Assessing the predictive potential of baseline monocyte and platelet counts in non-small cell lung cancer patients undergoing immune checkpoint inhibitor therapy**

TOSTES, K.[1]; SORROCHE, B.P.[1]; DIAS, T.C.[1]; RODRIGUES, N.D.C.[1]; DE OLIVEIRA, S.P.[1]; PAES, V.G.[1]; LIMA, J.A.[1]; DE SOUZA, V.G.[1]; DA SILVA, F.A.F.[2]; DE MARCHI, P.R.M.[3]; PINHEIRO, C.M.[1]; LEAL, L.F.[1]; ARANTES, L.M.R.B.[1]. 1. MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL, BARRETOS-SP, BRAZIL, MOLECULAR ONCOLOGY RESEARCH CENTER, BARRETOS CANCER HOSPITAL, BARRETOS-SP, BRAZIL BARRETOS - SP - BRASIL; 2. DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL, BARRETOS-SP, BRAZIL, DEPARTMENT OF CLINICAL ONCOLOGY, BARRETOS CANCER HOSPITAL, BARRETOS-SP, BRAZIL BARRETOS - SP - BRASIL; 3. ONCOCLÍNICAS, RIO DE JANEIRO-RJ, BRAZIL, ONCOCLÍNICAS, RIO DE JANEIRO-RJ, BRAZIL BARRETOS - SP - BRASIL.

Background: Lung cancer causes 1.8 million annual deaths, mostly due to non-small cell lung cancer (NSCLC) accounting for 85% of cases (Nature. 553:446-454, 2018). Immunotherapy using immune checkpoint inhibitors (ICI) has made significant progress, but reliable prognostic markers for guiding immunotherapy are still lacking (Cancer. 126:260-270, 2020). **Aim:** Improving the identification of immune markers in peripheral blood to differentiate NSCLC patients who respond to ICI therapy from non-responders. **Methods:** Biological samples and data will be collected prospectively from 55 patients with advanced NSCLC treated with ICIs at Barretos Cancer Hospital. The baseline Complete Blood Cell (CBC) values were analyzed, with a median value serving as the cutoff point. Peripheral blood mononuclear cells and plasma cytokines will be evaluated using flow cytometry, while FFPE biopsies will be assessed using NanoString technology. **Results:** This cohort is composed primarily by males (58.2%) and with an average of 62 years. Only 10.9% of the patients had never smoked. Adenocarcinoma was the most prevalent histological type (60.0%), followed by squamous cell carcinoma (34.5%). Here, 65.4% showed PD-L1 positivity and the most frequently recommended treatment was anti-PD-1 (78.2%). Patients with lower monocyte (<712.0/mm³) and platelet (<251.0K/mm³) counts exhibited a significant higher overall survival (OS) (p= 0.006 and p= 0.040, respectively) as well as improved progression-free survival (PFS) (p= 0.050 and p= 0.040). Furthermore, patients with an MP score (monocytes x platelets) <165.31K displayed higher rates of OS (p=0.039), PFS (p=0.017) and were correlated with ICI response, with an AUC of 0.767 (p=0.005). **Conclusion**: Baseline monocyte and platelet counts in NSCLC patients receiving ICI treatment could be assessed as predictive markers for clinical outcomes. Ongoing analysis seeks to validate their clinical application, enhancing prognostic capabilities. **Keywords:** Non-small cell lung cancer;Immunotherapy;Immune checkpoint inhibitors.

DO - 107 - **Functional characterization of two bone marrow-generated macrophage populations in vitro**

ETO, C.[1]; LUIZ, G.[1]; SILVA, H.M.[2]; MUNARI, E.L.[1]; BECK, B.K.[1]; MENDES, D.A.G.B.[1]; MASCARIN, L.Z.[1]; CANESSO, M.C.C.[3]; REIS, B.S.[3]; MUCIDA, D.[3]; SILVEIRA, G.F.[4]; BORDIGNON, J.[4]; DE MENEZES, Y.K.T.[1]; DA ROCHA, E.L.[1]; BAFICA, A.L.B.[1]. 1. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL; 2. MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE - ESTADOS UNIDOS DA AMERICA; 3. ROCKFELLER UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA; 4. INSTITUTO CARLOS CHAGAS - FIOCRUZ, CURITIBA - PR - BRASIL.

Based on expression of surface markers, recent studies have shown that bone marrow (BM)-generated macrophages contain two macrophage populations, which have not been functionally defined. Here we combined transcriptomics, phenotyping and functional approaches to characterize these two populations. Flow cytometry analysis indicated that these cells can be defined as two main phenotypes: $FSC^{hi}SSC^{hi}F4/80^{hi}CD11b^{hi}CD206^{hi}CD195^{lo}$ ($F4/80^{hi}CD11b^{hi}$) cells, which were the majority (80%) of the events and $FSC^{lo}SSC^{lo}F4/80^{lo}CD11b^{lo}CD206^{lo}CD195^{hi}$ ($F4/80^{lo}CD11b^{lo}$) cells, which corresponded to ~20% of the macrophages. At the transcriptomic level, $F4/80^{lo}CD11b^{lo}$ macrophages were found to display enrichment of proinflammatory, innate recognition and leukocyte migration pathways. Strikingly, $F4/80^{lo}CD11b^{lo}$ macrophages presented a significant increase in both binding and phagocytosis of *Mycobacterium tuberculosis* and *M. bovis*-BCG, which correlated with augmented secretion of TNF. Our results reveal a highly phagocytic BM-derived macrophage subpopulation in vitro. **Keywords:** macrophages;mycobacteria;heterogeneity.

DO - 108 - **Role of type 2 Innate Lymphoid Cells in the susceptibility to experimental cerebral malaria in mice**

GONÇALVES, L.B.; BARCELOS, P.M.; FERREIRA, G.N.P.; RANGEL, A.J.F.; DIAZ, B.L.. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

Malaria is a tropical infectious disease caused by different species of the protozoan genus Plasmodium, with *P. falciparum* being associated with the most severe form of the disease, cerebral malaria (CM), which is characterized by parasite sequestering in brain vessels, severe type 1 inflammation and neurodegeneration that can lead to coma, paralysis and even death. Innate immune cells are important responders against the parasite, and thus are able to shape the immune response phenotype, impacting on disease severity. Type 2 innate lymphoid cells (ILC2) are important producers of interleukin (IL)-13, being able to switch the immune response towards a type 2 phenotype, which could prevent CM development. It is well known that Type 1 inflammation is the main contributor to the onset of CM, and it has been shown that exogenous activation of ILC2 has protecting effects against CM development, but it is not known whether these cells are part of the mechanism behind disease susceptibility. Thus, the aim of this work is to characterize the role of ILC2 in mouse strains that are susceptible (C57BL/6J) or resistant (Balb/c) to CM development. Mice were infected with GFP-*P. berghei* ANKA intraperitoneally, and parasitemia was measured through blood smears and flow cytometry. Flow cytometry analysis of spleens of control or infected mice at 5 dpi showed an increase in the frequency of ILC2 and an overall greater increase in ICOS expression in ILC2 from infected Balb/c mice in comparison with C57BL/6J mice; no changes in ILCs were observed on mouse brains. T cell analysis of spleens of control or infected mice at 5 dpi displayed different frequencies of $CD8^+$ and $CD4^+$ positive cells within the T cell population on the spleens and brains of infected mice when compared to the control group. These data point towards a possible correlation between ILC2 and T cell activation in the periphery, which could be linked to modulation of the immune response affecting disease severity. **Keywords:** MALARIA;ILC2;INNATE IMMUNITY.

DO - 109 - **Lung impairment and Titan Cells in TLR9-/- mice infected by Cryptococcus gattii**

DA SILVA JUNIOR, E.B.[1]; DE OLIVEIRA, J.C.G.[1]; LIMA, I.D.[1]; DOS SANTOS, I.M.F.[1]; DE SOUSA, B.A.[1]; NASCIMENTO, D.D.O.[2]; DE LIMA, L.F.[1]; DECOTE-RICARDO, D.[2]; GUEDES, H.L.D.M.[1]; FREIRE-DE-LIMA, C.G.[1]. 1. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL; 2. UFRRJ, UFRRJ SEROPÉDICA - RJ - BRASIL.

Cryptococcus gattii is a basidiomycetous saprophyte yeast, that grows in the environment and within healthy humans and animals. The mechanisms that underlie host susceptibility have not been well understood. GXM and GalXM polysaccharides are the main important components of the large capsule that covers the yeasts and the major virulence factors described. After fungus inhalation by host, cryptococcal yeasts arrives at the lungs and cause a severe inflammation and subsequent dissemination in the central nervous system (CNS), leading to a meningoencephalitis. Pattern recognition receptors in resident and adaptative immune cells, such as TLRs, plays an important role in the pathology. Among the TLRs, the TLR9 is capable to recognize the non-methylated CpG dinucleotides, which are found on prokaryotes and viral genome. The TLR9 is most expressed in the reticulum (RE) of B cells and plasmacytoid dendritic cells (pDC). TLR9 activation result in type I IFN production for pDCs, leads the activation of NK cells, monocytes, effector T cells and pDCs. We aim to evaluate the importance of TLR9 in the control of the infection by *C. gattii* and, for that, we intratracheally inoculate 10^4 yeast cells in TLR9-/- and wildtype (WT) mice and evaluate after 3 weeks. Using lung histological sections stained with picrosirius red, alcian blue, congo red or hematoxilyn and eosin, we analyze histopathological aspects associated to cryptococcal infection. We observed that TLR9-/- infected mice have higher frequency of eosinophils in pulmonary mixed cellular infiltrate, severe bronchiolitis, thickened alveolar wall, alveolar congestion, atelectasis, bronchiectasis, generalized destruction of respiratory epithelium and titan cells with larger diameter. In addition to, TLR9-/- infected mice have a greater diffuse interstitial pulmonary fibrosis compared to WT infected mice. This way, with our preliminary results, we aim to elucidate the importance of TLR9 in *C. gattii* experimental infection. **Keywords:** Cryptococcus gattii;cryptococosis;innate immunity.

DO - 110 - **Protective antibodies against P. falciparum merozoite are induced during pregnancy and early life**

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Introduction: Malaria caused by *Plasmodium falciparum* (*Pf*) is a public health problem and its control strategies are limited. Therefore, it is crucial to develop new vaccines to control the disease. Studies have investigated merozoite surface proteins in malaria control. A TOLIMMUNPAL project will help to identify the most promising malaria vaccine candidate antigens. **Objective:** To assess the malaria protection conferred by antibodies (Abs) against merozoite antigens in Benin. **Methods:** 400 women and their infants were followed during pregnancy and the first two years of age. Malaria infections were recorded during follow-up and Abs against recombinant *Pf* merozoite antigen were quantified by ELISA at Antenatal Visits (ANV), at delivery, at birth, and until 24 months of age, including environmental factors. Statistical analyses were performed using logistic, linear and Cox-proportional hazard regressions. **Results:** We report that: i) anti-merozoite IgG levels were lower at delivery after two intermittent preventive treatments (IPTp) when compared to before IPTp intake ii) IgG Abs against MSP1, MSP2-3D7, and GLURP-R0 were associated with protection against peripheral and placental malaria infection during pregnancy, iii) previous malaria infection in pregnancy and placental malaria negatively influenced Abs transfer and iv) IgG Abs do not protect children, however, IgM against MSP1, MSP2-3D7, MSP2-FC27, and MSP3 show a more effective response against malaria after one year of life. **Conclusions:** The main finding suggests that IgM should be considered in vaccine designs during infanthood. MSP1, MSP2, and GLURP appear to be more promising candidates for malaria vaccines. *In vitro* functional studies on these Abs need greater attention. **References:** Sci Adv 2019;5:eaax4489. **Keywords:** protection;candidates;vaccine.

DO - 111 - **Higher responsiveness of Th17 and Tc-17 cells to ligands for TLR2 and TLR4 was associated with clinical relapses and in vitro corticoid resistance in multiple sclerosis patients**

SALES, M.D.C.[1]; DO SACRAMENTO, P.M.[2]; KASAHARA, T.D.M.[2]; DA SILVA, J.C.C.[2]; BILHÃO, R.D.O.[2]; DE ANDRADE, R.M.[2]; VASCONCELOS, C.C.[2]; BENTO, C.A.D.M.[2]. 1. UERJ, UNIRIO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIRIO, RIO DE JANEIRO - RJ - BRASIL.

The clinical activity of relapsing-remitting multiple sclerosis (RRMS) is defined by the occurrence of relapses. Elevated expression of toll-like receptors (TLRs) on circulating T cells has been observed in RRMS patients. The aim of the present study was to investigate whether frequency of $TLR2^+$ and $TLR4^+$ Th17/Tc-17-like cells and *in vitro* cytokine production by T-cells in response to TLR2 and TLR4 agonists was predictive of new relapses in RRMS during a 1-year follow up. In those cell cultures, the ability of corticoid to control cytokine production was also investigated. For this study, PBMC from 58 RRMS patients were stimulated with PMA and Ionomycin. After 4 h, the frequency of $IL\text{-}17^+CD4^+$ and $IL\text{-}17^+CD8^+$ T cells positive for TLR2 and TLR4 was determined through cytometry and the cytokine profile was determined by Luminex. High intensity of TLR2 and TLR4 expression on ($CD4^+$ and $CD8^+$) T cells, was predictive of new relapses in RRMS. Although treatment with disease-modifying therapy (DMT) significantly decreased the hyperresponsiveness of MS-derived T cells to ligands for TLR2 and TLR4, the levels of IL-1β, IL-6, IL-17 and GM-CSF released by $CD4^+$ T cells, and IL-1β, IL-6 and IL-17 released by $CD8^+$ T cells, in response to both TLR agonists were higher among relapsed patients. Interestingly, corticoid was less efficient at reducing IL-1β, IL-6 and IL-17 released by TLR-activated ($CD4^+$ and $CD8^+$) T-cells from clinically unstable MS patients. Collectively, the data suggested that persistence of circulating Th17- and Tc17-like cells expressing elevated levels of functional TLR2 and TLR4 in treated MS patients could indicate high disease activity and lower therapeutic efficacy. **Keywords:** Multiple sclerosis;Th17/Tc17;TLR.

DO - 112 - **Preliminary characterization of the impact of the absence of the host receptor Clec4F on the immune response in experimental cystic echinococcosis.**

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Cystic echinococcosis is a chronic infection caused by the larval stages (hydatids) of cestode parasites belonging to the species cluster *Echinococcus granulosus* sensu lato. Hydatids are bladder-like structures that attain large sizes within various internal organs of livestock ungulates and humans. Hydatids are protected by the massive acellular mucin-based laminated layer (LL). Parasite growth requires LL turnover, which leads to the liberation of abundant LL-derived particles to the host tissues. We have recently reported that in mice, LL materials circulate systemically and are selectively captured by Kupffer cells (KC), the main resident liver macrophages. This uptake is mostly dependent on the LL mucin glycans and on the C-type lectin Clec4F, only expressed in KC. We also reported a trend towards lower parasite burdens in *Clec4f*$^{-/-}$ mice in comparison to WT. We are currently characterizing the immune response in chronically infected WT and *Clec4f*$^{-/-}$ mice. At the 7-months timepoint analyzed, the two genotypes displayed similar numbers of neutrophils, eosinophils, monocytes, macrophages, and $CD8^+$T cells at the infection site (peritoneal cavity), with a trend towards higher $CD4^+$T cell numbers in *Clec4f*$^{-/-}$. Up-regulation of M2 (Relm-α) and suppressive markers (PD-L1 and/or PD-L2) in macrophage sub-populations at the infection site did not differ between genotypes. Regarding the systemic responses, no differences were observed between genotypes in terms of cytokine production by *ex vivo* antigen-stimulated splenocytes or antigen-specific IgM, total IgG or IgG subclasses. Interestingly, in terms of changes in the liver, we observed higher PD-L1 expression in KC of infected WT than infected *Clec4f*$^{-/-}$ mice. KC are known for inducing tolerance by mechanisms thought to require their expression of PD-L1. We therefore speculate that the Clec4F-dependent uptake of LL glycans by KC may enhance the capacity of these cells to promote regulatory responses in favor of the parasite. **Keywords:** Echinococcus granulosus;Clec4F;Immune response.

DO - 113 - **Density-based lipoprotein depletion improves extracellular vesicle isolation and enhance proteome analysis**

MERIJ, L.B.[1]; DA SILVA, L.R.[2]; LEITE, K.T.Q.[2]; NUNES, M.A.R.[2]; ANDRADE, F.B.[1]; MARTINS, S.D.T.[3]; NASCIMENTO, A.L.R.[4]; ROCHA, V.N.[1]; ALVES, L.R.[3]; BOZZA, P.T.[2]; TRUGILHO, M.R.O.[2]; HOTTZ, E.D.[1]. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, UNIVERSIDADE FEDERAL DE JUIZ DE FORA JUIZ DE FORA - MG - BRASIL; 2. INSTITUTO OSWALDO CRUZ, INSTITUTO OSWALDO CRUZ RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO CARLOS CHAGAS, INSTITUTO CARLOS CHAGAS CURITIBA - PR - BRASIL; 4. UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO, UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO RIO DE JANEIRO - RJ - BRASIL.

Extracellular vesicles (EVs) participate in many biological processes mediating cell communication, immune response and homeostasis. Previous attempts of isolating EVs from plasma have shown contamination with lipoproteins, which is a concerning bias on most EVs studies with clinical samples, once lipoproteins can also modulate metabolic and inflammatory responses. Here we aimed to isolate plasma EVs after depleting lipoproteins, improving sample purity and EV proteome. Density-based gradient ultracentrifugation (G-UC) was used as a first-step method for lipoprotein depletion from plasma, which was then subjected to serial centrifugation (SC) or size exclusion column (SEC) for EV isolation. Recovered EVs were analyzed by size, concentration, cellular source, ultrastructure and bottom-up proteomics. G-UC efficiently separated lipoproteins from the plasma, allowing subsequent EV isolation through SEC or SC. Besides lipoprotein depletion, EV isolation through G-UC+SEC resulted in lower contamination with plasma proteins. Combined analysis from EV proteomics, cholesterol quantification and apoB-100 detection confirmed the significant reduction of lipoproteins contamination from isolated EVs. Proteomic analysis identified similar gene ontology and cellular components in EVs regardless of lipoprotein depletion, which was consistent with similar EV cellular sources by flow cytometry and similar EV ultrastructure by transmission electron microscopy. Importantly, apolipoprotein depletion enriched the EV proteome by increasing the detection of less abundant proteins. Taken together our results demonstrated that the combination of G-UC+SEC provided lipoprotein-depleted EVs without interfering in EV cellular source, gene ontology and ultrastructure, allowing the obtention of highly pure EVs with potential implications for functional assays, proteomic and lipidomic analysis. Financial support: CNPq, CAPES, FAPEMIG. **Keywords:** extracellular vesicles;isolation;lipoprotein.

DO - 114 - **Anti-CAIX CAR T cells in the treatment of renal cell carcinoma patient-derived xenografts**

DE CAMPOS, N.S.P.[1]; PIVETTA, R.S.[1]; BESERRA, A.D.O.[2]; FONSECA, F.L.A.[3]; MEDINA, T.D.S.[2]; DOS SANTOS, T.G.[2]; SUAREZ, E.R.[1]. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, UNIVERSIDADE FEDERAL DE SÃO PAULO SÃO PAULO - SP - BRASIL; 2. A.C. CAMARGO CANCER CENTER, A.C. CAMARGO CANCER CENTER SÃO PAULO - SP - BRASIL; 3. CENTRO UNIVERSITÁRIO FMABC, CENTRO UNIVERSITÁRIO FMABC SANTO ANDRÉ - SP - BRASIL.

Introduction. T cells expressing chimeric antigen receptors (CAR T cells) have demonstrated remarkable clinical efficacy in treating different hematologic tumors. However, several challenges must be overcome to allow similar efficiency against solid tumors. Several hypoxic tumors and especially clear cell renal carcinoma (ccRCC) express high amounts of an enzyme called carbonic anhydrase IX (CAIX), which is considered an interesting tumor-associated antigen for CAR T cell development. This project aim the evaluation of anti-tumor effects of CAIX-targeted CAR T cells containing CD28 or 4-1BB as costimulatory domains and capable of inducing different levels of T cell exhaustion in a ccRCC patient-derived xenograft model (PDX). **Methods.** The lentiviruses will be produced by transient transfection, concentrated, titrated, and transduced into T cells CD4:CD8 2:1 purified from the mononuclear fraction of the blood of healthy donors. The resulting CAR T cells will be expanded, and their transduction levels will be accessed in the short and long term. The Anti-CAIX CAR T cells containing different co-stimulatory domains CD28 or 4-1BB will be evaluated in vivo in a ccRCC PDX model, determining the exhaustion status of tumor-infiltrating T cells. **Results.** Using two doses of ≅106 CAR T cells/kg dose, Anti-CAIX 4-1BB resulted in smaller tumors with slightly higher survival rates. However, the anti-CAIX construct with CD28 was unique in avoiding the occurrence of metastasis and significantly reduced the T cell population expressing all of the exhaustion markers analyzed. No significant difference in the expression of alanine transaminase (ALT), aspartate transaminase (AST) and creatinine was found among the groups, providing further evidence for the absence of hepatic and nephrotoxicity. **Conclusion.** This project has the potential to optimize the performance of CAR T against ccRCC. Supported by FAPESP (2018/17656-0). **Keywords:** CAR T;solid tumors;carbonic anhydrase IX.

DO - 115 - **Production of IFN-γ by CD4+ T cells reveals acquired immune response during asymptomatic Plasmodium vivax infection**

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Introduction: Malaria is a severe disease transmitted by the *Anopheles* vectors infected with *Plasmodium* protozoa. In Brazil, most cases are reported in the northern region and caused by *Plasmodium vivax* (*P. vivax*). *Plasmodium* infection does not induce long-term protection, but both humoral and cellular immune responses are essential for partial protection against malaria. The identification and measurement of antigen-specific T cell response are hampered mainly by the lack of well-defined immunogenic protein antigens of *P. vivax.* **Objective:** The aim of this study was to evaluate the IFN-γ response of $CD4^+$ T cells from symptomatic and asymptomatic individuals infected with *P. vivax* after stimulation with defined peptide pools. **Methods:** Peripheral blood mononuclear cells from 33 symptomatic before (SY-I) and 21 after (SY-R) treatment, 43 asymptomatic individuals infected with *P. vivax*, and 15 healthy donors (CTL) were stimulated *in vitro* for 24h with a library with 310 peptides from *P. vivax* (MPv310), 19 smaller peptides libraries (minipools m1-m19) or an equimolar dose of DMSO (negative control). Production of IFN-γ was analyzed by ELISpot assay. The plates were read by Mabtech Iris™ equipment and analyzed by Mabtech Apex™ software. **Results:** Both libraries were able to induce activation and production of IFN-γ in $CD4^+$ T cells from *P. vivax* infected individuals. Significant IFN-γ production was triggered by $CD4^+$ T cells from ASY when cultured with five out of 19 minipools (m1, m6, m9, m13 and m17). On the other hand, MPv310 and the minipools m4 and m5 induced IFN-γ production by $CD4^+$ T cells from only SY-R and SY-I, respectively. **Conclusion:** Our data indicate that defined peptide pools activate $CD4^+$ T cells from *P. vivax*-infected individuals, mainly in ASY, inducing the production of IFN-γ, which reveals the presence of antigen-specific $CD4^+$ T cells. **Keywords:** Peptides;;Plasmodium vivax;;Immunity;.

DO - 116 - **Comparison of adaptive immune responses induced by standard and fractional doses of 17DD-YF vaccine against yellow fever**

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The re-emergence of yellow fever (YF) urged new mass vaccination campaigns and, in 2017, the World Health Organization approved the use of the fractional dose (FD) of the YF vaccine due to stock shortage. The FD induced the production of neutralizing antibodies against the yellow fever (YF) virus and cytokine responses similar of that generated by the standard dose (SD). Our aim was to compare the viremia kinetics after immunization with both doses of the 17DD vaccine, their capacity to induce the production of antibodies, soluble mediators, and the expansion and activation of effector and memory T and B-cells. Study participants were selected from the population immunized with FD or SD in the campaigns against YF in the state of São Paulo, Brazil. Blood samples were taken before and 7 and 10-15 days after vaccination. Serum samples were also taken 30-45 days after vaccination. Cells were purified from blood clots with density gradient and phenotyped by flow cytometry. The production of soluble factors was measured using a 27-plex assay, and YF-specific IgM, IgG and neutralizing antibodies were measured by ELISA and mPRN-HR assays, respectively. Viremia was measured by reverse transcription quantitative real-time PCR. Similar viremia and levels of antibodies and soluble markers were induced early after immunization. However, a faster decrease in the latter was observed after SD. The FD led to a sustained expansion of helper T-cells and an increased expression of activation markers on T-cells early after vaccination. Although with different kinetics, expansion of plasma cells was induced upon SD and FD immunization. Integrative analysis reveals that FD induces a more complex network involving follicular helper T cells and B-cells than SD. Our findings substantiate that FD can replace SD inducing a protective immune response against YF. **Keywords:** Yellow fever;Vaccine;Fractional dose.

DO - 117 - **ANTI-HER2 CAR-T CELLS EVALUATION IN AN OVARIAN TUMOR PRECLINICAL MODEL**

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Chimeric antigen receptors (CARs) are molecules capable of redirecting immune cells against a specific tumor antigen; several studies choose the HER2 receptor as a good target due to its specific overexpression in solid tumors. The objective of this work was to evaluate and compare the efficacy in the production of two anti-HER2 CAR-T (4D5 and FRP5) from peripheral blood mononuclear cells (PBMCs), as well as their antitumor capacity in vivo. A constant expression of the two receptors was observed, reaching an average of 25% of cells expressing the CAR 12 days after genetic CAR transfer. On the eighth day of expansion, the central and effector memory phenotypes were also evaluated, in addition to exhaustion-related markers (PD1, TIM3 and LAG3) in CAR-T CD4+ and CD8+ populations. In both cases we observed a similar phenotype evidencing higher frequency of cells with central memory phenotype. For exhaustion-associated receptors, we observed a lower frequency of expression among CD8+ CAR-T cells expressing the FRP5 clone. For the CD4+ subset, we could observe an increase of the markers for either of the CAR constructs. In the in vivo assay, we observed non-tumor- related death. While 4D5 exhibited decrease on tumor volume, with one mouse reaching full regression, FRP5-based CAR wasn't able to control tumor burden. A new experiment with mice inoculated with tumors in earlier stages, induced complete remission in peritumoral treatment, and partially on intraperitoneal and intravenous treatment. The two evaluated CARs showed consistent expression in PBMCs from different donors, with a predominant central memory phenotype and low expression of inhibitory receptors. Functionally, the CAR anti-HER2 4D5 proved to be effective in the treatment in a solid tumor model of ovarian cancer, especially by the peritumoral route of administration. In the future, the immunosuppressive context in the effectiveness of the treatment and additional therapies will be evaluated. **Keywords:** HER-2;CAR-T;SOLID TUMOR.

DO - 118 - **LEISHMANIA BRAZILIENSIS-INDUCED INCREASE IN THE ACTIVITY OF ATP-BINDING CASSETTE TRANSPORTERS IN HUMAN MACROPHAGES, IS ASSOCIATED WITH PENTAVALENT ANTIMONY RESISTANCE**

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Introduction: *Leishmania* spp. are intracellular protozoan parasites causative of cutaneous leishmaniasis (CL). Recognized as a neglected tropical disease, it is endemic in the state of Bahia. In Brazil, pentavalent antimony (SbV),the main therapy used to treat all clinical forms of leishmaniasis, has become increasingly associated with treatment failure. ATP-binding cassette (ABC) transporters are xenobiotic efflux pumps whose activity has been associated with drug resistance in a variety of diseases. Current evidence suggests that some ABC transporters (e.g. MRP1 and MDR1) play a role in drug resistance in leishmaniasis and may decrease the cellular accumulation of SbV. Our hypothesis is that infection with *L. braziliensis* isolated from patients that failed therapy induce ABC transporters activity in human macrophages. **Methods and Results:** Human macrophages infected with *L. braziliensis* isolates obtained from lesions of CL patients that healed or failed treatment with SbV, were treated *in vitro* with SbV and MRP, MDR1 and BCRP inhibitors. Also, they were stained with a dye that serves as an indicator of these proteins' activity in the cell and measured by flow cytometry. The concentration of IL-10, IFN-ɣ, IL-5, IL-1ß and TNF were determined in culture supernatants by ELISA. **Conclusion:** Most *Leishmania* isolates showed some degree of *in vitro* resistance to SbV and increased the activity of ABC transporters. Inhibition of MDR1 and MRP activity decreased dye efflux and proinflamatory citokines in *L. braziliensis*-infected macrophages and may suggest the participation of these ABC transporter in resistance to SbV. **Funding:** NIH AI136862, Capes PRINT. **Keywords:** leishmania braziliensis;drug resitance;pentavalent antimony.

DO - 119 - **ANALYSIS OF THE IMMUNOMODULATORY ACTIVITY IN DIFFERENT LINEAGES OF MACROPHAGES BY NATURAL AND SYNTHETIC LEISHMANICIDAL COMPOUNDS**

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Macrophages are immune phagocytic cells involved in tissue defense and homeostasis and are the cells infected by Leishmania parasite in vertebrate host. Natural products are accessible and safe option with pharmacological properties and allows chemical modifications to improve their activity. We evaluated the immunomodulatory action of 3 natural products (-)-Guaiol, Piperine (AF1), 1,8-cineol and a synthetic derivative of Piperine, N4-cyclohexyl-1,2,4-triazol-3-thione (AF2) in murine P388D1 and RAW 264.7 and canine DH82 macrophages. The compounds have a dose-dependent leishmanicidal activity for promastigotes forms of *Leishmania amazonensis* with IC_{50} of 56.24 µM, 9.36 µM and 8.73 µM for (-)-Guaiol, AF1 and AF2 respectively and the cell lines showed viability above 70% after the treatment. Preliminary results demonstrate that AF2 and 1,8-cineol [50 µM] decreased the phagocytosis of promastigotes in DH82. The treatment with the compounds can alter the production of NO and ROS in all the three lineages. Cytometry analyzes of RAW not stimulated and stimulated with LPS+INF-γ demonstrated that the treatments did not affect the expression of MHC II+, CD80+ and CD86+. For P388D1 stimulated or not with LPS, there was a decrease in the population of MHCI + cells treated with AF2 [60 µM]. There was an increase on the population of MHCII+ cells treated with AF2, AF2+LPS and infected with *L.a* and treated with AF2. There was an increase in double positive populations treated with AF1, AF2 and AF2+LPS. Finally, qPCR demonstrate that RAW+LPS and treated with the compounds decreased the expression of IL-10 and IL12. AF1, AF2 and 1,8-cineole also decreased TLR4. In DH82+LPS, (-)-Guaiol and 1,8-cineol decreased IL-10 and AF2 and 1,8-cineol decreased IL12. Unstimulated P388D1 and treated with (-)-Guaiol increased IL-1β expression. AF1 led to reduced expression of TLR4 in cells after treatment. Preliminary results suggest that the compounds can modulate different macrophages lineage. **Keywords:** Macrophages;immunomodulation;natural and synthetic products.

DO - 120 - **GENE CONVERSION ANALYSIS IN HUMAN IMMUNOGLOBULINS**

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The humoral response plays a critical role in pathogen protection and in the development of immunological memory following vaccination. This response relies on the generation of a diverse repertoire of antibodies, which are produced through somatic recombination on B lymphocytes. Another mechanism contributing to immunoglobulin diversity is gene conversion (GC), which involves the transfer of segments from pseudogenes into functional genes when their similarity exceeds 80%. While GC has been predominantly identified in the chicken immunoglobulin (IG) locus, its occurrence in human IG remains poorly understood. Therefore, our study aimed to investigate the possibility of GC in the heavy chain of human IG (IGHV). Initially, IGHV segments from Homo sapiens were selected on the IMGT-Gene-DB database. We selected 183 genes, comprising 48 functional genes, 6 ORFs, 93 pseudogenes, and 36 orphon genes. These genes underwent multiple alignments using ClustalW Omega with standard settings. The analysis of the alignment results revealed the presence of three distinct clusters of identity among the samples. Notably, pseudogenes were in higher number than functional genes, and we also observed a significant identity between orphon and functional genes. To assess the GC rate between these genes, we employed the BrepConvert program with standard settings. Following annotation, genes were selected based on their identity between samples. Consequently, we identified 38 unique functional genes with a similarity exceeding 80% with 19 pseudogenes, indicating that they are potential candidates for gene conversion. Among these, 15 functional genes (IGHV1/3/4/5) displayed over 90% similarity with four pseudogenes (IGHV1/3/4/5). Based on our findings, we could observe that GC can occur within the IGHV1/3/4/5/7 families of IG. This observation can be attributed to the high similarity between these families, which could be contributing to the increased diversity of these genes. **Keywords:** Gene conversion;Immunoglobulin;Human.

DO - 121 - **DEVELOPMENT OF A MULTIANTIGEN RAPID TEST FOR MONITORING ASYMPTOMATIC INDIVIDUALS WITH SUBMICROSCOPIC PARASITEMIA OF Plasmodium vivax**

SAMPAIO, J.R.[1]; ALMEIDA, G.G.[2]; COELHO, F.F.[1]; SOARES, I.D.S.[3]; COSTA, C.M.[4]; VALIATE, B.[2]; OLIVEIRA, J.K.A.[1]; SILVA, A.M.V.[5]; PEREIRA, D.[5]; ANTONELLI, L.[2]; FERNANDES, A.P.[1]; GAZZINELLI, R.T.[1]. 1. CT VACINAS (UNIVERSIDADE FEDERAL DE MINAS GERAIS), CT VACINAS (UNIVERSIDADE FEDERAL DE MINAS GERAIS) BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO RENÉ RACHOU/FIOCRUZ MINAS, INSTITUTO RENÉ RACHOU/FIOCRUZ MINAS BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO SÃO PAULO - SP - BRASIL; 4. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL; 5. CENTRO DE PESQUISA EM MEDICINA TROPICAL, CENTRO DE PESQUISA EM MEDICINA TROPICAL PORTO VELHO - RO - BRASIL.

A large proportion of individuals infected with *Plasmodium vivax* do not show clinical signs of disease and test negative in thick smear microscopy and on currently available antigen capture diagnostic tests. Subpatent infections allow the individual to be a silent infectious reservoir that challenges malaria elimination efforts. To overcome this challenge, it is crucial to develop novel tools to enable efficient identification of individuals with submicroscopic parasitemia for therapeutic elimination of the parasite. In this study, we developed a rapid diagnostic test (RDT) by employing two different *P. vivax* surface antigens conjugated with colloidal gold nanoparticles and an anti-human IgG immobilized on the nitrocellulose membrane as test line. The RDT detect antibodies in both symptomatic and asymptomatic individuals infected with *P. vivax.* By analyzing the frequency of positive cases in an endemic area of Brazil we assessed the efficacy of our test. The RDT was validated in the laboratory (n=89 samples) and in the field (n=336 samples) in Candeias do Jamari and Porto Velho (Rondônia, Brazil), to determine the sensitivity and specificity comparing with thick smear (symptomatic individuals), ELISA and quantitative PCR. This prototype achieved a sensitivity of 88.4 % and specificity of 100 % in the laboratory, when evaluating symptomatic and asymptomatic individuals altogether. However, both the sensitivity and specificity were reduced when analyzing only asymptomatic individuals tested by RDT in the field. We are currently optimizing the RDT to diagnose asymptomatic patients in the field. **Keywords:** rapid test;malaria;immunodiagnosis.

DO - 122 - **Lectin-Fc fusion proteins demonstrate inhibitory activity against the emerging multiresistant pathogenic Candida auris**

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Candida auris, an emerging fungus was first discovered in Japan in 2009. The pathogen has rapidly spread worldwide and is now recognized as a significant nosocomial pathogen with high mortality associated rates ranging from 28% to 78%. Accurate identification of *C. auris* is crucial for selecting appropriate therapy, as most clinical isolates exhibit resistance to commonly used antifungal drugs recommended for other *Candida* species infections. In our pursuit of alternative treatments, our research group has previously developed three Lectin-Fc(IgG) proteins (WGA-Fc(IgG2a), Dectin-1-Fc(IgG2a), and Dectin-1-Fc (IgG2b)). These proteins can recognize and bind to chitin oligomers and β-1,3-glucan polysaccharides present in the fungal cell wall and have demonstrated antifungal capabilities *in vivo* and *in vitro* against models of Aspergillosis, Histoplasmosis, Cryptococcosis, and Candidiasis. In this work, we evaluated the antifungal capacities of the Lectin-Fc(IgG) proteins against *C. auris*. Results showed that Lectin-Fc(IgG) proteins recognized and bound to chitin and β-1,3-glucan on the fungi cell wall. Co-incubations with Dectin-1-Fc(IgG2a) (as low as 6.25µg/mL, Dectin-1-Fc(IgG2b) 1.56 µg/mL and WGA-Fc(IgG) (0.78µg/mL), efficiently inhibited fungal growth. After 24 hours, Dectin-1-Fc(IgG2b) attenuated biofilm formation as similar to 4µg/mL of Amphotericin-B. Lectin-Fc(IgG) proteins significantly enhanced the interaction rate and augmented the phagocytic cell's killing capacity, as evidenced by reduction of fungal viability. *In vivo* assessments in murine models of systemic candidiasis demonstrated that administration of Lectin-Fc(IgG) proteins reduced fungal burden and mortality, effectively neutralizing the infection, in line with previous reported antifungal functions. observations. Collectively, these results strongly support the notion that Lectin-Fc(IgG) proteins can work as opsonins, enhance phagocytic cell function and improve disease outcomes, favoring the host. **Keywords:** Lectin-Fc;fusion proteins;Candida auris.

DO - 123 - **Effects of butyrate on experimental psoriasis: a promising immunomodulatory approach**

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INTRODUCTION: Psoriasis is a chronic disease characterized by T cell activation, release of inflammatory mediators, and keratinocyte hyperproliferation. Resident memory T lymphocytes (TRM) are implicated in psoriasis reactivation through continuous cytokine release. Butyrate, an immune-regulating nutrient, is being studied for its potential therapeutic effects. However, its impact on TRM activation by dendritic cells in experimental psoriasis remains unclear. This study aimed to investigate the effects of oral administration of butyrate on experimental psoriasis. **METHODS:** Male C57BL/6 mice were divided into three groups: control (C), psoriasis (IMQ), and psoriasis treated with butyrate (IMQbut). Psoriasis was induced using a 5% imiquimod-based cream for 6 days, followed by reapplication from day 21 to day 26. The IMQbut group received 150 mM butyrate in their drinking water from day 7 to day 28. It was evaluated the skin thickness, animal weight, water consumption, feed intake, spleen area and weight, and gene expression of TRM markers in the skin. Statistical analysis was performed using one-way or two-way ANOVA followed by post-hoc Bonferroni test ($p < 0.05$). **RESULTS:** Topical application of imiquimod mimicked psoriatic inflammation, as evidenced by increased skin thickness, redness, and peeling on the back skin, along with increased spleen area and weight, and reduced body weight compared to animals treated with vaseline. Treatment with butyrate reduced skin thickness and the gene expression of CD103 and CD69, while increasing CD49a expression on the 28th day. **CONCLUSION:** Butyrate treatment had beneficial effects on psoriatic inflammation. It led to a reduction in skin thickness and modulated the expression of TRM markers, specifically decreasing CD103 and CD69 expression. These findings suggest that butyrate may have immunomodulatory properties that can attenuate the activation of TRM cells in psoriasis. **FUNDING:** FAPESP, CAPES (Finance Code 001) and CNPq. **Keywords:** Psoriasis;Butyrate;Tissue-resident memory T cell.

DO - 124 - **The immunosuppressive environment accompanying chronic cystic echinococcosis can be reproduced by repeated injection of particles from the parasite's acellular coat**

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The larval stage (hydatid) of the cestode *Echinococcus granulosu*s causes the chronic infection known as cystic echinococcosis, deploying strong regulatory mechanisms on host immune responses. The hydatid is protected by an acellular mucin-based layer called laminated layer (LL). During chronic infection the immune system is continuously exposed to particles shed from LL as a consequence of parasite growth. For this and other reasons, the LL and its shed materials have long been considered as candidates to contribute to the immune regulatory mechanisms deployed by this parasite. Our group previously characterized the local immune environment that accompanies the chronic phase (6 months p.i.) of intraperitoneal cystic echinococcosis in C57BL/6 mice, finding several signs of an immunosuppressive environment. In order to model chronic stimulation by LL-derived materials, we injected intraperitoneally in C57BL/6 mice a low dose (50 mg/mouse) of LL particles twice a week during 5 weeks. Twenty-four hours after the last injection, we observed changes in the peritoneal cavity that imitated strikingly those that accompany chronic infection. These changes included local M2-like monocytes/macrophages with up-regulated expression of Relm-α, Chil-3 (Ym1) and PD-L1. In addition, we observed expansion of separate regulatory ($FoxP3^+$) and PD-1 sub-populations within $CD4^+$ T cells, an increase in the levels of IL-1Ra and TGF-β proteins in the cavity fluid and a strong arginase activity in cellular extracts. This immunosuppressive environment appeared capable of imprinting an hyporesponsive state on T cells, since these displayed diminished capacity to proliferate ex-vivo in response to anti-CD3 antibody. In these assays, a Th2/Th17 biased response was also observed. The fact that all of the changes mentioned had been similarly observed in chronic infection suggests that LL-derived materials are major contributors to the immune-suppressive environment in cystic echinococcosis. **Keywords:** Echinococcus granulosus;immunosuppression;M2-like macrophage.

DO - 125 - **Impact of R70W and T124P Mutations on the Physicochemical Properties and Stability of CTLA-4: Insights from Computational Analysis**

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Primary immunodeficiency is a complex condition associated with compromised immune response. Mutations at exon 1 of the *CTLA-4* gene, particularly at positions R70W and T124P of the encoded molecule, were identified in the autosomal-dominant immune dysregulation syndrome, impacting the interaction with the CD80 and CD86 ligands. Considering that: i) single point mutations may impact on protein stability and dynamics, altering its 3D structure, and ii) may affect protein activity, in this study, we evaluated the *in silico* physicochemical properties and dynamic characteristics of the mutated and wild type CTLA-4 molecule. The 3D structure of the CTLA-4 homodimer was obtained from the Protein Data Bank (PDB, ID: 3OSK) and utilized as the reference to construct mutant structures, using MODELLER v.10.4 software. Assessment of surface hydrophobicity and electrostatics was performed using PyMol, while ENCoM algorithm implemented on the DynaMut server was employed to evaluate stability and dynamics of the tertiary structure as well as intra-atomic interaction prediction. Results demonstrated that the mutations altered the protein's physicochemical properties, affecting hydrophobicity and electrostatic potential. The R70W mutation led to loss of positive charge and increased hydrophobicity within a vital region for molecular interactions, whereas the T124P mutation did not affect the charge significantly. Consequently, these alterations may compromise CTLA-4's interaction with ligands. Moreover, vibrational entropy analysis revealed that the R70W mutation increased structural rigidity, while the T124P mutation enhanced flexibility in specific regions. Intramolecular interactions and hydrogen bonds were compromised in the T124P mutation. These findings indicate the influence of such mutations on CTLA-4's stability and structure, impacting protein function. **Keywords:** Immunogenetics;Cytotoxic T Lymphocyte Associated Antigen 4;Computational Biology.

DO - 126 - **Guanylate Binding Protein (GBP)-5 is involved in bacterial DNA sensing and inflammasome activation, however, only cooperation of multiple GBPs accounts for control of Brucella abortus infection**

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Guanylate-binding proteins (GBPs) are expressed in response to interferons and other pro-inflammatory stimuli. In mice, the most studied cluster of GBPs is on chromosome 3 (GBPchr3). It comprises the genes for GBP1-to-3, GBP5 and GBP7. In humans, all GBPs are in a single cluster on chromosome 1. *Brucella abortus* is a Gram-negative bacterium that causes brucellosis, a systemic debilitating zoonosis. Our group demonstrated that GBPchr3 is important to disrupt *Brucella*-containing vacuole (BCV) and GBP5 itself is important to *Brucella* LPS recognition. In this study, we investigated further the role of GBPs during *B. abortus* infection by using C57BL/6 wild-type, GBP2$^{-/-}$, GBP5$^{-/-}$ and GBPchr3$^{-/-}$ mice, and THP-1 human monocyte cell line. We found that all GBPs contained in the chromosome 3 are significantly upregulated during *B. abortus* infection in mouse bone marrow-derived macrophages (BMDMs). Of note, GBP5 presents the highest expression level in all time points evaluated up to 17 hrs of infection. However, only GBPchr3$^{-/-}$ cells presented increased bacterial burden compared to wild-type macrophages. *Brucella* DNA could be available for inflammasome activation after BCV disruption mediated by GBPs. In this regard, we investigated the inflammasome activation mediated by *B. abortus* DNA in Pam3Cys primed macrophages. We observed reduced IL-1β production in the absence of GBP2 or GBP5, as well as in GBPchr3$^{-/-}$ BMDMs. Similar result was shown by THP-1 macrophages with downregulation of GBP2 and GBP5 mediated by siRNA. In an *in vivo* perspective, we found that GBPchr3$^{-/-}$ mice had increased *B. abortus* burden and higher number of granulomas/cm^2 in the liver, indicating increased disease severity. Altogether, these results demonstrate that although GBP5 presents the fastest and highest expression in macrophages, the cooperation of multiple GBPs from murine chromosome 3 is required for full control of *Brucella abortus* infection. **Keywords:** GBPs;Brucella abortus;inflammasome activation.

DO - 127 - **Inflammatory response in patients diagnosed with Acanthamoeba keratitis**

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Acanthamoeba is widely distributed in nature, suggesting that humans are likely to be exposed to this protozoan at some point in their lives, having 50 to 100% of the population specific serum antibodies to *Acanthamoeba* antigens. Some parts of the eye are immunoprivileged and little is known about the immune responses that develop in *Acanthamoeba* keratitis (AK). For this reason, this study aims to evaluate *in vivo* activated and developed inflammatory responses during the course of AK, including the production of IgA and investigate *in vitro* response and activation of innate immunity inflammatory cells when challenged by *Acanthamoeba* isolates. For that, healthy donors and patients diagnosed with AK will be recruited. Blood and tears will be collected from participants to measure levels of lacrimal secretory cytokines and IgA using Luminex multiplex immunoassay and enzyme-linked immunosorbent assay (ELISA), respectively. Neutrophils and monocytes from venous blood will be challenged with *Acanthamoeba* clinical isolates. Infection and survival rate of inflammatory cells and *Acanthamoeba* isolates will be evaluated in vitro as well as inflammatory mediators. To date, six participants have been recruited (three donors and three AK patients). Preliminary data obtained with a buffy coat and THP-1 cells revealed that human cells are able to internalize and kill *Acanthamoeba polyphaga* (ATCC 30461) cysts and trophozoites. Moreover, infection with cysts resulted in the inflammasomes assembly, evaluated by ASC (Apoptosis-associated speck-like protein containing a CARD) punctas formation and cell death, thus suggesting an inflammasome mediated cytotoxic effect, which will be confirmed in patient samples. **Keywords:** Acanthamoeba;inflammation;immunoglobulins.

DO - 128 - **PROFILE OF CIRCULATING MICROVESICLES AS A PROGNOSTIC TOOL IN COVID-19**

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COVID-19 is an infectious disease caused by the SARS-CoV-2 virus and has been responsible for the morbidity and mortality of millions of people worldwide since 2019. Its rapid spread and potential severity, especially in risk groups, have made the search for complementary laboratory prognostic tools a key issue and a great challenge for science and medicine. To characterize the profile of circulating microvesicles (MVs) as a prognostic factor in COVID-19, samples from 39 patients with COVID-19 of both genders aged between 30 and 85 years were enrolled in the study and evaluated in 4 timepoints (Days 0, 7, 14, and 21 days after admission). Thirty-two individuals were also included to compose a healthy control group. There was a significant increase in total MVs in the COVID-19 group compared to healthy controls, mainly related to the increased number of neutrophil and endothelial cell-derived MVs. Moreover, at admission, there was a significant decrease in monocyte, T lymphocyte, and platelet-derived MVs in the COVID-19 group compared to healthy controls. Considering the clinical outcome of the COVID-19 patients, there was a significant increase in the total MVs, neutrophil and erythrocyte-derived MVs in the death group compared to the discharge group. Discriminant univariate analysis achieved 88% of accuracy in differentiating COVID-19 patients from healthy individuals by using total MVs and neutrophil, monocyte, and T lymphocyte-derived MVs, as well as 77% of accuracy in discriminating COVID-19 patients according to the clinical outcome based on total MVs and neutrophil-derived MVs. Finally, the classification proposed by a tree decision built after multivariate analysis demonstrated a 28% chance of death in patients with increased MVs derived from neutrophils and monocytes. These findings suggest that MVs can play an important role as biomarkers and be used as a promising tool for prognostic evaluation in COVID-19 patients. **Keywords:** Microvesicles; COVID-19;;cytometry; biomarker;;prognosis;

DO - 129 - **Kluyveromyces marxianus ORAL ADMINISTRATION FOR PREVENTION OF TUMORIGENESIS IN A COLITIS-ASSOCIATED CANCER MURINE MODEL**

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Colorectal cancer (CRC) is the third most prevalent cancer worldwide. It is known that chronic inflammation increases the risk of tumor development. Our group has demonstrated that *Kluyveromyces marxianus* CIDCA 8154 strain (Km 8154) has immunomodulatory properties and can control intestinal inflammation in an acute model. Therefore, here we evaluate if its administration impacts the process of tumorigenesis in a murine model of colitis-associated colorectal cancer by administration of azoxymethane/dextran sodium sulfate (AOM/DSS). After intraperitoneal injection of AOM 10 mg/kg, the AOM/DSS group were provided with 2,5% DSS in drinking water for 5 consecutive days, followed by 16 days of regular drinking water. This cycle was repeated 4 times. Mice were administered a yeast suspension on gelatin (1-3 10^8 UFC/cage) every 48 hours, control mice received gelatin without yeast. Mice were monitored daily and euthanized at week 13. Macroscopic inflammation parameters, such as colon length and liver weight, were measured. No difference in these markers nor in the weight fluctuation between the AOM/DSS groups with or without Km 8154 were found. We counted the presence of polyps in the colon, finding that there are fewer polyps in the group AOM/DSS+Km 8154 compared to the control AOM/DSS group ($p<0,05$). The distribution throughout the length of the colon and the characteristics of the polyps were similar in both groups. Adenocarcinoma-like structures were found in mice in both groups but one third of the animals from AOM/DSS group presented more than 4 polyps of >4mm^2 whilst none of the AOM/DSS+Km8154 group has more than 4 big polyps. We also administrated heat killed yeast, observing that it has not the same effect of the living form. Our results indicate that dietary administration of Km 8154 could be a positive intervention in prevention of inflammation-induced colorectal cancer, as we found fewer and smaller polyps in the group which has received probiotic yeast. **Keywords:** PROBIOTIC YEAST;INFLAMMATION;CANCER.

DO - 130 - **Cisplatin toxicity causes neutrophil-mediated inflammation in zebrafish larvae**

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Cisplatin is a type of chemotherapy drug used to treat various types of tumors. Although it is effective, it has several harmful side effects, including significant kidney toxicity, tissue damage, and inflammation. The inflammatory response triggered by cisplatin leads to the recruitment and infiltration of immune cells, particularly neutrophils, at the site of inflammation. Cisplatin has been widely used in experimental models, such as zebrafish (Danio rerio), to induce acute kidney injury (AKI) due to its accumulation in kidney tubular cells. However, current protocols in larval zebrafish focus solely on studying its AKI-inducing effects, neglecting its potential systemic consequences. In this study, we aimed to investigate the systemic inflammatory effects of cisplatin by directly administering it into the fish water. Our findings demonstrated that cisplatin causes dose-dependent mortality in 9 days post fertilization larvae, resulting in noticeable changes in morphology and decreased locomotion speed. Furthermore, the expression of pro-inflammatory cytokines, including IL-12, IL-6, and IL-8, significantly increased after 48 hours of cisplatin exposure. Interestingly, we observed a decrease in the number of neutrophils in the glomerular region of the pronephros, while there was an overall increase in neutrophil migration throughout the entire body of the zebrafish larvae. In summary, our study reveals that cisplatin can have systemic effects on zebrafish larvae, leading to morphological and locomotory abnormalities, elevated levels of inflammatory cytokines, and altered neutrophil distribution. Therefore, our experimental protocol can serve as a valuable tool for inducing systemic inflammation in zebrafish larvae, enabling further investigations into novel therapeutic interventions or the elucidation of mechanisms involving neutrophils. **Keywords:** neutrophil ;cisplatin;neutrophil .

DO - 131 - **IMPACT OF COLORECTAL CANCER CELL CO-CULTURE ON MACROPHAGE IMMUNOMODULATION AND PROLIFERATION**

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. The immune system plays a crucial role in CRC promotion and progression. Macrophages, an innate immune cell, can be classified into M1 (pro-inflammatory), and M2 (anti-inflammatory) phenotypes. In tumors, there are Tumor Associated Macrophages (TAM), but in CRC tumors their role remains unclear. Therefore, further investigation is necessary for potential treatment targets. Focusing on that, in this study, peripheral blood mononuclear cells (PBMCs) (CEP 2.476.898) were isolated from human blood. Monocytes were differentiated into macrophages using Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and co-cultured with CRC cell lines (HCT116 and Caco-2) or conditioned medium from cell lines. Analyses included necrotic cell death (propidium iodide) and nitric oxide production (inducible Nitric Oxide Synthase). Flow cytometry assessed membrane potential (RHO123), cytokine levels (CBA), cell proliferation (BrdU), and macrophage phenotype (HLA-DR, CD206, CD163). One-way ANOVA was used for statistical analysis. Macrophages exhibited a tropism for tumor cells and displayed an activated macrophage morphology. Necrosis was absent, indicating that both cell types were compatible for coexistence. Co-culture promoted proliferation in both tumor cells and macrophages, with significant CD14 upregulation in macrophages. Nitrite levels increased after 48 hours of co-culture. Additionally, pro-tumor cytokines TNF, IL-10, IL-6, and IL-1β were elevated. The most notable finding was the upregulation of markers CD206 and CD163, indicating M2 polarization and TAM association. Our results suggest that co-cultured macrophages induce type-2 polarization, contributing to tumor promotion in CRC. However, further analysis is needed to understand the interaction between CRC tumor cells and TAMs within the microenvironment. Acknowledgments: CNPq, CAPES, FAPERGS, and UFCSPA. **Keywords:** Colorectal cancer;Macrophages;Tumor microenvironment.

DO - 132 - **Role of Lipid Droplets on cognitive damage due to Zika virus infection**

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Zika virus (ZIKV) quickly became a serious public health problem because of its high morbidity/mortality associated. ZIKV exhibits tropism for central nervous system (CNS) cells, which would be responsible for developing the neurodegenerative and serum disorders characteristic of the infection, such as microcephaly and genetic impairment in adults. In general, diseases that affect the CNS tend to exhibit mitochondrial dysfunction and impairment of antioxidant defenses, which causes an increase in reactive oxygen species (ROS) and nitrogen (RNS) in brain tissue, as well as lipid peroxidation. Several viruses seem to use host-lipid metabolism as an enhancer of pathology and replication, especially the lipid droplets (LDs) organelles. LDs can protect cells from hypoxia and oxidative stress. However, dysregulation of lipid metabolism is usually associated with neurodegenerative diseases. Thus, the objective of this study is to investigate the impact of LDs on the development of long-term cognitive damage resulting from ZIKV infection, inflammation and neurodegeneration so as to better elucidate the mechanisms that generated them. Our findings reveal that in vivo treatment with iDGAT reduced viral load, weight loss, and mortality, in addition to preventing the development of cognitive impairment. iDGAT was able to significantly reverse serum and tissue levels of cytokines and chemokines, leukocytes' rolling, malondialdehyde, 3-nitrotyrosine, and 4-hydroxynonenal levels. Since the high expression of PSD-95 and synaptophysin, post and presynaptic proteins, we verified that ZIKV infection promoted the unbalancing of synaptic functions, which was reversed by iDGAT. Thus, it is noted that the modulation of lipid metabolism plays a neuroprotective and anti-inflammatory role, preventing cognitive impairment by preventing tissue damage, and offering support for therapeutic strategies in the face of neurocognitive dysfunction. **Keywords:** Zika virus;Lipid Droplets;Neuroinflammation.

DO - 133 - **Metabolic syndrome enhances IL-17+ and IL-6+ T Cells and the production of Th2 and Th17-related cytokines in obese individuals**

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Introduction: Metabolic syndrome (MetS) is an endocrine disorder that significantly enhances the risk of obesity-related diseases, such as cardiovascular events, diabetes and cancer. It's defined by the International Diabetes Federation (IDF) as an increase in waist circumference together with other metabolic disfunctions, such as hypertension, dyslipidemias and/or insulin resistance. Individuals with MetS have increased pro-inflammatory markers and altered frequencies of immune cells types, however studies in humans that analyze T cell phenotypes and cytokine production in individuals with MetS are scarce. Methods: Briefly, 15 individuals with MetS and 6 obese controls were recruited, with MetS diagnostic been defined by IDF criteria. From blood samples, peripheral blood mononuclear cells (PBMC) were obtained and stimulated for 20h with PMA+Ionomycin, for flow cytometry analysis, or for 3 days with PHA, for analysis of cytokine production, by Luminex. From plasma, cytokines and adipokines levels were measured by Luminex and ELISA, respectively. Results: Individuals with MetS had increased plasmatic levels of IL-1β, IL-6, IL-5 and IL-17, with no difference in levels of IL-10, GM-CSF, IFN-γ, IL-12, IL-8 or IL-23. In the supernatant of PHA stimulated cultures, higher production of IL-6, IL-5, IL-17 and GM-CSF was observed in patients with MetS. Those individuals also had increased frequencies of IL-6+ and IL-17+ CD4 T cells and IL-17+ CD8 T cells when compared with controls. Plasmatic levels of leptin were positively correlated with circulating levels of IL-6, IL-5 and IL-17, while the cytokine production of IL-6, IL-5, IL-17 and GM-CSF by the activated cells had a positive correlation with plasmatic leptin levels. Finally, plasmatic adiponectin levels had a negative correlation with cells production of IL-5 and IL-17. Conclusion: While preliminary, our results suggest that MetS is capable of enhancing both the frequency and production of pro-inflammatory markers. **Keywords:** T Cells;Metabolic Syndrome;Obesity.

DO - 134 - **Obesity induces lung endothelial glycocalyx shedding and LFA-1-dependent Th17 cell influx to the lungs promoting asthma exacerbation**

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Obesity is a risk factor to asthma exacerbation leading to lung neutrophil influx, which is the hallmark of severe asthma. Shedding of the endothelial glycocalyx (EG), a network of proteoglycans and glycoproteins bound on the endothelial cells, increases leukocyte migration. We hypothesized that pulmonary EG shedding as consequence of obesity-induced dysbiosis aggravates pulmonary inflammation during the comorbidity obesity and asthma by increasing neutrophil and Th17 cell influx. Female C57BL/6 mice were fed with high fat diet (HFD) for 12 weeks and submitted to ovalbumin (OVA)-induced asthma. Obese mice exposed to OVA (HFD+OVA group) exhibited an increase of neutrophils in the bronchoalveolar lavage fluid (BALF), Th17 cells in the lungs and mucus production compared to lean mice exposed to OVA (LFD+OVA group). HFD+OVA group also exhibited an increase of intestinal permeability, measured by serum LPS content and FITC-dextran assay, suggesting an imbalance on the microbiota. Increased Firmicutes and reduced Bacteroidetes detected in the feces of HFD and HFD+OVA groups support the presence of dysbiosis during the comorbidity obesity and asthma. Decreased lung EG thickness, determined by electron microscopy, was more accentuated in the HFD+OVA group compared to HFD and to LFD+OVA group. Neutrophils and Th17 cells of HFD+OVA group showed high LFA-1 (CD11a) expression, and LFA-$1^{+}CD4^{+}ROR\gamma T^{+}$ cells were positively correlated with neutrophils in the BALF. Deficiency of β_2 chain of LFA-1 ($CD18^{-/-}$ mice) in HFD+OVA group diminished lung inflammation, mucus production and Th17 cell influx. Our results show that obesity induces lung EG shedding, promoting increased neutrophil influx and LFA-1-dependent Th17 cell influx after OVA exposure. Our findings suggest that EG and LFA-1 might be therapeutic targets to reduce neutrophil influx during the comorbidity obesity and asthma. **Financial support:** FAPESP, grants 2017/21629-5; 2021/14343-3. **Keywords:** Endothelial glycocalyx;LFA-1;Th17 cells.

DO - 135 - **CHANGES IN MICROBIOTA MAY INDUCE HISTONE ACYLATIONS IN INTESTINAL EPITHELIAL CELLS DURING EARLY INFLAMMATION**

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INTRODUCTION: Intestinal epithelial cells (IECs) continuously respond and adapt to environmental stimuli to maintain intestinal homeostasis. This interaction can modify cellular processes, including gene expression, through histone post-translational modifications (HPTMs). Our laboratory observed elevated HPTMs (acetylation and crotonylation) in colonic IECs during early intestinal inflammation induced by 2.5% dextran sulfate sodium (DSS). However, the cause and consequences of these modifications remained unclear. **METHODS AND RESULTS:** In our study, we analysed gene expression in IECs and its association with two HPTMs: acetylation of lysine 8 on histone 4 (H4K8ac) and pan-lysine crotonylation (Kcr). The 401 up-regulated genes in IECs were linked with terms related to the response to lipopolysaccharide (LPS) and the onset of inflammation. Conversely, the 170 down-regulated genes were mainly associated with type I and type II interferon response. Fifteen up-regulated and five down-regulated genes showed a positive correlation with Kcr, while no gene was correlated with H4K8ac. Additionally, we observed alterations in the mucus layer and microbiota composition, including a reduction in microbiota diversity and an enrichment of bacteria from the *Izemoplasmataceae*, *Dethiosulfatibacteraceae*, and *Akkermansiaceae* families in the DSS-treated group. **CONCLUSION:** In summary, histone crotonylation increases during early inflammation and influences gene expression in IECs. The intestinal microbiota likely plays a significant role in this process, as IECs exhibit enhanced responsiveness to lipopolysaccharides, a component of abundant Gram-negative bacteria in this condition. Furthermore, the separation between luminal contents and the colonic epithelium seems to be reduced. Further experiments are needed to understand the signalling pathway and its implications during inflammation. **Keywords:** histone post-translational modifications;intestinal epithelial cells;microbiota.

DO - 136 - **PRODUCTION OF PROSTAGLANDINS AND FREE FATTY ACIDS IS PPAR-GAMMA-DEPENDENT IN MYCOBACTERIUM TUBERCULOSIS LIPID-STIMULATED MACROPHAGES**

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The cell wall of wild-type (WT) *Mycobacterium tuberculosis* (Mtb) and the Mtb strain disrupted in a 13-gene operon mce1 (Δmce1) varies by more than 400 lipid species. In a recent work it was observed that non-polar lipid extracts from Δmce1 enhanced the mRNA expression of lipid-sense nuclear receptors TR4 and PPAR-γ in murine macrophage relative to WT-Mtb cell wall lipids. Accumulated evidence over the last decade indicates that PPARs influence multiple facets of inflammation and immunity, thereby providing important crosstalk between metabolism and immune system. Here, we used GW9662, the PPAR-γ blocking agent, and applied the mass spectrometry-based targeted eicosanoid, and untargeted lipidomics and metabolomics analysis to assess the role of this nuclear receptor in regulating the lipid and metabolic alterations in Δmce1 Mtb lipid-induced murine macrophages. Under Δmce1-Mtb lipid stimulation, macrophages produced high levels of phosphatidylglycerol, diacyl and triacylglycerols, and low levels of free fatty acids (FFAs) compared to unstimulated control. In contrast, macrophage stimulated with Δmce1-Mtb lipid and PPAR-γ-blocking produced high levels of FFAs, as arachidonic acid (ARA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) among others. In the culture supernatants, Δmce1-Mtb lipid and PPAR-γ-blocking macrophages demonstrated a reduction in the levels of prostaglandin-E2 (PGE2), prostaglandin-D2 (PGD2), and prostaglandin-A2 (PGA2) molecules, when compared to Δmce1-Mtb lipid counterparts. Additionally, metabolomics analysis revealed a significant elevation in the level of 2-monopalmitin in Δmce1-Mtb and PPAR-γ-blocking lipid macrophages compared to Δmce1-Mtb lipid counterparts. Collectively, these results unveil an intricate mechanism involving PPAR-γ in macrophages, controlled by Mtb, in the synthesis of prostaglandins and free fatty acid molecules. **Keywords:** Tuberculosis;Lipids;PPAR-γ.

DO - 137 - **The role of neutrophil extracellular vesicles and their impact on Leishmania amazonensis infection**

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In the context of *Leishmania* infection, neutrophils (NOs) are the first cells to migrate to the affected tissue, where they can promote either the control of the parasite or exacerbate the infection. In addition to their microbicidal mechanisms, neutrophils also interact with macrophages, the parasite's final host cells. In response to different inflammatory stimuli, neutrophils release extracellular vesicles (EVs), which can regulate numerous physiological processes and contribute to tissue homeostasis or propagation of infectious agents. These particles have immunomodulatory properties and potentiate neutrophil migration. The participation of neutrophil EVs during *Leishmania amazonensis* (L.a) infection has not been explored. Our aim in this work is to characterize EVs released by neutrophils stimulated by this pathogen and study their impact on the infection. For that, human neutrophils were stimulated or not with *L. amazonensis* promastigotes for 1h at 37ºC. Exosomes and microvesicles were obtained by ultracentrifugation. Nanoparticle tracking analysis indicate that *L.amazonensis*-stimulated neutrophils release more microvesicles and exosomes than non-stimulated neutrophils. Furthermore, we observed that the stimulus with L.a did not affect the EV size distribution profile. Transmission electron microscopy show that samples isolated from both groups of neutrophils presented spherical and membrane-bounded morphologies. Then, we evaluated how these EVs could influence the response of other neutrophils. Our results demonstrate that vesicles stimulated by L.a increase ROS and NET production in relation to unstimulated vesicles, without affecting elastase activity or the phagocytosis process. As future perspective, we aim to characterize the content of these vesicles and perform in vivo experiments to understand the effect of these EVs on infection by *Leishmania* parasite. **Keywords:** Extracellular Vesicles;Neutrophils;Leishmania.

DO - 138 - **The TNF rs1800629 gene variant is associated with a reduced risk for COVID-19 severity**

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COVID-19 is a viral disease that affects thousands of people worldwide. Symptomatic individuals can present from a flu-like syndrome to pneumonia and systemic hyperinflammation, called cytokine storm. In this process, the levels of pro-inflammatory cytokines such as TNF and IL-6 are elevated, which designates worsening the patient's condition. It is suggested that host genetics is an elucidative factor for the symptom heterogeneity and the different course mechanisms observed in the disease, since some genes may influence its outcome. Thus, the study aimed to investigate the host TNF rs1800629 variant linked to the severity of COVID-19. The work was conducted with 265 severe and mild cases from regions of Brazil. The rs1800629-A variant was genotyped via RT-PCR. Serologic determination of IL-6 and TNF by ELISA was performed. The outcomes evaluated were COVID-19 severity, Intensive Care Units (ICUs), and death by logistic regression (PLINK 1.9). Analysis of cytokines compared to genotypes was performed in GraphPad Prisma using the Kolmogorov-Smirnov test, followed by Kruskal-Wallis or Mann Whitney. Our results revealed an association of rs1800629 with reduced risk for COVID-19 severity in the additive (OR=0.20, CI=0.06-0.6, p=0.007), dominant (OR=0.17, CI=0.63-0.05, p=0.006) and heterozygous (OR=0.17, CI=0.05-0.60, p=0.005) models. No associations between ICUS and death were observed. The A allele, which is more commonly observed in general populations, was found to be less frequent in our population.. Survival analysis showed no difference between the groups. The AA genotype showed lower TNF production than the AG or GG genotypes. No relationship was observed regarding IL-6 dosages. These findings suggest that rs1800629 present TNF may be a key factor in the non-aggravation of COVID-19 and the relationship with lower production of pro-inflammatory cytokine, thus inhibiting cytokine storm. **Keywords:** TNF;Genetic variant;COVID-19 severity .

DO - 139 - **ANALYSIS OF THE ANTI-INFLAMMATORY AND IMMUNOMODULATORY ACTIVITY IN VITRO OF BROMAC® ON PERIPHERAL BLOOD CELLS STIMULATED WITH SARS-COV-2**

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In the past three years, the COVID-19 pandemic has claimed nearly 7 million lives and more than 767 million cases have been reported worldwide. Ongoing research into therapeutic countermeasures for the clinical management of COVID-19 remains crucial. In this regard, BromAc® is a combination of bromelain and acetylcysteine (NAC), currently used for the palliative treatment of pseudomyxoma (Phase 3) and has been studied for repositioning in the treatment of COVID-19. BromAc® has already demonstrated *ex-vivo* mucolytic and anti-inflammatory activity in tracheal aspirate samples from patients with severe COVID-19. To examine the anti-inflammatory effect of BromAc®, an assay was standardized and performed using the inactivated SARS-CoV-2 virus in an *in vitro* system with peripheral blood cells in the presence or absence of the compound. Luminex® assays and flow cytometry were performed. BromAc® demonstrated anti-inflammatory activity, reducing the action of the cytokine storm, chemokines, growth factors and regulatory cytokines in the treated samples, in comparison with the samples stimulated with the virus. After stimulation, the cell suspension was incubated with antibodies for labeling subpopulations of lymphocytes, neutrophils, and monocytes. The results show that BromAc® was able to modulate the populations of $CD16^+$ neutrophils and $CD14^+$ monocytes observed after stimulation with iSARS-CoV-2, with a lower percentage being observed in the treated samples. BromAc® treatment has also been shown to increase the HLA-DR activation marker in $CD14^+$ monocyte populations. It is also possible to observe that the treatment with BromAc® decreases the production of TNF by the $CD19^+$ B cells, in comparison with the group stimulated with the inactivated virus. These results indicate a robust anti-inflammatory effect of BromAc® in an *in vitro* stimulation system with SARS-CoV-2, indicating its potential as a therapeutic strategy for COVID-19. **Keywords:** COVID-19;SARS-CoV-2;BromAc®.

DO - 140 - **Cisplatin toxicity causes neutrophil-mediated inflammation in zebrafish larvae**

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Cisplatin is a type of chemotherapy drug used to treat various types of tumors. Although it is effective, it has several harmful side effects, including significant kidney toxicity, tissue damage, and inflammation. The inflammatory response triggered by cisplatin leads to the recruitment and infiltration of immune cells, particularly neutrophils, at the site of inflammation. Cisplatin has been widely used in experimental models, such as zebrafish (Danio rerio), to induce acute kidney injury (AKI) due to its accumulation in kidney tubular cells. However, current protocols in larval zebrafish focus solely on studying its AKI-inducing effects, neglecting its potential systemic consequences. In this study, we aimed to investigate the systemic inflammatory effects of cisplatin by directly administering it into the fish water. Our findings demonstrated that cisplatin causes dose-dependent mortality in 9 days post fertilization larvae, resulting in noticeable changes in morphology and decreased locomotion speed. Furthermore, the expression of pro-inflammatory cytokines, including IL-12, IL-6, and IL-8, significantly increased after 48 hours of cisplatin exposure. Interestingly, we observed a decrease in the number of neutrophils in the glomerular region of the pronephros, while there was an overall increase in neutrophil migration throughout the entire body of the zebrafish larvae. In summary, our study reveals that cisplatin can have systemic effects on zebrafish larvae, leading to morphological and locomotory abnormalities, elevated levels of inflammatory cytokines, and altered neutrophil distribution. Therefore, our experimental protocol can serve as a valuable tool for inducing systemic inflammation in zebrafish larvae, enabling further investigations into novel therapeutic interventions or the elucidation of mechanisms involving neutrophils. **Keywords:** neutrophil ;cisplatin;neutrophil .

DO - 141 - **IMPACT OF COLORECTAL CANCER CELL CO-CULTURE ON MACROPHAGE IMMUNOMODULATION AND PROLIFERATION**

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. The immune system plays a crucial role in CRC promotion and progression. Macrophages, an innate immune cell, can be classified into M1 (pro-inflammatory), and M2 (anti-inflammatory) phenotypes. In tumors, there are Tumor Associated Macrophages (TAM), but in CRC tumors their role remains unclear. Therefore, further investigation is necessary for potential treatment targets. Focusing on that, in this study, peripheral blood mononuclear cells (PBMCs) (CEP 2.476.898) were isolated from human blood. Monocytes were differentiated into macrophages using Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and co-cultured with CRC cell lines (HCT116 and Caco-2) or conditioned medium from cell lines. Analyses included necrotic cell death (propidium iodide) and nitric oxide production (inducible Nitric Oxide Synthase). Flow cytometry assessed membrane potential (RHO123), cytokine levels (CBA), cell proliferation (BrdU), and macrophage phenotype (HLA-DR, CD206, CD163). One-way ANOVA was used for statistical analysis. Macrophages exhibited a tropism for tumor cells and displayed an activated macrophage morphology. Necrosis was absent, indicating that both cell types were compatible for coexistence. Co-culture promoted proliferation in both tumor cells and macrophages, with significant CD14 upregulation in macrophages. Nitrite levels increased after 48 hours of co-culture. Additionally, pro-tumor cytokines TNF, IL-10, IL-6, and IL-1β were elevated. The most notable finding was the upregulation of markers CD206 and CD163, indicating M2 polarization and TAM association. Our results suggest that co-cultured macrophages induce type-2 polarization, contributing to tumor promotion in CRC. However, further analysis is needed to understand the interaction between CRC tumor cells and TAMs within the microenvironment. Acknowledgments: CNPq, CAPES, FAPERGS, and UFCSPA. **Keywords:** Colorectal cancer;Macrophages;Tumor microenvironment.

DO - 142 - **Role of Lipid Droplets on cognitive damage due to Zika virus infection**

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Zika virus (ZIKV) quickly became a serious public health problem because of its high morbidity/mortality associated. ZIKV exhibits tropism for central nervous system (CNS) cells, which would be responsible for developing the neurodegenerative and serum disorders characteristic of the infection, such as microcephaly and genetic impairment in adults. In general, diseases that affect the CNS tend to exhibit mitochondrial dysfunction and impairment of antioxidant defenses, which causes an increase in reactive oxygen species (ROS) and nitrogen (RNS) in brain tissue, as well as lipid peroxidation. Several viruses seem to use host-lipid metabolism as an enhancer of pathology and replication, especially the lipid droplets (LDs) organelles. LDs can protect cells from hypoxia and oxidative stress. However, dysregulation of lipid metabolism is usually associated with neurodegenerative diseases. Thus, the objective of this study is to investigate the impact of LDs on the development of long-term cognitive damage resulting from ZIKV infection, inflammation and neurodegeneration so as to better elucidate the mechanisms that generated them. Our findings reveal that in vivo treatment with iDGAT reduced viral load, weight loss, and mortality, in addition to preventing the development of cognitive impairment. iDGAT was able to significantly reverse serum and tissue levels of cytokines and chemokines, leukocytes' rolling, malondialdehyde, 3-nitrotyrosine, and 4-hydroxynonenal levels. Since the high expression of PSD-95 and synaptophysin, post and presynaptic proteins, we verified that ZIKV infection promoted the unbalancing of synaptic functions, which was reversed by iDGAT. Thus, it is noted that the modulation of lipid metabolism plays a neuroprotective and anti-inflammatory role, preventing cognitive impairment by preventing tissue damage, and offering support for therapeutic strategies in the face of neurocognitive dysfunction. **Keywords:** Zika virus;Lipid Droplets;Neuroinflammation.

DO - 143 - **Metabolic syndrome enhances IL-17+ and IL-6+ T Cells and the production of Th2 and Th17-related cytokines in obese individuals**

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Introduction: Metabolic syndrome (MetS) is an endocrine disorder that significantly enhances the risk of obesity-related diseases, such as cardiovascular events, diabetes and cancer. It's defined by the International Diabetes Federation (IDF) as an increase in waist circumference together with other metabolic disfunctions, such as hypertension, dyslipidemias and/or insulin resistance. Individuals with MetS have increased pro-inflammatory markers and altered frequencies of immune cells types, however studies in humans that analyze T cell phenotypes and cytokine production in individuals with MetS are scarce. Methods: Briefly, 15 individuals with MetS and 6 obese controls were recruited, with MetS diagnostic been defined by IDF criteria. From blood samples, peripheral blood mononuclear cells (PBMC) were obtained and stimulated for 20h with PMA+Ionomycin, for flow cytometry analysis, or for 3 days with PHA, for analysis of cytokine production, by Luminex. From plasma, cytokines and adipokines levels were measured by Luminex and ELISA, respectively. Results: Individuals with MetS had increased plasmatic levels of IL-1β, IL-6, IL-5 and IL-17, with no difference in levels of IL-10, GM-CSF, IFN-γ, IL-12, IL-8 or IL-23. In the supernatant of PHA stimulated cultures, higher production of IL-6, IL-5, IL-17 and GM-CSF was observed in patients with MetS. Those individuals also had increased frequencies of IL-6+ and IL-17+ CD4 T cells and IL-17+ CD8 T cells when compared with controls. Plasmatic levels of leptin were positively correlated with circulating levels of IL-6, IL-5 and IL-17, while the cytokine production of IL-6, IL-5, IL-17 and GM-CSF by the activated cells had a positive correlation with plasmatic leptin levels. Finally, plasmatic adiponectin levels had a negative correlation with cells production of IL-5 and IL-17. Conclusion: While preliminary, our results suggest that MetS is capable of enhancing both the frequency and production of pro-inflammatory markers. **Keywords:** T Cells;Metabolic Syndrome;Obesity.

DO - 144 - **Obesity induces lung endothelial glycocalyx shedding and LFA-1-dependent Th17 cell influx to the lungs promoting asthma exacerbation**

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Obesity is a risk factor to asthma exacerbation leading to lung neutrophil influx, which is the hallmark of severe asthma. Shedding of the endothelial glycocalyx (EG), a network of proteoglycans and glycoproteins bound on the endothelial cells, increases leukocyte migration. We hypothesized that pulmonary EG shedding as consequence of obesity-induced dysbiosis aggravates pulmonary inflammation during the comorbidity obesity and asthma by increasing neutrophil and Th17 cell influx. Female C57BL/6 mice were fed with high fat diet (HFD) for 12 weeks and submitted to ovalbumin (OVA)-induced asthma. Obese mice exposed to OVA (HFD+OVA group) exhibited an increase of neutrophils in the bronchoalveolar lavage fluid (BALF), Th17 cells in the lungs and mucus production compared to lean mice exposed to OVA (LFD+OVA group). HFD+OVA group also exhibited an increase of intestinal permeability, measured by serum LPS content and FITC-dextran assay, suggesting an imbalance on the microbiota. Increased Firmicutes and reduced Bacteroidetes detected in the feces of HFD and HFD+OVA groups support the presence of dysbiosis during the comorbidity obesity and asthma. Decreased lung EG thickness, determined by electron microscopy, was more accentuated in the HFD+OVA group compared to HFD and to LFD+OVA group. Neutrophils and Th17 cells of HFD+OVA group showed high LFA-1 (CD11a) expression, and LFA-1^{+}CD4^{+}RORγT^{+} cells were positively correlated with neutrophils in the BALF. Deficiency of β_2 chain of LFA-1 (CD18$^{-/-}$ mice) in HFD+OVA group diminished lung inflammation, mucus production and Th17 cell influx. Our results show that obesity induces lung EG shedding, promoting increased neutrophil influx and LFA-1-dependent Th17 cell influx after OVA exposure. Our findings suggest that EG and LFA-1 might be therapeutic targets to reduce neutrophil influx during the comorbidity obesity and asthma. **Financial support:** FAPESP, grants 2017/21629-5; 2021/14343-3. **Keywords:** Endothelial glycocalyx;LFA-1;Th17 cells.

DO - 145 - **CHANGES IN MICROBIOTA MAY INDUCE HISTONE ACYLATIONS IN INTESTINAL EPITHELIAL CELLS DURING EARLY INFLAMMATION**

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INTRODUCTION: Intestinal epithelial cells (IECs) continuously respond and adapt to environmental stimuli to maintain intestinal homeostasis. This interaction can modify cellular processes, including gene expression, through histone post-translational modifications (HPTMs). Our laboratory observed elevated HPTMs (acetylation and crotonylation) in colonic IECs during early intestinal inflammation induced by 2.5% dextran sulfate sodium (DSS). However, the cause and consequences of these modifications remained unclear. **METHODS AND RESULTS:** In our study, we analysed gene expression in IECs and its association with two HPTMs: acetylation of lysine 8 on histone 4 (H4K8ac) and pan-lysine crotonylation (Kcr). The 401 up-regulated genes in IECs were linked with terms related to the response to lipopolysaccharide (LPS) and the onset of inflammation. Conversely, the 170 down-regulated genes were mainly associated with type I and type II interferon response. Fifteen up-regulated and five down-regulated genes showed a positive correlation with Kcr, while no gene was correlated with H4K8ac. Additionally, we observed alterations in the mucus layer and microbiota composition, including a reduction in microbiota diversity and an enrichment of bacteria from the *Izemoplasmataceae*, *Dethiosulfatibacteraceae*, and *Akkermansiaceae* families in the DSS-treated group. **CONCLUSION:** In summary, histone crotonylation increases during early inflammation and influences gene expression in IECs. The intestinal microbiota likely plays a significant role in this process, as IECs exhibit enhanced responsiveness to lipopolysaccharides, a component of abundant Gram-negative bacteria in this condition. Furthermore, the separation between luminal contents and the colonic epithelium seems to be reduced. Further experiments are needed to understand the signalling pathway and its implications during inflammation. **Keywords:** histone post-translational modifications;intestinal epithelial cells;microbiota.

DO - 146 - **PRODUCTION OF PROSTAGLANDINS AND FREE FATTY ACIDS IS PPAR-GAMMA-DEPENDENT IN MYCOBACTERIUM TUBERCULOSIS LIPID-STIMULATED MACROPHAGES**

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The cell wall of wild-type (WT) *Mycobacterium tuberculosis* (Mtb) and the Mtb strain disrupted in a 13-gene operon mce1 (Δmce1) varies by more than 400 lipid species. In a recent work it was observed that non-polar lipid extracts from Δmce1 enhanced the mRNA expression of lipid-sense nuclear receptors TR4 and PPAR-γ in murine macrophage relative to WT-Mtb cell wall lipids. Accumulated evidence over the last decade indicates that PPARs influence multiple facets of inflammation and immunity, thereby providing important crosstalk between metabolism and immune system. Here, we used GW9662, the PPAR-γ blocking agent, and applied the mass spectrometry-based targeted eicosanoid, and untargeted lipidomics and metabolomics analysis to assess the role of this nuclear receptor in regulating the lipid and metabolic alterations in Δmce1 Mtb lipid-induced murine macrophages. Under Δmce1-Mtb lipid stimulation, macrophages produced high levels of phosphatidylglycerol, diacyl and triacylglycerols, and low levels of free fatty acids (FFAs) compared to unstimulated control. In contrast, macrophage stimulated with Δmce1-Mtb lipid and PPAR-γ-blocking produced high levels of FFAs, as arachidonic acid (ARA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) among others. In the culture supernatants, Δmce1-Mtb lipid and PPAR-γ-blocking macrophages demonstrated a reduction in the levels of prostaglandin-E2 (PGE2), prostaglandin-D2 (PGD2), and prostaglandin-A2 (PGA2) molecules, when compared to Δmce1-Mtb lipid counterparts. Additionally, metabolomics analysis revealed a significant elevation in the level of 2-monopalmitin in Δmce1-Mtb and PPAR-γ-blocking lipid macrophages compared to Δmce1-Mtb lipid counterparts. Collectively, these results unveil an intricate mechanism involving PPAR-γ in macrophages, controlled by Mtb, in the synthesis of prostaglandins and free fatty acid molecules. **Keywords:** Tuberculosis;Lipids;PPAR-γ.

DO - 147 - **The role of neutrophil extracellular vesicles and their impact on Leishmania amazonensis infection**

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In the context of *Leishmania* infection, neutrophils (NOs) are the first cells to migrate to the affected tissue, where they can promote either the control of the parasite or exacerbate the infection. In addition to their microbicidal mechanisms, neutrophils also interact with macrophages, the parasite's final host cells. In response to different inflammatory stimuli, neutrophils release extracellular vesicles (EVs), which can regulate numerous physiological processes and contribute to tissue homeostasis or propagation of infectious agents. These particles have immunomodulatory properties and potentiate neutrophil migration. The participation of neutrophil EVs during *Leishmania amazonensis* (L.a) infection has not been explored. Our aim in this work is to characterize EVs released by neutrophils stimulated by this pathogen and study their impact on the infection. For that, human neutrophils were stimulated or not with *L. amazonensis* promastigotes for 1h at 37ºC. Exosomes and microvesicles were obtained by ultracentrifugation. Nanoparticle tracking analysis indicate that *L.amazonensis*-stimulated neutrophils release more microvesicles and exosomes than non-stimulated neutrophils. Furthermore, we observed that the stimulus with L.a did not affect the EV size distribution profile. Transmission electron microscopy show that samples isolated from both groups of neutrophils presented spherical and membrane-bounded morphologies. Then, we evaluated how these EVs could influence the response of other neutrophils. Our results demonstrate that vesicles stimulated by L.a increase ROS and NET production in relation to unstimulated vesicles, without affecting elastase activity or the phagocytosis process. As future perspective, we aim to characterize the content of these vesicles and perform in vivo experiments to understand the effect of these EVs on infection by *Leishmania* parasite. **Keywords:** Extracellular Vesicles;Neutrophils;Leishmania.

DO - 148 - **The TNF rs1800629 gene variant is associated with a reduced risk for COVID-19 severity**

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COVID-19 is a viral disease that affects thousands of people worldwide. Symptomatic individuals can present from a flu-like syndrome to pneumonia and systemic hyperinflammation, called cytokine storm. In this process, the levels of pro-inflammatory cytokines such as TNF and IL-6 are elevated, which designates worsening the patient's condition. It is suggested that host genetics is an elucidative factor for the symptom heterogeneity and the different course mechanisms observed in the disease, since some genes may influence its outcome. Thus, the study aimed to investigate the host TNF rs1800629 variant linked to the severity of COVID-19. The work was conducted with 265 severe and mild cases from regions of Brazil. The rs1800629-A variant was genotyped via RT-PCR. Serologic determination of IL-6 and TNF by ELISA was performed. The outcomes evaluated were COVID-19 severity, Intensive Care Units (ICUs), and death by logistic regression (PLINK 1.9). Analysis of cytokines compared to genotypes was performed in GraphPad Prisma using the Kolmogorov-Smirnov test, followed by Kruskal-Wallis or Mann Whitney. Our results revealed an association of rs1800629 with reduced risk for COVID-19 severity in the additive (OR=0.20, CI=0.06-0.6, p=0.007), dominant (OR=0.17, CI=0.63-0.05, p=0.006) and heterozygous (OR=0.17, CI=0.05-0.60, p=0.005) models. No associations between ICUS and death were observed. The A allele, which is more commonly observed in general populations, was found to be less frequent in our population.. Survival analysis showed no difference between the groups. The AA genotype showed lower TNF production than the AG or GG genotypes. No relationship was observed regarding IL-6 dosages. These findings suggest that rs1800629 present TNF may be a key factor in the non-aggravation of COVID-19 and the relationship with lower production of pro-inflammatory cytokine, thus inhibiting cytokine storm. **Keywords:** TNF;Genetic variant;COVID-19 severity .

DO - 149 - **ANALYSIS OF THE ANTI-INFLAMMATORY AND IMMUNOMODULATORY ACTIVITY IN VITRO OF BROMAC® ON PERIPHERAL BLOOD CELLS STIMULATED WITH SARS-COV-2**

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In the past three years, the COVID-19 pandemic has claimed nearly 7 million lives and more than 767 million cases have been reported worldwide. Ongoing research into therapeutic countermeasures for the clinical management of COVID-19 remains crucial. In this regard, BromAc® is a combination of bromelain and acetylcysteine (NAC), currently used for the palliative treatment of pseudomyxoma (Phase 3) and has been studied for repositioning in the treatment of COVID-19. BromAc® has already demonstrated *ex-vivo* mucolytic and anti-inflammatory activity in tracheal aspirate samples from patients with severe COVID-19. To examine the anti-inflammatory effect of BromAc®, an assay was standardized and performed using the inactivated SARS-CoV-2 virus in an *in vitro* system with peripheral blood cells in the presence or absence of the compound. Luminex® assays and flow cytometry were performed. BromAc® demonstrated anti-inflammatory activity, reducing the action of the cytokine storm, chemokines, growth factors and regulatory cytokines in the treated samples, in comparison with the samples stimulated with the virus. After stimulation, the cell suspension was incubated with antibodies for labeling subpopulations of lymphocytes, neutrophils, and monocytes. The results show that BromAc® was able to modulate the populations of $CD16^+$ neutrophils and $CD14^+$ monocytes observed after stimulation with iSARS-CoV-2, with a lower percentage being observed in the treated samples. BromAc® treatment has also been shown to increase the HLA-DR activation marker in $CD14^+$ monocyte populations. It is also possible to observe that the treatment with BromAc® decreases the production of TNF by the $CD19^+$ B cells, in comparison with the group stimulated with the inactivated virus. These results indicate a robust anti-inflammatory effect of BromAc® in an *in vitro* stimulation system with SARS-CoV-2, indicating its potential as a therapeutic strategy for COVID-19. **Keywords:** COVID-19;SARS-CoV-2;BromAc®.

DO - 150 - **POPULATIONS FROM DIFFERENT LOCATIONS IN BRAZIL SHOW DIFFERENCES IN IMMUNOLOGICAL SIGNATURES ASSOCIATED WITH SENESCENCE**

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Introduction: Various alterations in immune activity that occur during aging increase vulnerability to infectious diseases. This includes reduced T and B cell generation and activity, and a chronic low-grade inflammation called 'inflammaging' (Ann N Y Acad Sci. 908:244–254, 2006). Individuals of the same age may have different biological or epigenetic ages, impacting disease risk (Mech Ageing Dev. 207:111713, 2022). We previously showed that people from the endemic area Governador Valares-MG (GV) in Brazil exhibit accelerated epigenetic aging compared to São Paulo-SP (SP), a non-endemic area. Thus, this study aimed to compare immunosenescence parameters in individuals from GV, SP, and Belo Horizonte-MG (BH). **Methods:** We measured epigenetic age using DNA methylation-based clocks (metilclock 1.5.0 package for R). We assessed NK, T, and B cell immunophenotyping using flow cytometry, and measured 27 inflammatory mediators in plasma using a Multiplex assay (BioRad Bio-Plex® Pro Human Cytokine Standard) in healthy and COVID-19-infected individuals. **Results:** Our results show that both individuals residing in SP and individuals residing in GV have accelerated epigenetic aging compared to individuals residing in BH, as well as differences in immunophenotyping and presence of elevated secretion of inflammatory mediators. Furthermore, we demonstrate that the acceleration of biological aging in each city is correlated with different markers in both flow cytometry and the multiplex panel. When evaluating COVID-19 patients from the three cities, despite having similar clinical conditions, we also found differences in various markers of immunophenotyping and production of inflammatory mediators. **Conclusion:** Environmental, cultural, and genetic variations associated with our place of residence can result in diverse immunological signatures linked to immunosenescence and varied responses to infections like COVID-19. **Keywords:** Immunosenescence;remodeling;endemic area.

DO - 151 - **Study of Co-Expressed Genes in macrophages from diet-induced obese mice.**

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Obesity is a chronic inflammatory condition that is associated with the accumulation of fat in tissues. The white adipose tissue (WAT) is a major site of inflammation in obesity, and it secretes cytokines, chemokines, and adipokines that are regulated by gene expression in immune cells, such as macrophages. Despite the considerable research in this area, there is still a lack of understanding about the gene networks co-expressed in immune macrophages in obesity that could initiate this inflammatory profile. This study proposes an in silico methodology to construct a co-expressed gene network and identify the macrophage obesity hub genes. We conducted a comprehensive search for publicly available RNAseq data from immune cells in the WAT of obese mice, and obtained data from the GEO database (GSE126407). Co-expression networks identify groups of genes that exhibit similar expression patterns across multiple samples. The co-expression network can be represented as a gene-gene similarity matrix, which can be generated using the WGCNA package in R. In the first step, the pairwise relationships between genes are defined based on correlation measures or mutual information. These relationships describe the similarity between the expression patterns of each gene pair across all samples. To visualize the co-expression network, we used Cytoscape. Our analysis revealed that the most prominent module in the gene co-expression network, based on macrophage gene expression associated with obesity, consists of central genes with high connectivity, including Fam129b, Tmcc1, Sema4a, Notch2, and Igf2r. After identifying these highly connected genes, we utilized a simulated annealing algorithm from the RNetcarto package to identify hub connector genes within the modules. Through this approach, we identified several hub connector genes, such as Ctsd, Ctsk, and Hist1h1c.Co-Expression analysis of RNAseq reveals key genes controlling macrophages in obesity, providing insides into obesity. **Keywords:** Obesity;Macrophages;Co-Expression.

DO - 152 - **Serum biomarkers affects the seroconversion status of patients with autoimmune diseases after planned primary 17DD-YF vaccination**

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Yellow fever (YF) is a disease endemic in several tropical areas presenting a broad clinical spectrum with high mortality rate (50% in severe cases). It is well known that the large-scale vaccination is the most effective measure to disease control. However, it has been proposed that some vaccines are less effective in autoimmune diseases patients (AID). In this sense, the present study aimed to investigate the impact of the serum biomarkers on seroconversion status in AID patients upon planned primary 17DD-YF vaccination. For this purpose, 161 subjects were enrolled in a prospective study [AID patients – Rheumatoid Arthritis (RA=38), Spondyloarthritis (SpA=51), Systemic Lupus Erythematosus (SLE=21) and Sjögren's Syndrome (SS=30)] along with a group of healthy controls (HC=21). Analysis of plaque reduction neutralization test (PRNT) titers and seropositivity rates, viremia levels and serum biomarkers were carried out at distinct time points (D0/D3–4/D5–6/D7/D14–28). The results demonstrated lower PRNT titers and seropositivity rate (170 *vs* 448; 77 *vs* 95%) in AID as compared to HC, especially in SpA and SLE. No significant differences were observed in the viremia levels amongst groups. In general, a more prominent serum biomarker response was observed in AID as compared to HC. Remarkably, AID/PRNT(−) exhibited higher levels of several biomarkers at baseline as compared to AID/PRNT(+). While AID/PRNT(+) exhibited earlier increase in serum biomarkers at D3–4/D5–6, the AID/PRNT(−) displayed higher response at later time points (D7/D14–D28). Of note, a synchronic increase of IFN-γ at the peak of viremia (D5–6) was observed in HC and AID/PRNT(+) groups, whereas a later asynchronous IFN-γ response was reported for AID/PRNT(−) at D7. Altogether these data suggested that inflammatory status prior vaccination, low IFN-γ at viremia peak and the occurrence of asynchronous biomarker response after 17DD-YF vaccination interfere on the lack of neutralizing antibody response. **Keywords:** Yellow Fever;vaccine;autoimmune disease.

DO - 153 - **THE ROLE OF EXTRACELLULAR VESICLES IN RENAL CELL CARCINOMA PROGRESSION**

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Cancer represents a set of malignant neoplastic diseases and is a leading cause of death worldwide. Kidney cancer represents 4.6% of the total number of cancer diagnoses in 2020. It represents approximately 430,000 cases, which 90% correspond to Renal Cell Carcinoma (RCC). This neoplasm originates in the tubular epithelial cells of the nephron, the kidney functional unit. The 5-year relative survival is directly related to the stage of the tumor at diagnosis, with a drastic drop in the mortality rate when identified in the metastatic stage. Nowadays we are experiencing the “Golden Age” in relation to the state of the art in renal cancer research, but to overcome the alarming epidemiological data, it is still necessary to deepen the knowledge of this neoplasm biology. Extracellular vesicles (EVs) has emerged as a new paradigm of communication and crucial roles in physiological and pathological states. Despite evidence of their role in several tumors, little is known about EVs in the RCC context. Thus, this project aims to deep into the contribution of EVs in three main fields: (i) in hypoxia and angiogenesis, targeting HIF-1 pathway and its downstream signaling; (ii) in metastasis targeting epithelial-mesenchymal transition markers; and (iii) in immune evasion mediated by PD-1, Fas and cytokines that modulate the response towards a tumorigenic profile. For these studies, the technique of 3D culture has been implemented, allowing the mimicry of tumor architecture with greater similarity than the 2D culture. Thus, it is expected to bring to light new perspectives on the EVs contribution to the RCC development, in order to open doors for further studies and clinical applications such as the investigation of biomarkers and therapeutic targets.
Keywords: extracellular vesicles; renal cell carcinoma;tumor immunology.

DO - 154 - **ROLE OF CROTONATE IN STEM CELL GROWTH AND FUNCTION.**

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ABSTRACT: This study focuses on the crucial regulation of intestinal stem cell proliferation and differentiation for maintaining intestinal homeostasis. It highlights the role of short-chain fatty acids (SCFAs), particularly crotonate, in this regulation. **METHODS:** We conducted in vivo and in vitro experiments. In vivo, C57Bl/6 mice were orally supplemented with crotonate at a concentration of 150 mM for five days. Colonic and small intestine fragments were collected for proliferation and structure analysis. In the in vitro experiments, organoids derived from C57Bl/6 mice were treated with different concentrations of crotonate (0.1, 0.25, 0.5, 1.0, 2.5, and 5 mM) to evaluate clonogenicity and differentiation. The formation of organoids was observed over six days, and a separate set of organoids was replated at the different concentrations of crotonate as cited above for differentiation analysis at different time points (6h, 24h, 48h, 72h, 96h, and 120h). These experiments aimed to examine the consequences of crotonate supplementation on both in vivo and in vitro models. **RESULTS:** The in vivo experiments revealed that oral supplementation of crotonate enhanced proliferation in intestinal crypts but did not result in elongation of colonic crypts. In the in vitro experiments, crotonate increased the formation of organoids per crypt at all concentrations tested. Both the clonogenicity and differentiation experiments demonstrated that crotonate treatment generated larger organoids compared to the control group. These findings indicate a significant modulation of colonic stem cell function by short-chain fatty acids. **Keywords:** Crotonate;Stem Cell;Colonic Organoids.

DO - 155 - **Machine Learning Algorithm Using Blood Count As An Early Predictor Of COVID-19 Outcome**

PINTO, C.H.R.[1]; VENTURA, L.H.D.A.[1]; CAMATTA, G.C.[1]; SATO, H.I.[1]; COSTA, M.S.[1]; SILVEIRA-NUNES, G.[2]; GOMES, M.D.S.[3]; DO AMARAL, L.R.[3]; BERTARINI, P.L.L.[3]; TEIXEIRA, S.M.R.[1]; TUPINAMBÁS, U.[1]; CARVALHO, A.T.[4]; FARIA, A.M.C.[1]. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, UNIVERSIDADE FEDERAL DE JUIZ DE FORA GOVERNADOR VALADARES - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UNIVERSIDADE FEDERAL DE UBERLÂNDIA UBERLÂNDIA - MG - BRASIL; 4. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS BELO HORIZONTE - MG - BRASIL.

Although the SARS-CoV-2 infection already has some established risk groups, such as the elderly and people with comorbidities, it is still necessary to find biomarkers of disease outcomes to stratify patients' risk and improve clinical management. Novel antiviral medications are most effective if administered within the first five days of symptoms, helping patients with a high risk of developing the severe form of the disease to avoid hospitalization. Hence, the sooner patients who may benefit from these drugs are identified, the better their chances of survival. The complete blood count (CBC) is an affordable option to find biomarkers that predict the prognosis of COVID-19, since the infection can alter various blood parameters. Therefore, the goal of the present study is to identify a possible association between hematological parameters and different clinical forms of COVID-19 and use them as predictors of disease outcomes. We performed a CBC in blood samples from 296 individuals in Belo Horizonte, Brazil, and observed that in the first 4 days of symptoms the classic hematological COVID-19 alterations, such as lymphopenia, are not yet perceptible. However, the percentage of monocytes (MON%) and the granulocyte-to-lymphocyte ratio (GLR) were already altered during this timeframe in patients that displayed mild symptoms and later progressed to the need of hospitalization. We then performed ROC and TG-ROC Curves, as well as Decision Tree, a machine learning algorithm, to validate the accuracy of these hematological predictors and to establish their cutoff values. Thus, our findings demonstrate that COVID-19 patients with a MON% lower than 7.7% and a GLR higher than 8.75 are 86% of the time more likely to be hospitalized, suggesting that they can be important biomarkers in predicting disease outcomes and could be used to discriminate patients at hospital admission and manage therapeutic approaches, such as antivirals, in the beginning of infection. **Keywords:** Complete Blood Count;COVID-19;Machine Learning Algorithm.

DO - 156 - **COMPLEMENT SYSTEM IN TISSUE INJURY OF PATIENTS WITH LEPTOSPIROSIS-ASSOCIATED SEVERE PULMONARY HEMORRHAGIC SYNDROME**

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Leptospirosis is a neglected zoonosis, endemic in developing countries with tropical or subtropical climates. Approximately one million new cases are reported yearly with 5-10% deaths. Some patients develop a clinical condition called Leptospirosis-associated Severe Pulmonary Hemorrhagic Syndrome (LPHS), with a mortality rate higher than 50%. The etiopathology of LPHS remains to be elucidated. The deposition of C3 in the lungs of patients with LPHS has been previously reported, in human and animal models, suggesting that the activation of the Complement System may contribute to this pathology. With this background, this study aims to evaluate the possible activation of the Complement System in patients who died from LPHS and identify the pathways involved in this process. For this, samples from LPHS patients (n=11) were used and compared to patients who died of sepsis (n=5) and of acute myocardial infarction (n=5). Histopathological analysis was carried out in the lungs, kidneys, and liver of all groups and immunohistochemical assays were performed to analyze the deposition of Complement proteins: C1q, Factor B, MASP2, C3c, C4d, C5b-9 and the presence of anaphylatoxin receptors C3aR1 and C5aR. At the same time, immunoglobulins (IgG and IgM) deposition in the tissue and the presence of Leptospira antigens were also evaluated. Preliminary results of the lung of LPHS patients revealed the presence of C1q in the vascular endothelium, Factor B in the alveolar fibrin and lung epithelium, MASP2 in vascular endothelium and lung epithelium, and the Membrane Attack Complex (C5b-9n) in the hyaline membranes, vascular endothelium, and apoptotic cells. So far, these results suggest that all three Complement pathways were activated in patients with LPHS. We believe that understanding the contribution of Complement in the etiopathogenesis of LPHS can guide the future use of inhibitors as a therapeutic treatment for patients. **Keywords:** Pulmonary Hemorrhage;Leptospirosis;Complement System.

DO - 157 - **The absence of the TRPM5 ion channel is associated with changes in the activation and differentiation of T cells**

DA SILVA, J.V.H.; OLIVEIRA, S.D.S.; SILVA, L.M.; DA SILVA, E.M.; BOS, N.N.; CAMARA, N.O.S.. USP, USP SÃO PAULO - SP - BRASIL.

Transient receptor potential (TRP) ion channels are a family of ion channels composed of 28 members and subdivided into 6 subfamilies. These channels have diverse functions, both in the physiological and pathological context since they are highly expressed in different cell and tissue types. An example is the transient receptor potential melastatin 5 channel (TRPM5), which is a taste sensor for bitter, sweet and umami flavors, that influences the detection of organelles and is present in immune cells. TRPM5 is an ion channel that is impermeable to calcium. However, calcium is important for their activities, as they are activated by an increase in intracellular calcium. Since TRPM5 controls intracellular calcium concentration, we believe that it may influence T cell polarization. Therefore, our hypothesis is that the absence of TRPM5 would be associated with changes in T cell activation and differentiation, in a microenvironment conducive to differentiation into effector cells, exacerbating the inflammatory response. In vivo experiments involving female mice (half of these animals were wild - WT, and the other half were knockout for TRPM5 - KO) in a homeostatic state, revealed by flow cytometry in the spleen an increase in the number of CD4 T cells and alteration in their profile, reduction of B cells and Treg cells. Similar results were observed in the thymus in relation to the number of CD4 T cells. All procedures performed were approved by the Ethics Committee on Animal Use of USP (CEUA No. 5533161122 / March, 2023). Statistical analysis was performed in the GraphPad Prism 8.0 software, using Student's t test. Values with $p<0.05$ were considered significant. These results support our hypothesis, and indicate an influence of TRPM5 on the immune response, possibly contributing to the pathogenesis of different inflammatory diseases. **Keywords:** Calcium;Metabolism;Inflammation.

DO - 158 - **PROMOTION OF DENDRITIC CELL SUPPRESSION THROUGH EFFEROCYTOSIS OF GLIOBLASTOMA-APOPTOTIC CELLS**

OLIVEIRA, K.C.[1]; PEREIRA, L.D.S.[1]; PENTEADO, L.D.A.[2]; RAIMUNDO, B.V.B.[1]; RIBEIRO, D.M.[1]; LOPES, M.C.F.[1]; DE MEDEIROS, A.I.[1]. 1. SÃO PAULO STATE UNIVERSITY (UNESP)/ARARAQUARA, SÃO PAULO STATE UNIVERSITY (UNESP)/ARARAQUARA ARARAQUARA - SP - BRASIL; 2. SÃO PAULO UNIVERSITY (USP)/RIBEIRÃO PRETO, SÃO PAULO UNIVERSITY (USP)/RIBEIRÃO PRETO RIBEIRÃO PRETO - SP - BRASIL.

Recent findings have shown that treatment regimens can drive immunogenic (ICD) and non-immunogenic cell death (NICS). NICD inducers might promote the accumulation of apoptotic cells in the tumor microenvironment (TME), which can dramatically impact the dendritic cells (DCs) activation in the TME. DCs and macrophages play an important role during the clearance of dying cancer cells, called efferocytosis. Recent studies have shown that different metabolic pathways are activated in macrophages during efferocytosis of apoptotic cells (ACs) that support the tolerogenic profile acquired by these cells. In this context, the metabolic reprogramming of immune cells becomes a new alternative for treating tumors to generate an immunogenic response. Therefore, we hypothesized that efferocytosis of glioblastoma apoptotic cells (GBM-ACs) activates intracellular metabolic pathways promoting a tolerogenic DC phenotype contributing to tumor immunosuppression. The GBM cell line (U87MG) was irradiated with 100mJ of UV-C, following incubation for 3 h at 37°C. Then, the % of apoptotic cells was determined using anti-caspase-3 antibody. UV-C treatment induced 60% of cleaved caspase-3+ cells, compared with 7% on the untreated cells. Next, we evaluated the efferocytosis capacity, then GBM-ACs were labeled with PKH and co-cultured with BMDC for 18h, and approximately 90% of CD11c+ was able to phagocyte GBM-ACs. Internalization and digestion of GBM-ACs resulted in increased PD-L1 expression and a reduction in the mean fluorescence intensity of MHC-II and CD86 compared with BMDCs. Furthermore, the efferocytosis of GBM-ACs leads to a slight increase in IL-6 and IL-10 production compared to resting BMDCs. Taken together, these findings suggest that efferocytosis of GBM-ACs induces a tolerogenic state in DCs. Our next step will be to evaluate which metabolic pathways support this tolerogenic phenotype and whether metabolic reprogramming might be able to activate an efficient antitumor response. **Keywords:** Efferocytosis;Metabolic reprogramming;Glioblastoma.

DO - 159 - **Chronic ingestion of Whole Peanut Extract leads to allergic responses followed by desensitization in Ara h 1 and Peanut Protein Extract-sensitized mice**

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Peanut is a highly allergenic food that harbors at least 16 allergens. Allergic individuals may present IgE against one or multiple of these proteins. Although peanut allergy is associated with a low desensitization rate, studies have shown mice sensitized to peanut protein extract (PPE) undergo desensitization after chronic oral exposure to peanuts. However, little is known about how this process occurs in the context of single, major peanut allergens sensitization. Thus, we aimed to investigate the effect of chronic whole peanut extract (WPE) ingestion on Ara h 1, Ara h 2 or PPE-sensitized mice. C57BL/6 mice received an injection of Ara h 1, Ara h 2 or PPE adsorbed in aluminum hydroxide on days 0 and 14. On day 21, mice were orally challenged with WPE as the only option of drinking beverage for three weeks. Although sensitization with all three antigens led to specific IgG1 production, specific IgE was detected only in the context of Ara h 1 and PPE sensitization. Mice sensitized to Ara h 1 had high serum levels of specific IgE after one and two weeks of oral challenge, but it dropped at the third week. This pattern was not observed in PPE-sensitized mice. Interestingly, mice sensitized to Ara h 1 and PPE, but not Ara h 2, showed higher production of intestinal SIgA than control mice at the second week of WPE ingestion, but it also dropped at the third week. At this time point, Ara h 1-sensitized mice had higher frequency of Treg lymphocytes in the spleen and mesenteric lymph nodes (mLN) compared to control mice, whereas this effect was only observed in the mLN of PPE-sensitized mice. Both groups presented no alteration in the frequency of effector T helper cells in the spleen and mLN. Ara h 2-sensitized mice did not present changes in the frequency of T helper cells from both organs after oral challenge. Therefore, mice sensitized to Ara h 1 and PPE, but not Ara h 2, presented allergic immune responses followed by desensitization over the curse of WPE ingestion. **Keywords:** Food Allergy;Desensitization;Regulatory T cells.

DO - 160 - **Characterization of the intestinal microbiota of individuals with Covid-19 from endemic and non-endemic areas for infectious diseases and its association with the inflammatory profile**

CAMATTA, G.C.[1]; VENTURA, L.H.D.A.[1]; PINTO, C.H.R.[1]; TOMÉ, L.M.R.[1]; SILVA, L.C.F.[2]; MOREIRA, F.C.[1]; TORRES, L.[1]; SATO, H.I.[1]; SILVEIRA-NUNES, G.[3]; GOMES, M.D.S.[2]; LEITE, P.M.[3]; LIMA, M.R.[4]; NETO, A.G.[1]; TUPINAMBÁS, U.[1]; CARVALHO, A.T.[5]; FARIA, A.M.C.[1]. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UNIVERSIDADE FEDERAL DE UBERLÂNDIA UBERLÂNDIA - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, UNIVERSIDADE FEDERAL DE JUIZ DE FORA GOVERNADOR VALADARES - MG - BRASIL; 4. UNIVALE, UNIVALE GOVERNADOR VALADARES - MG - BRASIL; 5. INSTITUTO DE PESQUISA RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ, INSTITUTO DE PESQUISA RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ BELO HORIZONTE - MG - BRASIL.

During the Covid-19 pandemic, caused by the SARS-Cov-2 virus, several studies have shown marked changes in the intestinal microbiome in infected individuals. Although microbial composition remains stable throughout life, infections and the aging process can potentially interfere in its composition, and in the interaction with the host immune system. In the context of endemic areas for infectious diseases, the chronic antigenic load can accelerate immunessenesce and directly impact the gut microbiota. Thus, our aim was to analyze the fecal microbial composition of patients infected with SARS-Cov-2 from endemic and non-endemic areas for infectious diseases, and its association with the immunological profile. We collected blood and fecal samples from 92 individuals which were divided into two groups: healthy and Covid-19 patients, all of them submitted to RT-PCR and serological tests. Gut microbiome composition was determined by 16S rRNA sequencing and blood samples were used for quantifying 27 biomarkers (Bio-Plex® Pro Human Cytokine Assay). Our data showed that despite the similarity in alpha-diversity, the microbial composition of Covid-19 group from endemic area was distinct from all of the others at both phylum and genus levels. There was an increase in the opportunistic pathogenic taxa *Streptococcus*, while *Feacalibacterium* and *Parabacterioides*, associated with healthy aging and butyrate production, were reduced in this group. Although the overall inflammatory profile was similar, IL-10 was reduced in Covid-19 group from the endemic area comparing to the non-endemic and the control group. No difference in microbiome or inflammation was found between healthy groups. Altogether, these results point out a more expressive change in the microbial composition of individuals already exposed to previous infection, suggesting a potential loss of immune plasticity and resilience in the microbial composition when facing new pathogens, as a consequence of chronic infection. **Keywords:** Immunossenescence;Intestinal microbiota;Covid-19.

DO - 161 - **THE MICROBIOTA-DEPENDENT WORSENING EFFECTS OF MELATONIN IN GUT INFLAMMATION**

DA SILVA, J.L.[1]; BARBOSA, L.V.[1]; PINZAN, C.F.[1]; NARDINI, V.[1]; BRIGO, I.S.[1]; SEBASTIÃO, C.A.[2]; OLIVEIRA, J.E.[1]; BRAZÃO, V.[1]; DO PRADO JUNIOR, J.C.[1]; SARTORI, D.C.[1]; CARDOSO, C.R.D.B.[1]. 1. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAILO, UNIVERSIDADE DE SÃO PAILO RIBEIRÃO PRETO - SP - BRASIL.

Dysbiosis and disturbances in gut homeostasis may result in dysregulated responses, which are common in Inflammatory Bowel Diseases (IBD). These conditions may be refractory to the usual treatments and novel therapies are still necessary to reach a more successful regulation of intestinal immunity. The hormone melatonin (MLT) has been raised as a therapeutic alternative because of its known interactions with immune responses and gut microbiota. Hence, we evaluated the effects of MLT in experimental colitis that evolves with intestinal dysbiosis, inflammation and bacterial translocation. C57BL/6 mice were exposed to dextran sulfate sodium and treated with MLT. In the acute colitis, the hormone led to increased clinical, systemic and intestinal inflammatory parameters. During remission, continued MLT administration delayed recovery, increased TNF, memory effector lymphocytes and diminished spleen regulatory cells. MLT treatment reduced Bacteroidetes and augmented Actinobacteria and Verrucomicrobia phyla in mice feces. Microbiota depletion resulted in a remarkable reversion of the colitis phenotype after MLT administration, including a counter regulatory immune response, reduction in TNF and colon macrophages. There was a decrease in Actinobacteria, Firmicutes and, most strikingly, Verrucomicrobia phylum in recovering mice. Finally, these results pointed to a gut microbiota-dependent effect of MLT in the potentiation of intestinal inflammation. **Keywords:** Microbiota;IBD;Melatonin.

DO - 162 - **M. tuberculosis-infected airway epithelial cells activate macrophages that produce IL-1β and control lung bacterial load, inducing a balanced pulmonary inflammation**

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Alveolar epithelial cells (AEC), macrophages and dendritic cells (DC) are the first cells that interact with *Mycobacterium tuberculosi*s (Mtb), which still kill more than one million people worldwide annually. Although the interaction of a single cell population with Mtb has been evaluated, the interface of AEC and macrophages with Mtb remains to be investigated. We hypothesized that type II AEC (AEC-II) infected with Mtb generates a microenvironment that improves macrophage activation. Bone marrow derived macrophages (BMDM) were treated with supernatants of Mtb-infected AEC-II (MLE-15 mouse cell line), described as infected conditioned medium (ICM) or non-infected AEC-II (NICM) for 24 hours. BMDM treated with ICM showed increased secretion of IL-1b, TNF IL-10, and increased expression of arginase 1 compared to NICM treatment. Human monocyte-derived macrophages treated with ICM harvested from primary human epithelial cells (Normal Human Bronchial Epithelial Cells - NHBE) exhibited increased expression of CD206 compared to NICM treatment. BMDM infected with Mtb and treated with ICM restricted bacterial load and secreted higher IL-1β and IL-6 concentrations compared to non-treated BMDM. In attempt to confirm in vitro findings, we transfer ICM-treated BMDM (ICM-BMDM) followed by infection of C57BL/6 mice with $1x10^5$ CFU (Colony Forming Units) of $mCherry^+$ Mtb. Animals transferred with ICM-BMDM showed reduced pulmonary inflammation, although they exhibited a higher frequency of IL-1β-producing macrophages, compared to mice transferred with NICM-BMDM or animals left with no cell transfer. Both ICM-BMDM and NICM-BMDM cell transfer groups resulted in reduced bacterial load in the lungs and in a lower frequency of $mCherry^+$ Mtb macrophages in the ICM-BMDM group. Our results show that the crosstalk between Mtb-infected AEC-II and macrophages activates macrophages that produce IL-1b and control Mtb infection, inducing a balanced pulmonary inflammation. 2017/21629-5; 2019/24681-3. **Keywords:** Tuberculosis;Macrophage;Alveolar epithelial cells.

DO - 163 - **Salmonella enterica ΔihfABpmi: impact on melanoma regression and macrophage polarization**

SILVA, M.F.E.[1]; JORGE, G.P.[1]; GONTIJO, M.T.P.[2]; GOES, I.C.R.D.S.[1]; COSER, L.D.O.[1]; BROCCHI, M.[1]. 1. UNICAMP, UNICAMP CAMPINAS - SP - BRASIL; 2. DUKE UNIVERSITY MEDICAL CENTER, DUKE UNIVERSITY MEDICAL CENTER DURHAM - ESTADOS UNIDOS DA AMERICA.

Salmonella enterica Typhimurium is known as foodborne pathogen that can causes infectious diseases such as diarrhea and systemic infections*. S. enterica* has been used in development for a new treatment in various diseases like cancer. Cancer is a malignant disease and a search for efficient treatment has been made. *S. enterica* Typhimurium mutants, with attenuation, have been explored in cancer immunotherapy and has shown antitumor activity and is mainly attributed to the activation of the immune system. The administration of *S. enterica* in the tumor has been characterized with a macrophage infiltration. In the study we used two mutants of *S. enterica* Typhimurium and evaluated their therapeutics efficacy, the induction of macrophage and the phenotypic response in C57BL/6 mice with melanoma. To confirm the attenuation, we used *Galleria mellonella* model. Following, the animals were randomly divided in groups, and they're flank in the right side. We inoculated 10^5 CFU of *ΔihfABpm*i weekly, for two weeks and the control group injected with PBS weekly for two weeks. For phenotypic macrophage analysis we used flow cytometry, a suspension of isolated cells from the tumor tissue was prepared, the suspension was incubated with fluorochrome-labeled antibodies CD80-Pe, CD206-APC, CD11b-Percp, F4/80-Fitc and analyzed using the cytometer of NovoCyte. The mutant *ΔihfABpmi* showed attenuation model of the virulence and antitumor potencial. With the treatment with *ΔihfABpmi* reduced the proportion of M2-type macrophages and increased the proportion of M1-type macrophages compared to PBS group, the induction of M1-type macrophages showing the effectiveness of the mutant in eliminating the tumor. **Keywords:** Salmonella enterica;macrophage polarization;tumor.

DO - 164 - **EVALUATION OF THE ANTITUMORAL EFFECTS OF NICOTINAMIDE IN HUMAN INTESTINAL CANCER CELLS**

MOREIRA, L.F.S.[1]; MENEZES-SILVA, L.[1]; DA SILVA, E.M.[2]; PEREIRA, M.T.[1]; GOMES, J.M.M.[1]; HIYANE, M.I.[1]; .CAMARA, N.O.[1]. 1. UNIVERSIDADE DE SÃO PAULO, UNIVERSIDADE DE SÃO PAULO SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, UNIVERSIDADE FEDERAL DE SÃO PAULO SÃO PAULO - SP - BRASIL.

Introduction: A common characteristic of solid tumors is hypoxia, which occurs due to rapid cell replication with an oxygen consumption greater than the supply. During hypoxia, there is a metabolic reprogramming mediated by hypoxia-inducible factor 1α (HIF-1α) to adapt to low oxygen levels. This favors aerobic glycolysis, which can accelerate tumor proliferation, metastasis, and increased resistance to chemotherapy treatments. Nicotinamide (NAM) is a precursor of nicotinamide adenine dinucleotide (NAD), an essential coenzyme for mitochondrial metabolism. It has been described that NAM can induce the proliferator-activated receptor gamma co-activator 1α (PGC -1α), the main regulator of mitochondrial biogenesis. Thus, NAM has the potential to increase mitochondrial mass, driving oxidative metabolism. We believe that NAM treatment could perform metabolic reprogramming in HT-29 tumor cells through increasing mitochondrial mass caused by PGC-1α. **Methods**: For in silico analyses, we used the GEO NCBI database (GSE7319), to evaluate the effect of nicotinamide on several genes of the human HEK293 cells. We also intend to submit human intestinal cancer cells (HT-29) to different concentrations of NAM for 24, 48, and 72 h, associated with hypoxia conditions in vitro. We will assess cell viability, mitochondrial mass, mitochondrial membrane potential, and superoxide production, as well as the expression of genes related to cellular metabolic function. **Results and conclusion**: In silico data showed that nicotinamide reduced the expression of the genes linked to HIF-1α signaling and glucose metabolism, such as *ALDOB* and *GYG2*. A reduction in tumor-associated genes such as *ESR1* and *FGF7* was also observed, as well as an increase in the expression of the *CASC2* gene, involved in the antitumor immune response and in apoptotic processes. Thus, this will interfere with tumoral cell viability by increasing mitochondrial metabolism, ROS production, and tumor cell death. **Keywords:** mitochondria;metabolism;tumor.

DO - 165 - **Analysis of the light chain antibody repertoire of horses immunized with Loxosceles sp. venom**

DA SILVA, G.M.[1]; DE REYES, C.T.N.[1]; FERREIRA, M.C.E.[1]; BRAGA, M.N.D.M.[1]; SAMPAIO, Y.[1]; MANSO, T.C.[1]; MINOZZO, J.C.[2]; ANTUNES, B.C.[2]; FELICORI, L.F.[1]. 1. UFMG, UFMG BELO HORIZONTE - MG - BRASIL; 2. CPPI, CPPI CURITIBA - PR - BRASIL.

In Brazil in 2022, over 270,000 accidents involving venomous animals were reported, with 7% of them involving some species of spider. Loxosceles spiders, commonly known as brown spiders, cause most of these accidents, and their clinical manifestations are mainly characterized by dermonecrosis. The only available and WHO-recommended specific treatment is the administration of antivenom, which is produced by inoculating venom into horses. This therapy's success depends on the timing of administration after the accident, and its production involves the use of animals, which is an expensive process. This method has been used for over 100 years, but very little is known about the antibodies generated in horses at the molecular level. Thus, the objective of our work is to characterize the kappa (Igκ), and lambda light chains (Igλ), which are utilized in approximately 5% and 95% of equine antibodies, respectively. However, the complexity of this region has hindered its complete characterization until now, besides the sequencing of the horse immunoglobulin locus conducted in 2010 (EquCab2) and 2018 (EquCab3). Thus, our group sequenced the light chain antibodies from four previously immunized equines using a pool of Loxosceles sp. spider venom. By employing IgBLAST with both available annotations, the light chain gene segments were accurately annotated, leading to 80 and 65% of coverage of Igκ and Igλ. We also compared the mutation frequency after immunization, which corresponded to a rate of up to 9% and 8% in the V gene segment for Igκ and Igλ, respectively. The serological repertoire (Ig-Seq) was analyzed, and specific peptides were compared to the transcribed repertoire (RepSeq), in which most of the IGKV4-1 and IGLV8S1 family segments were found in both approaches. The molecular-level knowledge of immunoglobulins in the postimmunization repertoire provides insights to produce recombinant antibodies, offering potential improvements to the currently available treatment. **Keywords:** Loxosceles;Equine Antibody;Antibody repertoire.

DO - 166 - **IL-33 decreases IL-1β levels independent of IL-10 in cutaneous leishmaniasis due to Leishmania braziliensis infection**

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Cutaneous leishmaniasis (CL), due to *L. braziliensis* infection, is characterized by the presence of few or rare parasites, a predominance of lymphocytes and mononuclear phagocytes in the inflammatory infiltrate. Host immunological factors are known to play an important role in the pathogenesis of this disease. Studies on lesion tissue samples have confirmed the contribution of inflammation to ulcer development, as evidenced by the presence of Granzyme B produced by CD8+ and NK cells, in addition to metalloproteinases and inflammatory cytokines. Furthermore, an important role of IL-1β in CL pathogenesis has been documented in recent years. In humans, IL-1β concentrations were found positively correlate with lesion size, and previous research by our group has documented elevated expression of the NLRP3 inflammasome in monocytes obtained from the peripheral blood of CL patients, in addition to high levels of IL-1β in cultured peripheral blood mononuclear cells (PBMC) stimulated with SLA. IL-33 is a cytokine with regulatory properties, known to be involved in a Th1 to Th2 response shift, as it downregulates IFN-γ production and up-regulates IL-5 and IL-13. Mice deficient in ST2 (IL-33 receptor) have demonstrated better ability to control parasite growth in visceral leishmaniasis murine model. Here we evaluated whether IL-33 would be able to reduce inflammatory response in human CL. Our results show that CL individuals do not produce IL-33 and that the exogenous addition of recombinant IL-33 (rIL-33) contributed to the reduction of IL-1β production regardless of IL-10 production. In addition, the presence of IL-33 contributed to the destruction of the parasite. The ability of IL-33 to regulate IL-1β production opens perspectives for the potential use of this cytokine as an adjuvant immunotherapy to control inflammatory response in severe CL. **Keywords:** Leishmania braziliensis;IL-33;Cutaneous Leishmaniasis.

DO - 167 - **Behavioral and Neuroinflammatory Alterations in Mice with Graft-versus-Host Disease**

LINHARES, A.F.S.; BERG, B.B.; DA SILVA, M.C.M.; RUGGIO, G.M.F.; CAMPOS, Z.D.T.; DE OLIVEIRA, A.C.P.; ROMERO, M.G.M.E.C.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL.

Graft-versus-host disease (GVHD) is a complication that arises after hematopoietic stem cell transplantation. It occurs due to genetic disparities between the donor and recipient, leading to systemic inflammation and affecting multiple organs, including the central nervous system (CNS). However, several gaps in knowledge about GVHD in CNS remain. To address these gaps, this study aimed to investigate CNS alterations caused by GVHD. Male, isogenic C57BL/6j and BALB/c mice were used, with C57BL/6j undergoing total bone marrow ablation followed by allogeneic transplantation (GVHD group), while the control group received syngeneic transplantation. Behavioral tests were conducted followed by euthanasia for inflammation analysis. The results in the open field test and zero maze test indicating anxious-like behavior. However, no depressive-like behavior was observed in the forced swimming test. There were no differences between the two groups in the Maze in Y or Object Recognition tests, suggesting no impairment in short- or long-term memory. Regarding inflammation analysis, IBA1+ cells, CD4+ and CD8+ lymphocytes, co-localized with H2d (donor's MHC marker), were increased in various brain regions of sick group. Intravital microscopy of the cerebral microvasculature revealed an elevated adhesion of inflammatory cells to the brain venules in the mice submitted to GVHD. Moreover, ELISA measurements showed elevated levels of the chemokines CCL2, CCL3, and CCL5 in GVHD animals and Cytokines as TNF-α and IL-10. In conclusion, GVHD induced anxiety-like behavior and neuroinflammation in the CNS. The findings suggest the involvement of IBA1+ cells, donor lymphocytes and various cytokines and chemokines in the inflammatory response within the CNS. Bibliographic references: 1. **J Pediatr Hematol Oncol**., 43, 8:e1088-e1092, 2021. 2. **Transplant Cell Ther.**, 27, 1:6-20, 2021. 3. **World J Clin Cases.**, 9, 6:1359-1366, 2021. 4. **Frontiers in Immunology**,10, 93:1-12, 2019. **Keywords:** graft versus host disease;neuroinflammation;hematopoietic stem cell transplant.

DO - 168 - **STUDY OF T CELL-MEDIATED IMMUNITY IN EXPERIMENTAL COVID-19: INVOLVEMENT OF PURINERGIC SIGNALING**

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COVID-19 is a severe respiratory disease with the potential for new variants. Current vaccines primarily induce a short-lived humoral response, which may not be effective against reinfections. This study aimed to optimize the generation and maintenance of SARS-CoV-2-specific resident memory T cells (TRMs) in the lung. C57BL/6 mice were immunized with inactivated SARS-CoV-2 (~ 60 ng/ml) intravenously and intranasally (IV+IN) or only intranasally (IN). The IV+IN immunization consisted of one IV dose on day 0 and three IN doses on days 5, 8 and 11. The IN immunization consisted of three IN doses on the same days. The animals were sacrificed on day 16 (acute phase) or day 46 (memory phase) and the lungs and mediastinal lymph nodes were analyzed. We inoculated anti-CD45 fluorescent antibodies intravenously to discriminate cells located in the parenchyma and vasculature, as well as anti-ARTC2 nanobodies as a homeostatic regulator. We evaluated the response of activated $CD4^+$ and $CD8^+$ T cells in the lung and draining lymph node by flow cytometry, as well as the generation of TRM cells in the lung parenchyma. We observed that IV+IN immunization induced a robust response of TRM cells in the lung, which expressed high levels of P2RX7, which in known to be involved in the generation and maintenance of TRM cells. In addition, virus-specific IgG antibodies were detected in the lung cell supernatants. Thus, this immunization strategy is being applied in B cell-deficient mice that express the hACE2 cellular receptor for SARS-CoV-2 infection (BKO-hACE2), which were developed during this study. This approach will allow us to investigate the ability of lung TRMs to protect against SARS-CoV-2 without interference from the humoral response, showing the potential for a robust response mediated by immunization and development of tissue-specific memory. This project is supported by FAPESP (2015/20432-8; 2022/07800-1), CAPES (88887.663361/2022-00) and CNPq (303810/2018-1). **Keywords:** COVID-19;PROTECTION;P2RX7.

DO - 169 - **IMMUNOLOGICAL PROFILE OF PATIENTS VICTIMS OF ENVENOMING BY SNAKES OF THE GENUS BOTHROPS**

SILVA, M.D.S.[1]; SOARES, M.C.F.[2]; DA SILVA, C.P.[1]; SERRATH, S.N.[1]; LOPES, J.A.[1]; SETÚBAL, S.D.S.[1]; PALOSCHI, M.V.[1]; ZULIANI, J.P.[1]. 1. FUNDAÇÃO OSWALDO CRUZ, FIOCRUZ RONDÔNIA, FUNDAÇÃO OSWALDO CRUZ, FIOCRUZ RONDÔNIA PORTO VELHO - RO - BRASIL; 2. CENTRO DE MEDICINA TROPICAL DE RONDÔNIA CEMETRON, CENTRO DE MEDICINA TROPICAL DE RONDÔNIA CEMETRON PORTO VELHO - RO - BRASIL.

Snakebites are considered a serious public health problem on tropical countries and classified as a neglected tropical disease, due to the severity and incidence of envenoming. Bites by venomous snakes cause systemic alterations and local effects in the skeletal muscle with an intense hemorrhage, edema, necrosis, and inflammatory infiltrate due to venom actions on tissues and cells. The aim of this study was to evaluate the immunological profile of patients who are victims of envenoming caused by snakes of the genus *Bothrops*, treated at the Centro de Medicina Tropical de Rondônia (CEMETRON) in Porto Velho – Rondônia. Methodology: Based on clinical parameters patients were classified into mild, moderate, and severe cases. Blood samples from 12 patients (Ethical statement: CEPEM-RO/CAAE: 60691822.2.0000.0011) were collected immediately after the patient's arrival at the health unit and 24h after treatment with antivenom. Hemograms (lymphocytes, monocytes, and neutrophils), neutrophil-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), deoxyribonucleic acid (DNA), and leukotriene B4 (LTB_4) assays were conducted. Preliminary Results: Patients classified as severe cases tended to increase the number of neutrophils, monocytes, and lymphocytes, as well as NLR when compared to mild and moderate patients. There was an increase in LDH levels in severe cases 24h after administration of antivenom. The amount of DNA present in the plasma of severe cases increased considerably compared to other cases. LTB_4 release increased in the plasma of severe cases 24h after antivenom. Conclusions: Data obtained demonstrated that victims frequently suffer from a strong initial inflammatory response in severe cases. Along with the LTB_4 release, the large increase in neutrophils relative to the number of monocytes and lymphocytes may be responsible for the rise in the LDH and DNA release. The preliminary data is significant and supports the mouse model of this genus of snake venom envenoming. **Keywords:** Bothrops;mediators;snakebites.

DO - 170 - **Regulation of exhausted CD8+ T cell differentiation by IKZF transcription factors**

READING, S.[1]; MUNOZ, I.[1]; DE MENEZES, M.N.[1]; SAW, N.[1]; AMBANI, K.[1]; AHN, A.[1]; NUSSING, S.[1]; ROTH, S.[1]; SAMPURNO, S.[1]; RAMSBOTTOM, K.M.[1]; TRAPANI, J.A.[1]; GOOD-JACOBSON, K.[2]; JOHNSTONE, R.W.[1]; GOEL, S.[1]; BEAVIS, P.A.[1]; PARISH, I.A.[1]. 1. PETER MACCALLUM CANCER CENTRE, PETER MACCALLUM CANCER CENTRE MELBOURNE - AUSTRALIA; 2. MONASH UNIVERSITY, MONASH UNIVERSITY MELBOURNE - AUSTRALIA.

$CD8^+$ T cells are indispensable for pathogen and tumour clearance, although can induce immunopathology if left unrestricted. T cell exhaustion, progressively programmed in the context of persistent antigen exposure, ensures partial control while mitigating immunopathological risk. However, exhaustion is characterised by curtailed proliferative capacity, cytokine production and cytotoxic functions, which tumours and chronic pathogens exploit to persist. As such, transiently disrupting exhaustion has emerged as a therapeutic strategy for treating cancer. Exhausted cells transition through a range of differentiation states, from stem-like progenitors that mediate response to checkpoint blockade, through to terminal effector or exhausted cells. Understanding the molecular pathways moderating the exhausted states is essential for augmenting cancer immunotherapy. We identify IKZF transcription factors, Ikaros (IKZF1), Helios (IKZF2) and Aiolos (IKZF3), as key mediators of T cell exhaustion, using gene knock out mice and a CRISPR-mediated genetic knock out system in both chronic infection and tumour models. IKZF1 and IKZF3 are critical regulators of exhausted subset expansion, retention, phenotype and cytokine function. Strikingly, dual IKZF1 and IKZF3 ablation improves the anti-viral and anti-tumour response of exhausted T cells, potentially via the formation of an atypical progenitor population and the enrichment of functional exhausted subsets. Cumulatively, our data highlight a novel role for IKZF transcription factors in biasing exhausted T cell differentiation and provides potentially important clinical implications, directing future cancer therapies targeting T cell exhaustion. **Keywords:** T cell exhaustion;Immunotherapy;CAR T cells.

DO - 171 - **Immunosenescence transcriptional signature in American Tegumentary Leishmaniasis**

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Introduction: American Tegumentary Leishmaniasis (ATL) is a group of diseases that encompasses a wide range of disease manifestations. In one end, we have an anergic response, with features of cellular exhaustion, in patients that develop the diffuse cutaneous leishmaniasis form (DCL). The middle of this spectrum is characterised by a highly inflammatory and cytotoxic setting in localized cutaneous leishmaniasis lesions (LCL). These features can further increase, and patients develop mucocutaneous leishmaniasis (MCL) with detrimental outcomes. We have previously shown that LCL lesions present a strong immunosenescence signature associated with highly differentiated CD8+ T cells, and now aim to extend these findings. **Methods and Results:** In this study, RNA sequencing data from previous studies of patients with LCL, MCL and DCL, and healthy controls, were analysed. We focused on the expression of genes related to senescence features that may contribute to disease outcomes. In addition to being increased in LCL, most senescence markers were further increased in MCL (CD57, p21, p16, p38, p53, Sestrin 2, ATF5) and lower or unchanged in DCL patients (except for ATF5 and CD57). In addition, MCL was associated with an increased signature of CD8 T cells, consistent with previous findings. **Conclusion:** In this study, we compare three different manifestations of ATL and highlight their differences when comparing senescence features. Higher senescence features were found in the MCL group, which is consistent with a higher inflammatory and cytotoxic environment. Of importance, MCL lesion samples were acquired before progression to MCL, indicating that early establishment of immunosenescence processes might contribute to impair disease resolution. **Financial Support:** This study was financially supported by Fundação de Amparo a Pesquisa do Espírito Santo – FAPES/Newton Fund and Medical Research Council (Grant 72939273/16); Coordination for the Improvement of Higher Education – CAPES. **Keywords:** Immunosenescence;Leishmaniasis;Immunopathogenesis.

DO - 172 - **Serum TNF levels and impact of Benznidazole therapy in patients with severe chronic Chagas' heart disease**

DA SILVA, T.D.E.S.P.[1]; ALVARADO-ARNEZ, L.E.[1]; ALVES, S.M.M.[2]; BATISTA, A.M.[3]; MELO, G.[2]; CARRAZZONE, C.V.[2]; PACHECO, A.G.[1]; MORAES, M.O.[1]; OLIVEIRA JR, W.[2]; LANNES-VIEIRA, J.[1]. 1. INSTITUTO OSWALDO CRUZ, INSTITUTO OSWALDO CRUZ RJ - RJ - BRASIL; 2. PROCAPE, PROCAPE RECIFE - PE - BRASIL; 3. UNIVERSIDADE ESTÁCIO DE SÁ, UNIVERSIDADE ESTÁCIO DE SÁ RJ - RJ - BRASIL.

Chagas disease (CD) is a neglected tropical disease caused by infection by the protozoan parasite *Trypanosoma cruzi*. CD has two distinct phases: (i) the generally asymptomatic acute phase, and (ii) a chronic phase with different clinical outcomes. Decades after infection, one third of the patients develop chronic chagasic cardiomyopathy (CCC), an inflammatory and fibrotic process that may progress to heart failure (HF) with reduced left ventricular ejection fraction. Furthermore, an increase in circulating TNF levels is observed in patients with CD, and the presence of cells producing this cytokine seems to be associated with HF in patients with the cardiac form of CD. Benznidazole is the drug of first choice for the treatment of CD in the acute phase, but its indication in the chronic phase is controversial. Cardioprotective medicaments are also recommended for the treatment of CCC, according to the observed clinical abnormalities. Here, we evaluated the association of TNF levels and its relationship with the cardiac form of CD, as well as the possible immunomodulatory role of antiparasitic (Bz) and cardioprotective treatments. For this, we carried out a case-control study involving 402 patients with positive serology for CD, residing in Pernambuco/Brazil. Patients were classified as non-cardiopathic (stage A; 109) and severe form of Chagas' heart disease (stage C; 132) and serum TNF levels were quantified using ELISA DuoSet. Serum TNF levels are increased in patients C, compared to patients A. Then, we questioned the impact of therapies (Bz and cardioprotective drugs) in group C on systemic inflammatory profile, assessing TNF levels. Serum TNF levels were reduced in the group of C patients treated with Bz years before treatment with cardioprotective, drugs compared with patients C that received only cardioprotective medicaments. Thus, we aim to contribute to the proposal of rational treatment schemes for chronic CD. **Keywords:** Chagas disease;Cardiomyopathy;Benznidazole.

DO - 173 - **PHENOTYPIC PROFILE OF UNCONVENTIONAL T CELLS IN PATIENTS WITH COVID-19**

CARVALHO, N.O.[1]; DE OLIVEIRA, G.[1]; DOS SANTOS, T.M.A.F.[1]; MIRANDA, V.H.S.[1]; ALVES, C.C.[1]; PEREIRA, A.A.S.[1]; PRADO, R.O.[1]; CARVALHO, K.A.B.[1]; CAMPI-AZEVEDO, A.C.[1]; ANTONELLI, L.R.D.V.[1]; PASCOAL-XAVIER, M.A.[1]; COELHO-DOS-REIS, J.G.[2]; MARTINS-FILHO, O.A.[1]; PERUHYPE-MAGALHÃES, V.[1]; DE CARVALHO, A.T.[1]. 1. INSTITUTO RENÉ RACHOU, INSTITUTO RENÉ RACHOU BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL.

T cells play a critical role in antiviral immunity. In COVID-19, disease severity is associated with reduction of circulating cells and phenotypic-functional alterations. Unconventional T cells (UTCs), comprise three cellular subsets: T cells γδ, iNKT, and MAIT cells, that recognize non-peptidic antigens in an unrestricted way to the MHC. These cells present potent cytotoxic function have biological features between innate and adaptive immunity. In this context, the role of these cells in COVID-19 will help in understanding mechanisms involved in SARS-CoV-2 infection. The phenotypic profile (activation, regulation, exhaustion, and memory) of unconventional $CD161^{+}$T cells was evaluated by flow cytometry technique using peripheral blood samples from 86 patients with COVID-19, confirmed by RT-PCR. Twelve healthy individuals without previous infection or COVID-19 vaccination were included as a control group. The peripheral blood samples were obtained at three timepoints: at study admission (D0), D7, and D14-28 days after study admission. At admission, our data demonstrated that the number of total lymphocytes and $CD161^{+}$ T-cells were reduced in COVID-19 patients. Furthermore, an activation phenotype profile with increased CD69 expression followed by increased expression of exhaustion markers such as PD-1, LAG-3, and TIM-3 were observed. In addition, higher levels of $CD8^{+}$ and CD4/CD8 DN $CD161^{+}$T cells subsets expressing activation (CD69 and CD38), cytotoxicity (CD107A), exhaustion (PD-1, LAG-3, and TIM-3), and memory ($CD45RO^{+}CD27^{-}$) markers was observed in D0 in COVID-19 patients than the control group. To understand and obtain more detailed and specific information about the phenotypic profile of UTCs, we will perform a functional analysis after *in vitro* antigen-specific stimulation with SARS-CoV-2. Considering the obtained results, we hope to provide better knowledge in the field of unconventional T cells during the pathogenesis of COVID-19. **Keywords:** COVID-19;unconventional T cells;immune response.

DO - 174 - **Gasdermin-D restricts Leishmania infantum replication in macrophages and in a mice model of visceral leishmaniasis**

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Visceral Leishmaniasis (VL) is a significant public health issue, causing substantial morbidity and mortality worldwide. Innate immune receptors detect Leishmania parasites, initiating intracellular signaling that activates crucial molecules involved in the inflammatory response. While the activation of the inflammasome has been recognized as important for controlling parasite replication in cutaneous leishmaniasis, its role in visceral leishmaniasis remains unclear. One key protein associated with pyroptosis following inflammasome activation is Gasdermin D (GSDMD). GSDMD forms pores in the cell membrane, leading to cell lysis and the release of cytokines from the IL-1R family into the extracellular space. In this study, we investigated the involvement of GSDMD during *Leishmania infantum* infection both *in vitro* and *in vivo*. Our findings demonstrate that GSDMD is cleaved into a 25 kDa fragment and secreted during the early stages of *L. infantum* infection *in vitro*. Moreover, macrophages and mice lacking GSDMD ($GSDMD^{-/-}$) showed increased susceptibility to infection, underscoring the role of GSDMD in host resistance against visceral leishmaniasis. Importantly, the observed GSDMD-mediated resistance does not rely on the pro-inflammatory cytokine IL-1b, suggesting the involvement of alternative mechanisms that require further investigation. Taken together, our results indicate that GSDMD could be involved in host resistance to *L. infantum* infection, contributing to unrevealing the disease pathogenesis. **Keywords:** visceral leishmaniasis;Gasdermina-D;Leishmania infantum.

DO - 175 - **Anti-VLA-4 antibodies for multiple sclerosis treatment: rational design and study of their mechanisms of action by high-content cell imaging**

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Multiple Sclerosis (MS) is a chronic inflammatory disease that affects the Central Nervous System (CNS). T lymphocytes are primary players in the MS pathogenesis, as these cells excessively infiltrate the CNS and react to self-antigens. Very Late Antigen-4 (VLA-4) has been assessed as a key protein involved in T cell trafficking towards the inflamed brain, making it a potential therapeutic target for MS. Natalizumab, an anti-VLA-4 antibody, has been largely used for MS immunotherapy. However, 30% of patients do not respond to natalizumab treatment and severe side effects are associated to it. Herein, high-content cell imaging (HCI) was applied to evaluate the effects of natalizumab on T cell functions and reveal biomarkers to predict natalizumab treatment efficacy. Next, epitope-specific anti-VLA-4 antibodies were developed to assess the effect of distinct VLA-4-targeting strategies on T cell biology. Our data show that natalizumab inhibits cytoskeleton remodeling towards cell polarization, reduces cell motility and decreases formation of conjugates with antigen-presenting cells. $CD8^+$ T cells from MS patient were found to be the most susceptible cell population to the *in vitro* natalizumab exposure. Cell thickness, actin texture and symmetry were captured by HCI as potential markers to predict patient outcome to natalizumab treatment. Two of the designed antibodies, a Single-chain variable fragment (scFv) natalizumab-based Anti-Epitope B (SNAE) and an IgG4 Anti-MIDAS (AM) presented potential applications in MS therapy. SNAE demonstrated stable interface interaction and had a similar effect to natalizumab without affecting actin enrichment. Differently, AM reduced IFN-γ production without interfering with cell spreading and actin enrichment. Interestingly, the latter antibody might be considered a mild VLA-4 antagonist. Altogether, our study provides an original pipeline for the rational design and testing of improved VLA-4 targeting antibodies for MS treatment. **Keywords:** T lymphocytes;VLA-4;Multiple sclerosis.

DO - 176 - **Topical 11β-HSD1 inhibition ameliorates cutaneous wound healing in type 1 diabetic mice model**

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Impaired wound healing is a rife complication in diabetic patients and is related to a high rate of lower limb amputations worldwide. The sustained low-grade inflammation promotes endocrine alterations such as glucocorticoid axis disruption, overregulating cortisol (humans), and corticosterone (rodents) production in a systemic/local manner. In excess, glucocorticoids directly affect wound healing, and keratinocytes and fibroblasts have the whole enzymatic machinery to produce glucocorticoids. This local via is a potential target for pharmacological strategies to improve wound healing in diabetic patients. The 11β-HSD1 is an enzyme widely expressed in the skin that reactivates glucocorticoid locally. In this work, we aimed the topical inhibition of 11β-HSD1 with Metyrapone (MTP), a drug approved to treat orally Cushing Syndrome patients. To access our treatment efficiency, we used the full-thickness wound model in C57BL/6 type 1 diabetic mice induced by a single dose of alloxan. Then, we topically treat the animals with 100mg/kg of MTP immediately after wounding for 14 days. Metyrapone topical treatment improves wound healing in diabetic mice ($P < 0.01$), and reduced corticosterone levels in skin homogenates ($P < 0.05$) without altering glucocorticoid serum levels. On day 7 post wounding, the treated group also presents the downregulation of IL-1α, IL-1β, IL-6, CXCL1, TNF, and RORγT genes. On the other hand, MTP-treated mice upregulated FOXP3 gene expression. The MTP topical treatment promoted significative changes in the skin milieu, diminishing neutrophil infiltration ($P = 0,05$) and modifying immune cell trafficking to the draining lymph nodes where neutrophil ($P < 0,05$), macrophages ($P < 0,01$), $CD4^+$ lymphocytes ($P < 0,05$) and $CD8^+$ lymphocytes ($P < 0,001$) were drastically reduced. *In vitro*, MTP improved fibroblast migration with any changes in cell proliferation. Together our finds denote the potential MTP topical treatment in chronic diabetic wound management. **Keywords:** wound healing;glucocorticoids;chronic inflammation.

DO - 177 - **Immunosenescence profile and age acceleration are associated with severe COVID-19**

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Since the onset of COVID-19 cases, the elderly were more susceptible to the most severe clinical forms of the disease. The inflammatory explosion of COVID-19 has mediators related to the inflammging of aging. Studies have shown how certain infections and co-infections can help to intensify and accelerate the biological aging process, especially in endemic areas for multiple infections. We collected and isolated plasma and PBMCs from volunteers residing in endemic and non-endemic areas with 1 to 9 days of symptoms. We performed a Luminex Bio-Plex for 27 soluble mediators, immunophenotyping of T, B and NK cells and a DNA methylation analysis. The results a probable signature of mediators in volunteers with COVID-19 in relation to other flu syndromes (negative control), and the expression of these mediators follows the severity of the disease. In the elderly, we evaluated this same signature and observed an increase in the elderly in endemic areas. Furthermore, we identified an increase in a serum marker of aging (CXCL9) in adults hospitalized with COVID-19, suggesting that immunosenescence would be associated with more severe forms of COVID-19. We found concordant data on the cellular profile with increased markers of depletion (PD-1, TIGIT and ICOS) and senescence (CD57 and KLRG-1) in individuals with severe COVID-19. A concordant profile was identified in the population of B cells with an increase in the population of memory and antibody-producing cells proportional to the severity of the disease. Finally, we found an acceleration of biological age in the analysis of DNA methylation in 7 of the 8 aging clocks analyzed. We observed that volunteers from endemic areas showed a more intense difference in this acceleration. Thus, we conclude that the results obtained suggest that the acceleration of aging and the senescence/exhaustion profile of individuals are associated with the severity of COVID-19. **Keywords:** Aging;COVID-19;Inflammaging.

DO - 178 - **Involvement of different B and TFH cell subtypes as a biological marker associated the risk of multiple sclerosis progression.**

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Multiple sclerosis (MS) is an inflammatory and demyelinating autoimmune disease of the central nervous system (CNS) characterized by progressive damage to the myelin sheath in genetically susceptible individuals, affecting a socioeconomically productive age group. Most patients (>80%) progress with acute episodes of neurological disability followed by total or partial clinical remission, known as relapsing-remitting MS (RRMS), which, over time, will progress to the secondary progressive form (SPMS). Although there is no cure, the RRMS form can be controlled with the use of different drugs that target Th1 and Th17 cells. Unfortunately, there are no therapeutic options for the SPMS form and this could mean that other immune cell subtypes are involved. Recently, B cells have been linked to progression, and efficient activation of B cells depends on their ability to interact with a subset of $CD4^+$ T cells called follicular helper T cells (T_{FH}). In this sense, the aim of this study is to analyze the behavior of different B cell subsets and T_{FH} as a function of MS progression. Therefore, a phenotypic characterization of B and T_{FH} cells in peripheral blood mononuclear cell cultures from 36 patients with low or high risk of disease progression was conducted by flow cytometry after stimulation for 4h with PMA and ionomycin. Besides, plasma levels of CXCL13 were quantified by ELISA. Our results demonstrate that patients at high risk of progression show a preferential expansion of $T_{FH1}IL\text{-}21^+PD\text{-}1^+$ and $T_{FH1.17}IL\text{-}21^+PD\text{-}1^+$ cells, *Naive* $IL\text{-}10^-IL\text{-}17^{++}$ B cells and more functional plasmoblasts $IL\text{-}10^-IL\text{-}17^{++}$, in association with high plasma level of CXCL13. In contrast, we observed a high frequency of different B cell subtypes associated with immune regulation ($IgD^+HLA\text{-}DR^-$) among low-risk patients. Despite the need to increase the number of patients, our findings suggest that monitoring the different T_{FH} and B cell subsets can become an effective biomarker to predict the severity of MS. **Keywords:** Multiple Sclerosis;TFH cells;B cells.

DO - 179 - **Profile of cell populations and soluble immunological mediators in Bothrops atrox envenomations**

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Bothrops atrox envenomations are common in the Brazilian Amazon. The venom of *B. atrox* is highly inflammatory, which results in severe local complications, including the formation of blisters. Moreover, there is little information on the immune mechanisms associated with this condition. Thus, a longitudinal study was carried out to characterize the profile of the cell populations and soluble immunological mediators in the peripheral blood and blisters in *B. atrox* patients according to their clinical manifestations (mild [MILD] and severe [SEV]). A similar response in both *B. atrox* patient groups was observed, with an increase in inflammatory monocytes, NKT, and T and B cells, as well as CCL2, CCL5, CXCL9, CXCL10, IL-1 and IL-10, when compared with the group of healthy blood donors. After the administration of antivenom, the participation of patrolling monocytes and IL-10 in the MILD group was observed. In the SEV group, the participation of B cells was observed, with high levels of CCL2 and IL-6. In the blister exudate, a hyperinflammatory profile was observed. In conclusion, our results explore and demonstrate that cell populations and soluble mediators are important components of this inflammatory response; with a distinct profile between groups; which was differentiated according to the clinical evolution after *Bothrops atrox* snakebite. We revealed the involvement of cell populations and soluble mediators in the immune response to B. atrox envenomation at the local and peripheral level, which is related to the onset and extent of the inflammation/clinical manifestation. **Keywords:** Bothrops snakebite;Immune response;Blister.

DO - 180 - **The inflammasome in response to L. infantum infection in mouse models of infection**

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Leishmaniasis are tropical neglected diseases that affect more than 12 million people around the world. Leishmania spp cause different clinical manifestations, such as cutaneous leishmaniasis (CL), resulting in ulcerative skin lesions; mucosal lesions, mucocutaneous leishmaniasis (MCL); and visceral leishmaniasis (VL), affecting liver, spleen and bone marrow. The diseases are caused by obligated intracellular parasites and may be fatal when visceral leishmaniasis (VL) is established. VL cause more than 30.000 deaths per year, becoming VL as second cause of parasite-associated death. Thus, development of functional and standardized in vivo infections and the study of immune responses are very important to allow and facilitate vaccines development and the production of effective new drugs to better treat the disease. The role of NLRP3 inflammasome is already described during Leishmania infection. Previous works have demonstrated the importance of this platform during CL. It is already known that NLRP3 inflammasome activation play a crucial role in the control of L. amazonensis infection. Moreover, it is already known that NLRP3 inflammasome activation and IL-1β production are important to establishment of VL after L. donovani-transmitted by natural vector. These data could suggest NLRP3 inflammasome as an important mechanism also to the control during L. infantum infection. In this work, we demonstrated that the NLRP3 inflammasome is activated in liver of L. infantum infected mice and contributes for host resistance. Mice deficient for NLRP3 contained higher parasite loads when compared with C56Bl/6 control mice. Furthermore, we showed that L. infantum infected NLRP3 deficient mice had impaired granuloma formation and reduced macrophages in the liver. **Keywords:** Leishmaniasis;Inflamassome;Tropical neglected diseases.

DO - 181 - **Effects of hydrolyzed heterologous collagen on cutaneous wounds at 7 days after injury.**

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Skin lesions can become a health problem when they do not close in a timely manner, leading to exacerbated inflammation and consequently problems in scarring. Our research group has demonstrated that it is possible to reduce inflammation and improve wound healing by injecting tolerated proteins following oral ingestion. Here we investigated the effects of oral tolerance of hydrolyzed heterologous collagen peptides (HHCol) on cutaneous wound healing. Mice (8 weeks- N=6) of the oral tolerant (OT) group received by gavage HHCol at a concentration of 20mg/animal diluted in water, for 5 consecutive days. The saline group received water *ad libitum*. An excisional lesion was made on the back of the animals with a dermatological punch (7mm-diameter), 7 days after the end of oral tolerance induction. Saline was then applied and a micropore was placed for 5 days. Euthanasia was 7 days after injury, the skin was fixed in Carlson's formalin and stained in Hematoxylin & Eosin (HE). In the macroscopic analysis of the scar area there was no significant difference between the saline and TO groups. In the qualitative analysis it was observed that the TO group has a smaller granulation tissue, with advanced re-epithelialization having a well-formed epithelium tongue. Also demonstrating a more accelerated extracellular matrix (ECM) deposition and greater presence of nerve bundles than the saline control group. In the quantitative analysis, a reduction in the number of leukocytes and an increase in the number of fibroblasts and neo-vessels were observed in the TO group compared to saline. We conclude that HHCol ingestion improves skin wound repair, with better ECM deposition, increased fibroblast number and angiogenesis, 7 days after injury. This fact can be related to the systemic effects of oral and immunological tolerance, as collagen is a protein present in our body and especially in the skin. However, further studies are needed. **Keywords:** oral tolerance;collagen peptides;cutaneous wound healing.

DO - 182 - **Changes in inflammatory markers of endothelial and brain damage and their association with longer hospital stay and worse clinical outcome of hospitalized severe COVID-19 patients.**

TEIXEIRA, P.C.[1]; DORNELES, G.P.[1]; NEVES, C.L.A.M.[1]; SANTANA FILHO, P.C.[1]; RIBEIRO, R.L.[1]; ROTTA, L.N.[1]; THOMPSON, C.E.[1]; PERES, A.[1]; RODRIGUES JUNIOR, L.C.[1]; DA FONSECA, S.G.[2]; ROMÃO, P.R.T.[1]. 1. UFCSPA, UFCSPA PORTO ALEGRE - RS - BRASIL; 2. UFG, UFG GOIÂNIA - GO - BRASIL.

SARS-CoV-2 infection is characterized by a hyperinflammatory and dysregulated immune response, which can induce damage to multiple organs.Changes in inflammatory markers of endothelial and brain damage may contribute to the neurological disorders in patients with COVID-19 and be associated with a higher risk of in-hospital mortality and a lower likelihood of hospital discharge.**Objective:**To assess the systemic levels of inflammatory mediators of endothelial and brain damage in hospitalized patients with severe COVID-19,without neurological symptoms (COVID-19) and with neurological symptoms (NeuroCOVID-19),and compare them to patients who have respiratory symptoms but were negative for COVID-19(Controls).**Methods:**Patients were recruited at Hospital São Camilo(Esteio-RS) between June/December of 2020. Whole blood samples of unhealthy–non-COVID-control patients (n=5) and patients with COVID-19 (n=30) were collected after hospital admission (T1).Additionally, samples were collected from COVID-19 (n=15) and NeuroCOVID-19 (n=15)patients between 0 and 72 h before discharge or death (T2).**Results:**On admission (T1),reduced levels of BDNF were observed in the NeuroCOVID-19 group compared to the control; and elevated ICAM-1,CCL26 and VEGF levels in the COVID-19 and NeuroCOVID-19 groups in comparison to controls.NeuroCOVID-19 patients showed higher S100B levels compared to controls.Interestingly, S100B, ICAM-1, CCL26 and VEGF levels were elevated at T2 in the NeuroCOVID-19 group (last 72 hours before discharge/death). Higher levels of S100B, CCL26,and reduced levels of BDNF in NeuroCOVID-19 group at T2 compared to the COVID-19 group were also observed.NeuroCOVID-19 patients had a longer hospital stay when compared to the COVID-19 group. **Concl.:**The results indicate that changes in systemic levels of inflammatory mediators of endothelial and brain damage may contribute to the severity of COVID-19,which may be related to longer hospital stays and higher risk of in-hospital mortality. **Keywords:** COVID-19, SARS-CoV-2 ;;Inflammatory mediators;;Neurological symptoms.

DO - 183 - **Bordetella pertussis adenylate cyclase toxoid (CyaA) carrying fragments of Pneumococcal surface protein A (PspA) as a vaccine candidate against Streptococcus pneumoniae**

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Streptococcus pneumoniae (pneumococcus) is an important human pathogen that causes diseases such as pneumonia, sepsis and meningitis. Vaccines that target capsular polysaccharides are available on the market, but their efficacy is limited to the serotypes included in the formulations. As an alternative, protein antigens are studied for the development of serotype-independent vaccines. The Pneumococcal Surface Protein A (PspA) is an important candidate and has proven to be effective in animal models, but it presents some antigenic variability leading to its differentiation into 6 clades, grouped into 3 families. Antibody cross-reactivity among clades from the same family is reported, indicating that a broad-coverage formulation may be obtained by the combination of a few PspA molecules and the induction of potent humoral responses. In this work, we have tested a delivery system based on the adenylate cyclase toxoid (CyaA) from *Bordetella pertussis*: upon binding to CD11b receptors, CyaA drives genetically grafted antigens into antigen presenting cells, triggering specific humoral and cellular responses. CyaA was engineered to express fragments of PspAs from clades 2 and 4, which belong to the families most frequently observed in isolates. The resulting proteins – CyaA-A2 and CyaA-A4 – were tested through immunization experiments in mice. A mixture containing both proteins induced high levels of anti-PspA antibodies that were able to bind *in vitro* to pneumococci expressing PspAs from clades 2, 3, 4 and 5; the antibodies were also able to induce complement deposition onto the surface of these bacteria. When tested in invasive pneumococcal challenges, this formulation protected the animals against isolates expressing PspAs from clades 2, 4 and 5. Moreover, a single antigen containing both PspA fragments (CyaA-A2-A4) produced equivalent results. Our findings indicate the potential of the CyaA-PspA proteins as candidates for a broad-coverage vaccine against pneumococcus. **Keywords:** Pneumococcal vaccine;Pneumococcal surface protein A;Antigen presenting system.

DO - 184 - **Analysis of the immune profile of response to pediatric cancer in tissue, blood and metastatic site by multiparametric flow cytometry**

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Immunotherapy has revolutionized cancer treatment by enhancing the understanding of the immune response in pediatric cancer. This study aimed to describe the intratumoral and systemic immune response in pediatric cancer patients at diagnosis and during follow- up to support treatment decisions. A total of 500 samples from 349 patients (136 girls, 212 boys; median age 7 years) were analyzed using multiparametric flow cytometry (CFM) at 5 referral hospitals in Rio de Janeiro and 6 international centers. The Solid Tumor Orientation Tube (STOT) was employed for diagnostic guidance, enabling the identification of various immune cell populations in the sample. Through CFM analysis, B, T, NK lymphocytes, monocytes, neutrophils, and neoplastic cells were identified. The analysis revealed a significant decrease in B lymphocyte infiltrate in neoplastic samples compared to reactive cells (3% vs. 9%, p=0.017) and a significant increase in CD8+ T lymphocyte infiltrate associated with higher neoplastic cell percentages (p=0.02). Furthermore, abdominal and thoracic samples exhibited a significant predominance of lymphocytic infiltrate, whereas pelvic and soft tissue samples showed granulocytic infiltration (p=0.01 and p=0.002). In terms of pediatric cancer classification, neuroblastic tumors demonstrated a T lymphocyte immune response profile (61% vs. 25.7%, p=0.04), while soft tissue tumors exhibited a neutrophilic predominance (12% vs. 48.6%, p=0.007). Wilms tumors showed a significant increase in NK cells (10.5% vs. 2.4%, p=0.001), whereas germ cell tumors had a neutrophilic predominance (48% vs. 17%, p=0.014). In conclusion, flow cytometry analysis of the intratumoral immune infiltrate provides specific information on immune cell distribution based on tumor infiltration, affected site, and pediatric cancer type, contributing as an additional tool for stratification and therapeutic guidance. **Keywords:** Immune Landscape;Flow Cytometry;Pediatric Solid Tumours.

DO - 185 - **IMMUNOSENESCENCE PROFILE IN CIRCULATING T CELLS FROM PATIENTS WITH COVID-19**

SARMENTO, I.V.[1]; LOPES, P.D.O.[1]; DE MOURA, R.G.[1]; COVRE, L.P.[2]; DE MIRANDA, P.H.[1]; DE CASTRO, C.H.D.F.[1]; FARDIN, J.M.[1]; DE OLIVEIRA, A.L.[1]; MILL, J.G.[1]; FARIA, A.M.C.[3]; AKBAR, A.[2]; GOMES, D.C.D.O.[1]. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO VITÓRIA - ES - BRASIL; 2. UNIVERSITY COLLEGE LONDON, UNIVERSITY COLLEGE LONDON LONDON - REINO UNIDO; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL.

An exacerbated inflammatory environment, as observed in COVID-19, can lead to alterations in T cell differentiation and functionality, promoting the expansion of senescent cells characterized by the progressive loss of costimulatory receptors (CD27, CD28), DNA damage, activation of p38 MAPK, and diminished proliferative capacity. While the accumulation of senescent cells has been implicated in the immunopathogenesis of various diseases, its role in COVID-19 remains poorly understood. All procedures in this study were approved by the Ethics Committee and the Hospital Universitário Cassiano Morais (HUCAM/UFES) reference number # 32171120.0.0000.5071. In this study, we examined the phenotypic profile of T cells in patients with COVID-19 categorized as oligosymptomatic, moderate, and severe. We also assessed senescent phenotype characteristics within the circulating T cells (CD3, CD4, CD8). We also evaluated the memory profile, senescence markers (CD45RA, CD27, CD28, CD57, KLRG1), DNA damage (p-γH2Ax), and p38 MAPK expression using flow cytometry. Our findings revealed reduced cell viability in the moderate and severe groups, along with changes in the frequencies of CD4+ and CD8+ T cells, increased DNA damage, and elevated p38 MAPK expression. Furthermore, severe patients exhibited an accumulation of highly differentiated populations compared to the oligosymptomatic group, as evidenced by the CD28-CD27- and terminal memory cells (T EMRA) subsets. Thus, individuals with severe COVID-19 manifested a senescent T-cell phenotype, which may contribute to disease severity as an exacerbating factor. **Keywords:** COVID-19;Immunosenescence;T Cells.

DO - 186 - **CUTANEOUS LEISHMANIASIS IN THE EARLY PHASE OF DISEASE: HIGH INFLAMMATORY RESPONSE AND UNRESPONSIVINESS TO IL-10 AT LESION SITE**

NASCIMENTO, M.T.; LAGO, J.; LEAL, D.; ARRUDA, S.M.; GUIMARÃES, L.H.; DE CARVALHO FILHO, E.M.; CARVALHO, L.. FIOCRUZ-BA, FIOCRUZ-BA SALVADOR - BA - BRASIL.

Introduction: Pentavalent antimony is the main drug used to treat leishmaniasis in Brazil and high therapeutic failure rate is observed in *L. braziliensis* transmission areas. During CL strong inflammatory response with high levels of TNF and IL-1β is observed. Also, an important role for T CD8+ T and NK cells in the pathogenesis of CL has been documented. Before the ulcer develops, most CL individuals present a large regional lymphadenopathy, followed by an exoulcerative papule and are characterized as early cutaneous leishmaniasis patients (ECL). Recently, it has been demonstrated in the lesions of ECL an intense cytotoxic activity. Our aim was to investigate the presence of inflammatory markers and the possible regulatory mechanisms of the exacerbated inflammatory response observed at lesion site of ECL patients. **Methods and Results:** Skin biopsies from healthy subjects (n=8), ECL (n=11) and CL (n=10) were obtained and maintained in cultures for 72 hours. Levels of PDGF-BB, VEGF, FGF, GM-CSF, CCL2, CXCL9, CXCL10, IL-2, IL-15, granzyme B, perforin, TNF, IL-6, IL-1β and IL-10 were determined in supernatant cultures, by Luminex or ELISA. Additionally, skin biopsies from ECL and CL were stained with anti-IL-10 receptor (IL-10R) antibody by immunohistochemistry. We observed that cells from lesion from ECL patients produce PDGF-BB, VEGF, FGF, GM-CSF, granzyme B, perforin, TNF, IL-6, IL-1β in similar levels to those from CL, but they exhibit higher levels CCL2, CXCL9, CXCL10, IL-2, IL-15 and IL-10 than CL. Interestingly, we identified that in the lesion site of ECL there is a decrease number of positive cells for IL-10R when compared to CL. **Conclusion:** Our results suggest that the decrease regulatory mechanisms at the site of injury in ECL patients allows an exacerbated inflammatory response to be maintained, with elevated levels of CCL2, CXCL9, CXCL10, IL-2, IL-15 contributing to tissue damage, ulcer development and therapeutic failure in ECL patients. **Keywords:** Early Cutaneous Leishmaniasis;Inflammation;L; braziliensis.

DO - 187 - **The effects of Tenofovir, Lamivudine, and Dolutegravir antiretroviral therapy on serum levels of cytokines and chemokines in People Living with HIV**

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Introduction: The prolonged inflammatory status observed in human immunodeficiency virus (HIV) infection is associated with cytokines and chemokines. Antiretroviral treatment (ART) reduces viral load and restores CD4+ T lymphocyte levels, improving quality of life and the immune response to the virus. Thus, the objective of this study was to measure the serum levels of cytokines and chemokines in individuals diagnosed with HIV who were undergoing ART using the regimen tenofovir (TDF), lamivudine (3TC), and dolutegravir (DTG). Methods: Serum samples from 29 ART-naïve HIV patients (TDF/3TC/DTG) were collected. Two cohorts of patients formed four groups according to the time of post-treatment follow-up: Patients evaluated before treatment (aT2M) and patients treated after 2 months of treatment (pT2M); e Patient evaluated before treatment (aT4M) and patients treated after 4 months of treatment (pT4M). Cytometric bead array (CBA) used to measure soluble factor levels. Wilcoxon and Mann-Whitney tests used to calculate differences between groups. Results considered statistically significant when $p<0.05$. Results: When comparing the pT4M group to the pT2M group, we found that serum levels of IFN-γ ($p=0.02$), TNF ($p=0.04$), and IL-10 ($p=0.04$) were lower in the pT4M group. Only individuals in the aT2M versus pT2M groups showed a decrease in serum IL-4 levels ($p=0.04$). The levels of IL-2 and IL-6 did not differ amongst the groups studied. In the chemokine analysis, we found that those with pT4M had lower CXCL9 levels than people with pT2M ($p=0.009$). CXCL10, CXCL8, CCL5, and CCL2 levels did not differ amongst the groups studied. Conclusion: With prolonged usage, ART modifies cytokine and chemokine levels, resulting in a decrease in the inflammatory response (IFN-γ, TNF, and CXCL9), which contributes to a reduction in the IL-10 anti-inflammatory response as a compensatory impact. **Keywords:** cytokines;chemokines;HIV.

DO - 188 - **Analysis of P2X7 receptor and CD39 ectonucleotidase in the pathogenesis of acute respiratory distress syndrome associated with malaria**

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Acute respiratory distress syndrome (ARDS) is a clinical pathology that may be associated with malaria. In this injury condition, the extracellular adenosine triphosphate (eATP) molecules can be recognized by purinergic P2X7 receptor (P2RX7) expressed in immune cells to initiate an inflammatory response. Otherwise, eATP can be phosphohydrolyzed by CD39 and CD73 ectonucleotidases to generate adenosine, which modulate the immune response to an anti-inflammatory state. In this work, we investigated the role of P2RX7 and CD39 in the pathogenesis of malaria-associated ARDS. C57BL/6 (WT) mice were infected with *Plasmodium berghei* NK65 parasites (10^6 infected erythrocytes/animal) and treated with the P2RX7 inhibitor BBG (Brilliant Blue G) (45 mg/kg) on the 3rd, 5th, 7th and 9th days post-infection (dpi). At the 6th dpi, no significant difference was observed in clinical and respiratory parameters between the BBG-treated and untreated groups. Furthermore, similar numbers of activated $CD4^+$ and $CD8^+$ T cells, as well as comparable P2RX7 and CD39 expression, were observed at this time point. Remarkably, BBG-treatment increased the survival of infected mice (100.0%) comparing to untreated group (0.0%). Additionally, CD39 knockout mice (*Entpd1*$^{-/-}$) were infected to compare the pathology with WT mice. We found that 33.3% of the *Entpd1*$^{-/-}$ group exhibited malaria-associated ARDS, while 66.7% of the WT group showed manifestations of the disease, such as a high clinical score and respiratory changes (reduced frequency, increased respiratory pauses and decrease tidal volume). These results suggest that both P2RX7 and CD39 contribute to development of malaria-associated ARDS. Further studies are in progress to confirm these findings and elucidate the mechanism underneath the role of these molecules in disease pathology. This project is supported by FAPESP (2022/00858-4; 2015/20432-8), CAPES (88887.659095/2021-00) and CNPq (303810/2018-1). **Keywords:** Pulmonary malaria;CD39 ectonucleotidase;P2RX7.

DO - 189 - **Study of CD4 T lymphocyte response in patients with moderate and severe COVID-19**

PEREIRA, M.V.A.[1]; GALVANI, R.G.[1]; XAVIER, F.[1]; OLIVEIRA, K.D.S.[1]; DE VASCONCELOS, Z.F.M.[2]; BONOMO, A.C.[3]. 1. OSWALDO CRUZ FOUNDATION (FIOCRUZ), OSWALDO CRUZ FOUNDATION (FIOCRUZ) RIO DE JANEIRO - RJ - BRASIL; 2. FERNANDES FIGUEIRA INSTITUTE (FIOCRUZ), FERNANDES FIGUEIRA INSTITUTE (FIOCRUZ) RIO DE JANEIRO - RJ - BRASIL; 3. OSWALD CRUZ FOUNDATION (FIOCRUZ), OSWALD CRUZ FOUNDATION (FIOCRUZ) RIO DE JANEIRO - RJ - BRASIL.

COVID-19, has emerged as a global pandemic, presenting a significant threat to public health and requiring urgent research efforts to understand its pathogenesis. As the disease progresses quickly, understand the immune response in patients with good disease progression can help to improve specific treatment of the disease. Recent studies have highlighted the role of cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), in the pathogenesis of COVID-19, with elevated levels observed in severely infected patients, suggesting their potential as therapeutic targets for mitigating the cytokine storm associated with the disease. However, which cells produce those cytokines are still unknown. Although CD8 T cells are directly responsible for viral clearance, T CD4 cells are central players in orchestrating the immune response. They act as immune regulators, providing help to B cells for antibody production and facilitating the activation of cytotoxic T CD8 cells. Thus, in this work we investigated the role of CD4 T cells in COVID-19 in a cohort of 93 patients with moderate, severe, and severe COVID-19 who died. PBMCs, collected in 2020 (after infection and before vaccination) were stimulated with protein S and cytokines produced by CD4 T cells were measured by multiparametric cytometry. Patients from the moderate group showed higher production of TNF-α and IFN-y when compared to the severe group. The group of patients who died as a result of the disease showed a tendency towards a decrease in TNF-α and IFN-y. The data show a protective role of these cytokines and suggest that TCD4 play a significant role in the immune response to SARS-CoV-2 and the pathogenesis of COVID-19. Further research is needed to elucidate the complex interplay between TCD4 cytokines, viral infection, and disease outcomes. Targeting these cytokines could shed light in developing more effective therapeutic strategies for managing COVID-19. **Keywords:** COVID-19;T CD4 LYMPHOCYTES ;VIRAL INFECTION.

DO - 190 - **Involvement of Annexin A1 in modulating immune response, susceptibility, and tissue homeostasis during severe experimental malaria**

MELO, N.; RABELO, R.A.N.; PEREIRA, R.D.D.; SILVA, L.P.; TEIXEIRA, S.L.; NONATO, A.; LEITE, P.G.; OLIVEIRA, F.B.R.; SANTOS, L.L.O.; JÚNIOR, C.M.Q.; RUSSO, R.D.C.; SOARES, A.T.C.; GALVAO, I.; MACHADO, F.S.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL.

Plasmodium berghei ANKA (PbA) infection in mice is similar to human severe malaria in many aspects, such as cerebral malaria and respiratory distress. Annexin A1 (AnxA1) is a protein involved in the modulation of inflammation and plays an important role in the resolution of sterile and infectious diseases, but its function during *Plasmodium* sp infection is unclear. Herein, we investigated the AnxA1 in the development of pathogenesis in a murine model of severe malaria. BALB/c (WT) and AnxA1 knockout (KO) mice were infected with 10^5 PbA-parasitized red cells. Parasitemia and clinical score were evaluated daily, from 3rd day post infection (DPI). Histological, flow cytometry, hematological, vascular permeability, western blot and lung function analyses were performed on the 8^{th} DPI. PbA infection modulated AnxA1 expression in the brain, lung, and liver of infected WT mice. The absence of AnxA1 increased the susceptibility of PbA-infected mice, presenting early and worse clinical scores and decreasing survival rates. Moreover, infected AnxA1 KO mice showed a reduction in circulating leukocytes, platelets and eosinophils compared to WT counterparts. Deficiency of AnxA1 also resulted in lung in imbalanced cytokine production and expressive vascular permeability and injury. Notably, AnxA1 KO mice reduced the lung function presenting worse parameters related to compliance, resistance, and pressure volume. Additionally, infected KO mice show an imbalance in cytokines followed by worsened histopathological damage in the liver and brain compared to WT. Ultimately, KO mice failed in orchestrating the immune response in the spleen during infection, which may explain, along with the other results, the susceptibility of AnxA1 deficient mice. Collectively, the results suggest that ANXA1 may be an important molecular target for modulating the immune response during *Plasmodium* infection. Financial supported: CNPq, CAPES, and FAPEMIG. **Keywords:** Malaria;Plasmodium berghei ANKA;Imunorregulação.

DO - 191 - **Distinct antibody profiles discriminate disease severity of COVID-19**

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Introduction: Antibodies in hospitalized COVID-19 patients have been shown to display meaningful participation in the severity of clinical outcomes. We evaluated anti-nucleocapsid (NP) IgM, IgA and IgG, anti-receptor-binding domain (RBD), and anti-Spike IgA and IgG antibodies and the presence of neutralizing antibodies (nAb), in a cohort of unvaccinated COVID-19 patients in the first 30 days post-symptom onset (PSO) and a subset was followed for 6 months or more. **Methods:** Based on the disease severity criteria of COVID-19 infection, peripheral blood was collected from mild (n=37), moderate (n=43), severe (n=63), critical (n=14), fatal (n=36) groups, and 27 uninfected individuals. An indirect enzyme-linked immunosorbent assay was used for quantification of anti–NP IgM, IgA, IgG, anti-RBD, and anti-Spike IgA and IgG antibodies, and a cytopathic effect-based virus neutralization test was performed for nAb**.** Statistical significance was considered when $p<0.05$. **Results:** The median age was 58 years, and the most common comorbidities were hypertension (33.2%) and diabetes mellitus (23.8%), and 37.8% (n=73) required intensive care unit (ICU). Anti-NP IgG was higher in the moderate ($p=0.0002$) and severe ($p<0.0001$) groups than in the mild group. The seropositivity of nAb was 81% and there were no differences between severe, critical, and fatal groups. For individuals admitted to the ICU, who died presented lower levels of anti-NP IgG and IgA but higher levels of anti-spike and anti-RBD IgA levels compared to survivors. In summary, the levels of anti-SARS-CoV-2 antibodies in the first 30 PSO are associated with distinct COVID-19 outcomes, and this data may discriminate death from survival in individuals admitted to the ICU, suggesting that anti-Spike and anti-RBD IgA may play some deleterious effect, in contrast with the potentially protective effect of anti-NP IgG and IgA. **Keywords:** covid-19;immune response;antibodyes.

DO - 192 - **Recombinant influenza virus carrying surface protein A (PspA) of Streptococcus pneumoniae as a bivalent vaccine platform against flu and pneumococcal pneumonia**

CARDOSO, K.F.[1]; DE SOUZA, L.R.A.[1]; SANTOS, B.S.[1]; DE CARVALHO, K.R.A.[1]; KANO, F.S.[1]; MIYAJI, E.N.[2]; PASCOAL-XAVIER, M.A.[1]; ARAÚJO, M.S.S.[1]. 1. FIOCRUZ MINAS (IRR), FIOCRUZ MINAS (IRR) BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO BUTANTÃ, INSTITUTO BUTANTÃ SÃO PAULO - SP - BRASIL.

S. pneumoniae and influenza virus are respiratory tract pathogens that cause high mortality worldwide. The influenza virus causes flu, while pneumococcus causes pneumonia and meningitis, also secondary infections in flu patients, worsening their clinical condition. The licensed pneumococcal vaccines are serotype-specific, resulting in serotype replacement by non-vaccine serotypes. Therefore, aiming to develop a bivalent vaccine against both these pathogens, we constructed, by reverse genetics, a recombinant influenza virus carrying the pneumococcal PspA protein (Flu-PspA virus). Thus, this work aimed to evaluate the efficacy of a heterologous *prime-boost* protocol with the Flu-PspA virus and the rPspA protein, in the murine model via intramuscular route. For this, C57BL/6 mice were primed with Flu-PspA and boosted with rPspA+Alum (vaccine group). In the control groups, animals were primed with control virus (Flu-CT) and boosted with rPspA+Alum or Alum; or two doses of PBS. Later, fourteen days after the last immunization, anti-PspA and anti-influenza serum IgG antibodies were quantified by ELISA. Then, twenty-one days after the last dose, animals were lethally challenged with 7xLD50 of the invasive strain ATCC6303 or with 100xLD50 of influenza A/PR8/34 virus, and survival was monitored for ten days. In addition, three days after the lethal challenge, the bacterial load present in bronchoalveolar lavage (BALF) was quantified by titration, and inflammatory cytokines were quantified by CBA. Thus, we observed that the animals of the vaccine group showed high levels of anti-PspA and anti-influenza IgG antibodies in serum, 100% protection after lethal challenges with pneumococcus and influenza, two to three $\log_{10}$ reduction of bacterial load, and lower levels of inflammatory cytokines in BALF. Therefore, this heterologous *prime-boost* protocol showed high protection and immunogenicity, being a promising bivalent vaccine strategy against influenza and pneumococcal pneumonia. **Keywords:** Recombinant influenza virus;Bivalent Vaccine;Streptococcus pneumoniae.

DO - 193 - **: Podocyte-derived extracellular vesicles as biomarkers of kidney injury in systemic lupus erythematosus**

ALVES, L.S.D.S.[1]; FONSECA, S.[1]; LEMOS, A.P.[1]; CARVALHO, C.[2]; GAUDIO, R.[3]; LINO, K.[3]; MARTINS, J.[2]; CASTRO, M.J.C.[1]; MEDEIROS, T.[1]; SILVA, A.A.[1]. 1. GRADUATE PROGRAM IN PATHOLOGY, FACULTY OF MEDICINE,UFF, GRADUATE PROGRAM IN PATHOLOGY, FACULTY OF MEDICINE,UFF NITERÓI - RJ - BRASIL; 2. MULTIUSER LABORATORY FOR RESEARCH SUPPORT IN NEPHROLOGY AND MEDICAL SCIENCES, UFF, MULTIUSER LABORATORY FOR RESEARCH SUPPORT IN NEPHROLOGY AND MEDICAL SCIENCES, UFF NITERÓI - RJ - BRASIL; 3. RHEUMATOLOGY SERVICES, HOSPITAL UNIVERSITARIO ANTONIO PEDRO,UFF, RHEUMATOLOGY SERVICES, HOSPITAL UNIVERSITARIO ANTONIO PEDRO,UFF NITERÓI - RJ - BRASIL.

Introduction: Urinary extracellular vesicles (uEVs) are released by urinary tract cells and reflect early renal injury. About 40-75% of patients with systemic lupus erythematosus (SLE) develop kidney dysfunction (lupus nephritis) with mainly podocyte damage. Thus, in SLE, changes in uEVs may suggest the onset of endothelial injury, renal inflammation, and vascular dysfunction. **Aims:** To evaluate podocyte-derived uEVs as early biomarkers of kidney injury in SLE. **Methods:** Cross-sectional study performed with SLE patients recruited from the outpatient clinic of the Hospital Universitário Antônio Pedro (Niterói, Rio de Janeiro) from March to December 2022. The SLEDAI-2K index was used to measure disease activity and the R-SLEDAI index was used to identify renal dysfunction associated with active SLE. uEVs were evaluated by nanoscale flow cytometry, considering size ~100-900 nm, positivity for Annexin V, and podoplanin. A multiplex assay was used to quantify urinary levels of cytokines, chemokines and growth factors. **Results:** We studied 52 SLE patients (42.6±14.7 years, 94.2% female). When comparing groups according to disease activity, patients with active SLE (SLEDAI-2K ≥5) and renal dysfunction (R-SLEDAI ≥4) presented higher counts of podocyte uEVs (P=0.001 and P=0.0008, respectively). We also identified that patients with active SLE presented higher (P <0.05) urinary levels of interleukin (IL)-1β, -6, -8, -17 and chemokines CCL-2, CCL-5, CCL-11, and CXCL-10. Lastly, we observed that urinary levels of immune mediators (CCL-2, CCL-3, CCL-4, CCL-5, CCL-11, CXCL-10, IL-1β, IL-8, IL-9, IL-15, IL-17, and IFN-γ) were associated with total uEVs in all SLE groups. **Conclusions:** These findings suggest that podocyte-derived uEVs are associated with SLE activity and renal dysfunction possibly reflecting renal inflammation. **Keywords:** systemic lupus erythematosus;extracellular vesicles;inflammation;.

DO - 194 - **Prognostic value of myeloid-derived suppressor cells in Acute Myeloid Leukemia**

SANT'ANA, A.N.[1]; DIAS, C.K.[1]; NUNES, V.B.D.S.[1]; FARIAS, M.G.[2]; ALEGRETTI, A.P.[2]; PORTELA, P.[2]; CALVACHE, E.T.[2]; DAUDT, L.E.[2]; MICHALOWSKI, M.[2]; PAZ, A.A.[2]; FIGUEIRÓ, F.[1]. 1. UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL, UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL PORTO ALEGRE - RS - BRASIL; 2. HOSPITAL DE CLÍNICAS DE PORTO ALEGRE, HOSPITAL DE CLÍNICAS DE PORTO ALEGRE PORTO ALEGRE - RS - BRASIL.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population that impairs innate and adaptive immunity, facilitating tumor's immune escape and cancer progression. Despite the lack of standard markers to definitely immunophenotype MDSCs, especially for the polymorphonuclear (PMN-MDSC) subset, these cells appear to impact Acute Myeloid Leukemia (AML) prognosis. Moreover, the maturation stage of PMN-MDSCs is unknown in AML and a matter of discussion. The aim of this study was to assess the impact of enrichment of each neutrophil maturation stage, the frequency of PMN-MDSCs and monocytic MDSCs (M-MDSCs), as well as the MDSC/T lymphocyte ratio in relation to AML relevant clinical features and prognostic markers. For that, we performed a retrospective analysis of bone marrow standard flow cytometry immunophenotyping files acquired during the patients' diagnosis. We observed that the frequency of immature CD11b-CD16- neutrophils is decreased in patients with a good response to induction therapy, whereas low CD11b-CD16-/CD11b+CD16+ neutrophil ratio predicts shorter survival in AML patients. Also, the frequency of M-MDSCs and PMN-MDSCs, as well as their T lymphocyte ratio are increased in patients with adverse risk. The same was observed for M-MDSCs and M-MDSC/T lymphocyte ratio in FLT3-ITD carrier patients. Furthermore, based on clinical significance, we point CD36 as a potential marker of PMN-MDSCs in AML. Our study highlights significant findings regarding increase in MDSC subsets associated with poor prognostic factors. **Keywords:** MDSCs;Acute Myeloid Leukemia;Prognosis.

DO - 195 - **ADENOSINE FROM THERMOGENIC ADIPOCYTES AS A MODULATOR OF THE MACROPHAGE PHENOTYPE**

PEREIRA, N.; OLIVEIRA, F.; SALERNO, G.; ALVES, J.M.; DE MELO, P.; LEIRIA, L.O.S.. FMRP/USP, FMRP/USP RIBEIRÃO PRETO - SP - BRASIL.

Activation of brown adipocytes (BA) increases the concentration of intracellular cAMP. The intracellular accumulation of cAMP leads to its efflux through MRP4 channel; however, this phenomenon is still unclear for BA. cAMP/AMP is converted into adenosine by the CD73 enzyme. Adenosine is essential for BA thermogenic activity, and it has an anti-inflammatory role. Based on that, it is hypothesized that activation of BA accumulates cAMP and effluxes it through MRP4. Additionally, extracellular cAMP leads to adenosine production, which contributes to the pro-thermogenic function in brown adipose tissue (BAT) resident macrophages. AMP levels in the extracellular milieu increased when BA is stimulated with isoproterenol (β-adrenergic agonist) as well as MRP4 gene expression. Macrophages kept in *transwell* culture inserts with BA increase the gene expression *of Arg1, Ym1, Fizz1, Cd36* when BA is stimulated with isoproterenol. This effect was reversed when an MRP4 pharmacological inhibitor was added to BA. Macrophages that receive conditioned media from activated BA present high OXPHOS activity, as indicated in ScRNAseq analysis of macrophages from BAT activated by cold. Moreover, the expression of CD73 increased in macrophages that received conditioned media from activated BA, reinforcing the crosstalk between the efflux of metabolites from BA and macrophages. The absence of adenosine receptor A2a in macrophages impaired the phenotype induced by activated BA. The downregulation of *Arg1, CD36, and Nte5e* (CD73) gene expression in $A2a^{-/-}$ macrophages occurs even when they were treated with activated BA-conditioned media. These results suggest that the efflux of metabolites from activated BA can modulate the resident macrophage phenotype through extracellular adenosine production, promoting an anti-inflammatory and pro-thermogenic environment. This elucidates how the process of thermogenesis is regulated and contributes to studies about metabolic diseases. **Keywords:** Thermogenesis ;metabolic regulation;anti-inflammatory.

DO - 196 - **The mTORC1 and mTORC2 pathways as regulatory key in CD4+ T function – the role of physical fitness and body adiposity**

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The level of cardiorespiratory fitness ($\dot{V}O_{2max}$) is considered a potential modifier of the inverse association between visceral obesity (VAT) and mortality. These factors can modulate and modify nutrient sensors, such as mTORC, which play important roles in lymphocyte function and differentiation. Thus, the objective was to examine the impact of the mTORC1 and mTORC2 pathways on the regulation of Treg and Th17, and inflammatory responses according to different levels of $\dot{V}O_2$max and VAT. Nineteen young adults (10 men and 9 women; age: 25.1±4.5 years) were classified into high$\dot{V}$O2max-lowVAT (>55 mL.kg.min; VAT: ≤3.1 cm) and low$\dot{V}$O2max-highVAT (>35 mL.kg.min; VAT: ≥5.1 cm). $CD4^+$ T lymphocytes were isolated by negative selection (EasySep Kit) and differentiated into Treg (CellXVivoTM+2ng.mL TGF-β); and Th17 (20ng.mL of IL-6+2ng.mL of TGF-β). To inhibit mTORC1 and 2, 100nM of rapamycin or 50nM of Torin-1, respectively (incubated for 96 hours at 37°C and 5% CO_2). TNF-α, IL-6, IL-10, and IL-17 concentrations were determined by ELISA. Data are presented as median and interquartile range (IQR). We adopted gender as a covariate. A two-way analysis of covariance followed by Tukey's post hoc test (P<0.05). Individuals with high$\dot{V}O_{2max}$-lowVAT showed higher production of IL-10 from Treg cell (p^{adj}=<0.001) compared with low$\dot{V}O_{2max}$-highVAT individuals (p^{adj}=<0.001). Individuals with high $\dot{V}O_{2max}$-lowVAT showed lower production of IL-10 from Treg inhibited with rapamycin (p^{adj}=<0.001) and from Treg inhibited with Torin-1 (p^{adj}=<0.001) compared with Treg control. Individuals with low $\dot{V}O_2$max-highVAT showed higher production of IL-17 in Th17 control (p-^{adj}=<0.001, β=0.99) compared with $CD4^+$ control. There were no significant differences in Th17 TNF-α production between groups. In conclusion, gender, as well as low$\dot{V}O_{2max}$, and highVAT, play a crucial role in the inflammatory response of $CD4^+$, decreasing anti-inflammatory response. **Keywords:** T lymphocytes;immunometabolism;cardiorespiratory fitness.

DO - 197 - **CHARACTERIZATION OF AN ANIMAL MODEL TO STUDY THE ROLE OF FERROPORTIN IN MYELOID LEUKOCYTES DURING MYCOBACTERIUM TUBERCULOSIS INFECTION**

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The absence of an effective vaccine and therapy that quickly eliminates the pathogenic agent from the host organism contribute to tuberculosis (TB) being one of the most lethal infectious diseases in the world. The iron metabolism in infected cells has been suggested as a promising target for the development of host-directed therapies. The regulation of intracellular iron levels occurs through the action of ferroportin (FPN) that releases iron to the extracellular space. Thus, the decrease in its expression on the membrane of infected cells may contribute to intracellular iron loading, which may impact the control of bacterial replication. Our aim was to develop an animal model genetically deficient for FPN in macrophages in order to investigate the role of FPN during Mtb infection. Our results showed that Mtb infection of bone marrow-derived macrophages (BMDM) induced a significant increase in FPN in the presence of iron supplementation. We also identified that infected alveolar macrophages (AM) and interstitial macrophages (IM) displayed higher FPN expression compared to non-infected cells. Thus, we developed an animal model genetically deficient for FPN in AM and IM (FPN LyzMCre^{+}) to investigate the role of FPN in the containment of bacterial replication. FPN-deficient BMDM displayed higher bacterial load and cell death compared to (WT) cells. However, in vivo, FPN LyzMCre^{+} and WT animals presented similar pulmonary bacterial loads 4 weeks post-infection, despite the levels of cell death and iNOS expression were higher in FPN LyzMCre^{+} mice. Taken together, our results indicate that FPN absence results in increased susceptibility to bacterial replication in macrophages in vitro. However, a deeper investigation using this model is necessary for a better understanding of the role played by FPN during experimental TB, which may identify possible new targets for anti-TB therapies. **Keywords:** : Mycobacterium tuberculosis;ferroportin;iron.

DO - 198 - **Effect of Zileuton in modulating immune response and protecting lung function during an experimental acute respiratory syndrome (SARS) induced by a betacoronavirus**

PEREIRA, R.D.D.; RABELO, R.A.N.; MELO, N.; TEIXEIRA, S.L.; JÚNIOR, C.M.Q.; DE SOUZA-COSTA,, L.P.; SANTOS, F.R.D.S.; UMEZU, H.L.; FERREIRA, R.; DA SILVA, G.S.F.; CRUZ, J.D.S.; TEIXEIRA, M.M.; LITWINSKI, V.V.C.; MACHADO, F.S.. UFMG, UFMG BELO HORIZONTE - MG - BRASIL.

Host uncontrolled inflammatory response is the main cause for severe COVID-19. Zileuton (Zi), a selective inhibitor of the 5-lipoxygenase enzyme, is involved in the production of pro and anti-inflammatory/resolving lipid mediators, that may have protective effects. Herein, the effect of Zi treatment during a murine model of SARS was investigated. C57BL/6 female mice were infected with 3x10^{3} PFU of MHV3 and treated or not with Zi at different doses (1.5, 3, 15 or 30 mg/kg). The results demonstrated that MHV3-infected mice treated with 15 or 30 mg/kg of Zi presented significant improvement of the clinical score compared to infected untreated animals. The selected treatment with 30 mg/kg delayed the weight loss and promoted 25% survival. At 3 days post infection(dpi), although there was observed no difference in the lung viral load, the MHV3+Zi group showed reduced inflammatory score and higher IL-10 production when compared to the MHV3 group. The treatment increased the expansion of neutrophils (NEs) producing IL-10, reduced dendritic cells (DCs) producing IL-10 and TNF-α in the lung. In the spleen, there was a reduction of NE, DCs, and CD4+ T cells producing IL-10 and TNF-α compared to the infected untreated group. At 5dpi, treatment did not change the viral load, but promoted an increase in Th2/Treg, T CD8+ Treg/IL-10 producing cells, in addition to a reduction in Th1 cells in the lung. Zi increased in spleen the macrophages producing IL-10 and TNF-α, NE producing TNF-α, Th17, and CD8+ T cells producing IFN-γ, as well as maintained the Treg cells population. Furthermore, Zi treatment protected the mice cardiopulmonary function. When we infected K18-ACE2 animals with SARS-COV2, Zi significantly improved clinical score, weight loss and lung inflammatory score when compared to untreated animals. Overall, our data, so far, suggest that Zi treatment protects the development of severe/lung disease cases during SARS induced by betacoronavirus. **Keywords:** Infections;Severe acute respiratory syndrome coronavirus;SARS-CoV-2.

DO - 199 - **Behavioral alterations in Toxoplasma gondii infection of murine model are associated with neuroinflammation**

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The protozoan *Toxoplasma gondii* is an intracellular parasite and the causative agent of toxoplasmosis. The parasite may invade any cell type and tissues showing tropism for the central nervous system (CNS), with sustained neuroinflammation during the acute and chronic phase in mouse models of infection and may cause neurological alterations in immunocompromised persons. *T. gondii* infection has been related to mental illnesses such as schizophrenia, depression and other behavioral changes. In the present study, we evaluated the behavioral alterations in a model of chronic infection, assessing anxiety, depression and exploratory behavior, and the relationship with neuroinflammation and parasite cysts in brain tissue areas, blood-brain-barrier (BBB) integrity and cytokine in the brain and serum. Female C57BL/6 mice were infected with 5 cysts of the ME-49 type II *T. gondii* strain and analyzed as independent groups at 30 and 60 days postinfection (dpi). Anxiety, depressive-like behavior, and hyperactivity were detected in all analysis points in association with the presence of parasite cysts and neuroinflammation, linked to BBB disruption and independently of the presence of cysts in the brain tissue areas. Behavioral changes paralleled elevated concentration of tumor necrosis factor (TNF), interferon-gamma (IFNγ) and CC-chemokine (CCL2/MCP-1) in the serum, the presence of inflammatory foci with meningoencephalitis and perivascular inflammatory cuffs composed of mononuclear inflammatory cells in all analyzed areas of the CNS (cortex, hippocampus, cerebellum). Further, these behavioral alterations paralleled the upregulation of expression of TNF and CC-chemokines in the brain tissue. Our data suggest that the persistence of parasite cysts induces neuroinflammation, and BBB disruption, thus allowing leakage of cytokines of circulating plasma that may contribute to behavioral changes (anxiety, depressive-like behavior, and hyperactivity) in chronic *T. gondii* infection. **Keywords:** Toxoplasma gondii;neuroinflammation;cytokines.

DO - 200 - **Effect of C3 in the inflammatory response and renal fibrosis in the chronic leptospirosis murine model**

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Leptospirosis is a neglected zoonosis caused by pathogenic *Leptospira* spp., and estimated to affect approximately 1 million people and leading to 60.000 deaths every year. Acute leptospirosis can induce liver, kidney and lung dysfunction, leading to death and, in case of recovery, predispose patients to chronic and end-stage kidney disease. The Complement System (CS) is important to eliminate pathogens that enter the bloodstream, but pathogenic *Leptospira* spp. can evade the CS by cleaving its proteins, acquiring host CS regulators or producing their own CS protein inhibitors. Here, we report the role of C3 during leptospirosis in C57BL/6 wild-type (WT) and C3 knockout (C3KO) male mice 15- and 30-days post infection (d.p.i.). Mice were infected with 10^8 *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 (LIC). All mice survived the infection and carried LIC in the kidneys, but not in the liver. The absence of C3 did not influence the amount of fibrosis or expression of antimicrobial related genes. LIC induced the production of specific IgM and IgG3 in both mice strains, however C3KO mice produced less IgM after 30 d.p.i., while total IgG and the respective IgG subtypes levels were similar when compared to WT. The LIC colonization was not enough to maintain high levels of serum inflammatory cytokines compared to the WT control, however LIC-colonized mice had lower quantity of M-CSF, sICAM-1, IL-16 an C5/C5a. The infection diminished the number of early effectors T helper and T cytotoxic cells, an effect that was enhanced by the absence of C3. Overall, even though C3 did not impact the leptospiral load in the kidney during chronic leptospirosis, this protein may interfere in the number of effector T cells that may help controlling the disease. **Keywords:** Leptospirosis;Complement C3;Renal fibrosis.

DO - 201 - **Probiotic Yeast Treatment Attenuates Irinotecan Induced Intestinal Mucositis in Mice and in-vitro Model.**

GIRARDI, J.I.; COMPAGNUCCI, M.M.F.; ERREA, A.J.; RUMBO, M.. INSTITUTO DE ESTUDIOS INMUNOLOGICOS Y FISIOPATOLOGICOS, INSTITUTO DE ESTUDIOS INMUNOLOGICOS Y FISIOPATOLOGICOS LA PLATA - ARGENTINA.

Irinotecan, a chemotherapeutic drug used in colorectal metastatic cancer therapy, often causes severe intestinal damage, leading to drug-induced mucositis. This condition can necessitate the discontinuation of chemotherapy, impacting disease progression. In this study, we aimed to evaluate the protective effects of the probiotic yeast Kluyveromyces marxianus CIDCA 8154 (Km8154) against irinotecan-induced mucositis. We also investigated the effects of heat-killed (HK) yeast administration. In-vitro: Reporter CACO-2 cells, which express the luciferase gene under the CCL20 promoter, were treated with irinotecan and supplemented them with Km8154. The inflammatory response was measured by luciferase activity and cell oxidative stress by flow cytometry with the H2-DCFDA probe. In-vivo: Irinotecan 75mg/kg was administered to BALB/c mice for 4 days to induce drug-associated mucositis. The Km8154 intervention group received daily oral administration of the yeast at a concentration of 10^9 UFC/ml until the endpoint. After 7 days, mice were euthanized, and intestinal samples were obtained. Clinical, histopathological, and biochemical parameters were evaluated. Our in-vitro studies demonstrated that irinotecan treatment activated the reporter Caco-2 cells, but co-treatment with Km8154 reduced luciferase production significantly ($p<0.01$). Km8154 co-treatment also showed a slight reduction in irinotecan-induced oxidative stress in the cell line ($p=0.0519$). In-vivo studies revealed that both live yeast and HK treatment improved histopathological and diarrheal scores in mice treated with irinotecan. They also protected against intestinal damage as indicated by the villus/crypt ratio ($p<0.001$) and intestinal shortening ($p<0.01$). Additionally, HK treatment prevented weight loss induced by irinotecan ($p<0.05$). These findings suggest that the probiotic yeast Km8154 and HK offer protection against irinotecan-induced intestinal mucositis, as demonstrated in both in-vitro and in-vivo models. **Keywords:** Immunology;Mucosa;Probiotic.

DO - 202 - **INVOLVEMENT OF INFLAMMASOMES IN THE PATHOGENESIS OF LEPROSY NEUROPATHY**

DE ATHAIDE, M.M.[1]; SANTOS, A.F.R.[1]; CALVO, T.L.[1]; ROSA, T.L.S.A.[1]; MOREIRA, M.D.B.P.[1]; RODRIGUES, M.M.J.[1]; ROSA, P.S.[2]; LARA, F.A.[1]; FAPERJ, R.O.P.-.[1]. 1. INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO LAURO DE SOUZA LIMA, BAURU - SP - BRASIL.

Leprosy is the most common treatable cause of neuropathy in the world. Neural involvement can occur before, during, or after multidrug therapy, and especially during reactional episodes when neuritis occurs. Neuritis causes loss of function in the nerves and can be irreversible if left untreated. Disabilities caused by peripheral nerve injuries greatly affect these patients' lives, and the pathophysiological mechanisms underlying nerve damage remain unclear. Our previous study showed that leprosy type 1 reaction (T1R) outcome is associated with an impairment of autophagy in skin lesion cells. Furthermore, higher serum levels of IL-1β were demonstrated in multibacillary patients that developed reaction during treatment when compared with patients that did not develop reaction as well as in T1R patients. Moreover, an increase in serum levels of IL-1β was demonstrated in patients with leprosy neuropathy. The aim of the present study was to investigate the involvement of inflammasomes in the pathogenesis of leprosy neuropathy. The transcriptomic analysis of primary Schwann cells (SC) revealed that *Mycobacterium leprae* infection triggers a positive regulation of inflammasome genes like NLRP1 and PYCARD. Analysis of RNAseq in nerve fragments from Pure Neuritic Leprosy (PNL) patients showed that inflammasome pathway is enriched when compared to nerves from patients with another peripheral neuropathy. Increased expression of ASC and IL-β were observed in the perineurium from PNL patients. Both live and dead *M. leprae*, as well as different mycobacterial components were able to increase IL-1β levels in supernatants from ST88-14 SC lineage. Live and dead *M. leprae* increased IL-18 levels in supernatants from ST88-14 in relation to non-stimulated cells. Our results suggest that *M. leprae*, as well as different mycobacterial antigens, may activate the inflammasome pathway in SC and may be involved in the pathogenesis of leprosy neuropathy. **Funding:** CNPq, FAPERJ, INOVA, INCT-NIM. **Keywords:** inflammasomes;leprosy neuropathy;neuritis.

DO - 203 - **Evaluation of Immunogenicity and Long-Term Efficacy of Immunization using KHARON1 Knock-Out Parasites as an Immunoprophylactic Strategy for Visceral Leishmaniasis in Hamsters**

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Leishmanization was a successful immunization strategy against human leishmaniasis. Modern molecular techniques allow the selection of attenuated, non-pathogenic living parasites, inducing safer and long-lasting protective immunity. The deletion of the KHARON 1 gene in Leishmania infantum resulted in an attenuated strain that can infect mice and trigger a robust immune response. In the present study, we evaluated the production of intracytoplasmic cytokines in hamster splenocyte cultures and the vaccine efficacy in hamsters immunized with the KH1-deficient strain (receiving one or two doses via subcutaneous or intradermal routes) and challenged with a virulent strain of L. infantum. The animals were evaluated 8 months after the experimental challenge through the production of IFN-γ, TNF, and IL-10 cytokines using flow cytometry, in addition to parasitism in the spleen and liver by qPCR (Quantitative Polymerase Chain Reaction). The results indicate that immunization with knock-out parasites induces a mixed response, with production of pro-inflammatory and anti-inflammatory cytokines. Animals that received leishmanization through both routes (subcutaneous and intradermal) showed a higher percentage of IFN-γ and TNF-producing cells compared to the non-immunized and challenged group (control group). Regarding IL-10 cytokine, a reduction in the percentage of cells producing this cytokine was observed in all immunized groups compared to the control group. A reduction in hepatic parasite burden was observed in the groups that received two doses in both routes. The group that received two doses via the subcutaneous route, also showed a lower spleen parasite burden compared to the control group. The present study indicates that leishmanization is immunogenic and capable of reducing parasitism in immunized animals, highlighting the immunoprophylactic potential of this strategy. Supported by: UFOP, Fiocruz Minas, BIOT-FIOCRUZ, CCA-UFOP, FINEP, FAPEMIG, CAPES, CNPq, and INCT-DT. **Keywords:** Visceral Leishmaniasis;leishmanization;hamster.

DO - 204 - **DEVELOPMENT AND VALIDATION OF A MULTIPLEX RAPID DIAGNOSTIC TEST FOR HEPATITIS B AND D**

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Hepatitis D is an infectious disease caused by the hepatitis D virus (HDV), a defective virus that depends on the surface antigen HBsAg of the hepatitis B virus (HBV) for infectivity. It is estimated that 300 million people are infected with HBV worldwide and 15 to 20 million of them are also infected with HDV. In Brazil, 150 new cases were notified in 2019, however, this number may be underestimated. The virus is responsible for the most severe form of viral hepatitis, which may rapidly progress to cirrhosis and liver failure, increasing the need for an early diagnosis. The diagnosis of hepatitis D is performed through serological tests after the patient has received a positive HBsAg result. Currently, few diagnostic tests are approved for HDV infection, and no rapid diagnostic test (RDT) or multiplex test for the detection of both infections are available anywhere. Here, we report the development of a multiplex RDT for the simultaneous detection of anti-HDV IgG antibodies and HBsAg. For hepatitis D detection, a new recombinant antigen was designed after bioinformatic analysis of the delta antigen of HDV (HDAg). The protein was expressed in *E. coli* and purified by affinity chromatography. The multiplex RDT is composed of two test lines, one with a monoclonal antibody (mAb) anti-HBs immobilized (detection of HBsAg) and the other with an anti-human IgG antibody (for anti-HDV IgG), and a mixture of mAb anti-HBs and the recombinant HDAg conjugated to colloidal gold as conjugate. The multiplex ICT was validated with a sera panel from patients from an endemic area, positive for both infections and characterized with a commercial ELISA for hepatitis D. The ICT showed a sensitivity of 91.9% (95% CI: 83.4-96.2%) for HBsAg and 91.3% (95% CI: 73.2-98.45%) for anti-HDV IgG and specificity of 100% (95% CI: 91.0-100.0%) and 97.8% (95% CI: 92.3-99.6%), respectively. In conclusion, the prototype developed is a promising tool to improve the diagnosis of both HDV and HBV infection. **Keywords:** Rapid diagnostic test;Hepatitis D;Hepatitis B.

DO - 205 - **Schistosoma mansoni infection alters inflammation, pathogenesis, and parasite load in Leishmania amazonensis-coinfected BALB/c mice**

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The overlapping transmission areas of Neglected Tropical Diseases render several people threatened by coinfections. Still, the consequences of *Schistosoma-Leishmania* coinfections remain poorly explored. Interactions between schistosomiasis and tegumentary leishmaniasis may lead to enhanced susceptibility to infection, regulatory response profiles, and treatment failure. However, no study has addressed how previous *S. mansoni* infections can affect pathogenesis and immune response against *L. amazonensis*, the main etiologic agent of diffuse leishmaniasis. To explore this, BALB/c mice were divided into four groups: non-infected, infected with *L. amazonensis*, infected with *S. mansoni*, and coinfected with *L. amazonensis* after 8 weeks of *S. mansoni* infection. Weight, footpad thickness, and mortality were assessed weekly for 10 weeks. *S. mansoni* and *L. amazonensis* parasite loads were evaluated. Cytokine levels were measured by ELISA in popliteal lymph node cell-culture supernatant and footpad homogenate. There was no change in mortality, weight gain, and *S. mansoni* parasite load between groups. However, in coinfected mice, *L. amazonensis* induced smaller, slow-growing cutaneous lesions, accompanied by a 20-fold increase in *L. amazonensis* parasite load. In footpad homogenates, CXCL2 and IL-5 levels were increased in coinfected mice. In cell-culture supernatant, IL-5 concentration was also increased in coinfected mice. IL-12p70 production was only increased in *Leishmania* soluble antigen-stimulated cells from coinfected mice, with no difference between groups. IL-17 was detected in the supernatant of cells from all groups but the non-stimulated coinfected mice. *S. mansoni* infection affects the inflammatory processes involved in the response against *L. amazonensis*, leading to decreased lesion sizes and increased *in vivo* amastigote proliferation. Further studies are required to understand the involved mechanisms, especially regarding eosinophils and phagocytic mononuclear cells. **Keywords:** Schistosomiasis;Leishmaniasis;Coinfection.

DO - 206 - **Identification of Loxosceles Venom Specific Equine Antibodies**

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Alternative ways to produce anti-venom serotherapy, such as using synthetic antigens for immunization or producing monoclonal antibodies, have been explored in many studies. However, the composition of the immunoglobulins in this therapy remains unknown. In this study, we conducted a comprehensive serological characterization of the plasma from four horses that underwent hyperimmunization with *Loxosceles* spider venom. In our analysis, we assessed the reactivity of the plasmas toward the venom and determined the frequency of immunoglobulin sub-classes. We did a purification of immunoglobulins from the plasma, digested these molecules with pepsin, and selected the anti-venom F(ab')2. These fragments were trypsinized and sequenced through mass spectrometry coupled to liquid chromatography. The peptides were mapped to a dataset of sequences of the variable region of the heavy chain of immunoglobulins (IGHV) prepared from the B cells transcriptomes for each sample. The samples contained, on average, 142.21 mg/mL of IgG, mainly IgGT and IgGb subclasses. After purification using protein G affinity, IgGb became the most frequent subclass, followed by IgGa and IgGT, confirming protein G's selectivity. Mass spectrometry identified an average of 3257 peptides per sample, with at least 48% mapped to IGHVs. The complete Ig-heavy chain sequences were identified by peptides from their CDRH3 regions, leading to the discovery of 163 heavy chains of antibodies that potentially bind to venom components. The spotted sequences have a higher frequency of charged residues on the CDRH3 compared to the whole repertoire but a similar frequency of V and J gene segments usage and somatic hypermutation rate. This study represents a pioneering effort in identifying and characterizing circulating antibodies (heavy chain) against *Loxosceles* venom. These findings are promising for the production of recombinant antibodies *in vitro*, which expands the possibilities for diagnostic and therapeutic use. **Keywords:** Antibodies;Venom;Repertoire.

DO - 207 - **IMMUNOMODULATORY POTENTIAL OF THE RESVERATROL ANALOG AR23, IN BONE MARROW-DERIVED MACROPHAGES.**

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Introduction: Resveratrol is a natural compound found mainly in grapes and their derivatives, exhibiting important biological activities, such as anti-inflammatory and immunomodulatory effects, among others. Despite these activities, the use of resveratrol *in vivo* is limited due to its low bioavailability. However, the resveratrol molecule is an ideal prototype for the synthesis of analogous compounds capable of overcoming this issue. Thus, the present study aimed to evaluate the immunomodulatory activity of the compound AR23, an analogue of Resveratrol, in bone marrow-derived macrophages (BMDM). **Methods and Results**: First, the viability of BMDM (CEUA: 020/2018) treated with compound AR23 (25 and 50µM) was evaluated using the MTT method. Then, the influence of AR23 on the expression of costimulatory molecules (CD80 and CD86) was determined by flow cytometry on LPS-stimulated cells. Finally, the action of the compound on the production of cytokines IL-6 and IL-12 was determined in stimulated cells (ELISA). The results showed that AR23 did not present prominent cytotoxicity, maintaining cell viability above 70%, as recommended by ISO10993-5:2009. Furthermore, when compared to control (LPS-stimulated cells), AR23 treatment at a concentration of 25µM was able to decrease CD80 expression by 16.4±5.53% and CD86 by 32.67±1.39 % while at a concentration of 50µM, it inhibited CD80 expression by 24.77±5.01%. Regarding cytokines, treatment with AR23 (25µM and 50µM) was very effective, reducing IL-6 production by 81.96± 6.24% (25µM) and 96.91±1.95% (50µM) and IL-12 in 98.78±2.11% (25µM) and 99.31±0.09% (50µM). **Conclusion:** the results demonstrate that AR23 has anti-inflammatory and immunosuppressive potential on BMDM, being able to impact important mediators of the immune response. New experiments are being carried out in order to confirm this potential. **Keywords:** Immunomodulation;Resveratrol;Macrophages.

DO - 208 - **Etiopathogenesis of Experimental Leptospirosis-Associated Pulmonary Hemorrhagic Syndrome in C3H/HeJ mice**

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Leptospirosis is a neglected disease caused by *Leptospira*. Most infected individuals (90%) are asymptomatic or present mild symptoms common to other acute febrile illnesses. Some patients may develop a severe form known as Leptospirosis-Associated Pulmonary Hemorrhagic Syndrome (LPHS), characterized by an aggressive clinical evolution and high lethality (~50%). The etiopathogenesis of LPHS is still poorly understood and very little is known regarding the contribution of the immune response to local tissue damage. In addition, previous studies have shown the deposition of C3 from the Complement System (CS) in the alveolar septal of LPHS-patients. In this study, we infected male and female C3H/HeJ mice ($TLR4^{-/-}$) – susceptible to leptospirosis - with distinct doses of pathogenic *L. interrogans* Fiocruz L1-130. We observed a lower survival rate, associated with greater severity of pulmonary hemorrhage and jaundice in infected male when compared to infected female mice. Next, we inoculated C3H/HeJ male animals with sublethal and lethal doses of L1-130 to evaluate the circulating leukocyte populations in different days of infection. We registered a reduction in the lymphocyte population (at 7 dpi) and an increase of granulocyte numbers (at 3 dpi and 7 dpi). In parallel, infected mice carried less circulating platelets at 3 dpi and 7 dpi when compared to non-infected animals. Hemolytic assay and C5a concentration (ELISA) were performed to analyze the CS activation in the serum and the bronchoalveolar lavage samples, respectively. A decreased CS hemolytic activity and lower C5a levels were observed in infected mice when compared to negative control. Based on these findings, we suggest a potential immunomodulatory role of this pathogen in the immune response during LPHS, which may contribute to pathological aggravations. Further experiments will be performed to better understand the leukocyte populations' variation and the CS involvement in LPHS. **Keywords:** Complement System;Leptospirosis;Leptospirosis-Associated Pulmonary Hemorrhagic.

DO - 209 - **REGULATORY T CELLS (TREG) AND GALR2 EXPRESSION INFLUENCES ORAL SQUAMOUS CELL CARCINOMA (OSCC) PROGRESSION**

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Prognosis of OSCC is worse in the presence of an increased infiltration of immune cells with a suppressor profile. Treg cells are prototypical immune suppressor cells. In leukocytes, GALR2 is the only one of three known receptors for the neuropeptide Galanin expressed. Disputed evidence indicates that GALR2 is an oncogene in OSCC. This study evaluated the prevalence of Treg and active CD4 and CD8 cells and the expression of GALR2 and FOXP3 in primary tumors of OSCC patients. Samples of primary tumors and venous whole blood were collected from 16 OSCC cases (n=8 T1/T2 and n=8 T3/T4) at the moment of surgical resection of primary tumors. Whole blood samples were also collected from 5 healthy donors. PBMCs were isolated and flow cytometry was performed to identify CD3+CD4+FOXP3+, CD3+CD4+CD69+ and CD3+CD8+CD69+ cells. RT-qPCR was performed using RNA extracted from aliquots of the same PBMC and tumor tissue samples of a subgroup of these patients to determine expression of GALR2 and FOXP3. There was a trend towards greater expression of GALR2 in PBMCs from patients with larger tumors (T3/T4). Patients whose tumors had a higher expression of GALR2 also had a higher prevalence of Tregs in the circulating blood *(p<0.001).* Larger (T3/T4) tumors tended to present increased expression of FOXP3 mRNA. In the circulating blood of these same patients there was a trend of increased prevalence of CD3+CD4+FOXP3+ cells. Patients with T3/T4 tumors had more circulating Tregs than healthy donors (*p=0.04*). OSCC cases were divided into high (n=5) and low (n=6) FOXP3 mRNA expression in tumor tissues. Increased prevalence of circulating CD3+CD4+CD69+ cells was observed in low FOXP3 cases, whereas high FOXP3 cases had increased percentage of CD3+CD8+CD69+ cells (*p=0.02).* These data indicate a correlation between FOXP3 and GALR2 mRNA in tumors and circulating leukocytes. There is also a direct association between GALR2 expression in tumors and the prevalence of circulating Tregs. **Keywords:** Treg Cell;GALR2 Galanin Receptor;Oral Squamous Cell Carcinoma.

DO - 210 - **Increased levels of soluble TNF receptor (sTNFR1) are associated with severity and mortality from COVID-19**

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COVID-19, caused by SARS-CoV-2, remains a big challenge for global public health. The profile of the immune response and the influence of inflammation on the progression of the disease, among the associated factors, we have the tumor necrosis factor (TNF), a complex pro-inflammatory cytokine, which has several receptors involved in systemic inflammatory processes; soluble receptors are known for their antagonistic role to TNF. Thus, knowing the role of these guidelines gives us a better understanding of the immunopathogenesis of the disease. The aim of this study was to verify the serum levels of the soluble receptor (sTNFR1) in patients with COVID-19. A total of 131 patients confirmed for SARS-CoV-2 infection were separated into three groups: patients in the ward without oxygen (O2) support, group A (14); ward patients with O2 support, group B (85), and patients in the intensive care unit (ICU), group C (32), with the receptor dosed by cytometric bead array (CBA). The results showed that sTNFR1 is associated with disease severity, being found in higher values in patients with COVID-19 with the most severe condition when compared to patients with mild manifestations and may be a possible biomarker of disease progression. By associating the receptor level with the degree of pulmonary involvement, higher values of sTNFR1 were observed in patients with a lower degree of involvement, suggesting that inflammatory processes mediated by TNF are not necessarily associated with the primary site of infection. When evaluating the parameters of patients who died versus recovered, there was a significant increase in this receptor in the group of deaths. These drugs showed an important influence of the soluble receptor, sTNFR1, on the inflammatory processes involved in the pathogenesis of COVID-19, therefore, this receptor can be a potential biomarker of disease severity and mortality. **Keywords:** COVID-19;Receptor;sTNFR1.

DO - 211 - **TLR2 IS ESSENTIAL FOR REGULATORY T-CELL INDUCTION AND PROTECTION MEDIATED BY Lactococcus lactis EXPRESSING HSP65 IN TYPE 1 DIABETES**

OLIVEIRA, J.E.; RODRIGUES, V.F.; PEREIRA, Í.S.; BARBOSA, S.C.; MACHADO, M.S.G.; PEREIRA, J.A.; PACHECO, T.C.F.; MASSON, A.P.; SARTORI, D.C.. FMRP-USP, FMRP-USP RIBEIRÃO PRETO - SP - BRASIL.

Heat-shock proteins (HSPs) are chaperones highly expressed after cellular stress, which acts by degrading misfolded proteins. Heterologous HSPs are being associated with probiotics and recently used as therapeutic strategies to induce tolerance in autoimmune disease models. Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β cells, leading to hyperglycemia. New therapeutic strategies are important to avoid the complications associated with T1D. Here, we evaluated the effects of recombinant HSP65 on bone marrow-derived dendritic cells (BMDC) and evaluated the role of TLR2 in the protective effects of *L. lactis* expressing HSP65 in T1D induced by streptozotocin (STZ) (CEUA 007/2020). We demonstrate that BMDC stimulated with rHSP65 have increase in percentage and expression of MHC-II and CD80 in $CD11c^+$ cells after 24h of stimulation when compared to control group. Furthermore, rHSP65 increased cDC2 percentage and higher expression of MHC-II and CD80 after 24h of stimulation. In addition, rHSP65 also increased cDC1 percentage, with intermediate levels of MHC-II and CD80 expression. Interestingly, rHSP65 induced increased production of IL-10 by BMDC when compared to LPS-treated cells. *In vivo*, we observed that diabetic mice feeding with *L. lactis*-HSP65 had lower hyperglycemia when compared to the diabetic group. Furthermore, administration of *L. lactis*-HSP65 increased cDC1 $TLR2^+$ in the cecal lymph node (CLN) and regulatory T cells in the pancreatic lymph nodes (PLN) compared to diabetic mice without probiotic. In *Tlr2*$^{-/-}$ mice, we observed that *L. lactis*-HSP65 loses its ability to reduce T1D, which is associated with a reduction of cDC1 $XCR1^+$ in the CLN when compared to the control group. PLN from *Tlr2*$^{-/-}$ mice that received *L. lactis*-HSP65 showed fewer T regs and reduced PD-1 expression in these cells. Our data show that *L. lactis*-HSP65 has an immunoregulatory role in T1D through of a mechanism that is dependent on TLR2. **Keywords:** HSP65;T1D;TLR2.

DO - 212 - **GALR2 EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) INFLUENCES THE ASSOCIATED IMMUNE RESPONSE**

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HNSCC are highly heterogeneous cancers and this is linked to the variability in therapeutic responses irrespective of clinical stage. This heterogeneity encompasses their immune landscape as well; and tumors can exploit multiple suppressive/subversive mechanisms to evade immunosurveillance and promote tumor progression and metastasis. The anti-tumor immune response is influenced by multiple aspects, including tumor-derived immune-modulating biomarkers, such as GALR2 expression/activity. There is conflicting evidence on GALR2 as an oncogene or tumor suppressor. GALR2 is the only galanin receptor expressed by peripheral blood mononuclear cells (PBMC). We collected tissue samples and peripheral blood from HNSCC patients at the primary resective surgery. All samples were identified by demographic characteristics, gender, age, history of smoking and alcohol use, location of the primary tumor and TNM classification. RT-qPCR was performed to evaluate gene expression of GALR2, Galanin, PD-1, PD-L1, CTLA4, FOXP3, TBX21, and GATA3 in the tumor and PBMC from same patients. PBMC were stained with antibodies for CD56 and CD107 and flow cytometry analysis were performed. The cases were separated into two groups according to tumor size (T1/T2 and T3/T4) and GALR2 expression levels in the primary tumor: 75% percentile (higher) GALR2 expression (n=9) and 25% percentile (lower) GALR2 expression (n=10). Results suggest an increased expression of PD-1, CTLA4 and GATA3 in high GALR2 tumors, indicating a suppressive (Th2) and/or anergic T cell response. Prevalence of both CD56+ cells in the PBMC of high GALR2 patients and of CD56+CD107+ cells is significantly reduced ($p<0.01$). These results indicate that increased expression of GALR2 in HNSCC is associated with both local and systemic immunosuppression. **Keywords:** GALR2 expression;Head and Neck Squamous Cell Carcinoma;Immunosuppression.

DO - 213 - **ASSOCIATION BETWEEEN INCREASED INTESTINAL PERMEABILITY AND DECREASED POPULATION OF CELLS EXPRESSING IL-17 IN THE ILEUM IN OBESITY MODEL**

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Obesity can trigger intestinal inflammation characterized by increased permeability to bacterial products and intestinal dysbiosis, in addition to inferring an inflammatory condition in visceral adipose tissue (VAT) named metainflmammation, increasing susceptibility to associated diseases, such as type 2 diabetes mellitus. This work aims to investigate the therapeutic use of the probiotic *Lactococcus lactis* that expresses IL-17 in a model of diet-induced obesity. To verify Th17 cytokine protein expression kinetics, female C57BL/6 mice were fed standard diet (SD) or high fat diet (HFD) for 6, 12 or 18 weeks. After 6 weeks of HFD, the mice show more body weight gain, fasting hyperglycemia and increase in the weight of the VAT. After 12 weeks of HFD, there was a significant increase in body weight gain, increase in VAT weight and adiposity index, as well as an increase in intestinal permeability compared to SD-fed mice. Furthermore, a lower concentration of IL-17 was observed in the pancreas, a reduction in IL-22 in the ileum and a reduction in $CD4^{+}IL\text{-}17^{+}$ T cells in the ileal lymph node of mice with diet-induced obesity. After 18 weeks of consuming a HFD, the mice showed greater weight gain and adiposity index, however there was no difference in intestinal permeability and Th17 cytokine concentrations in the ileum when compared to the control group. To verify the effects of the probiotic *L. lactis* that expresses IL-17, female C57BL/6 mice were fed with SD or HFD for 18 weeks, the administration of the probiotic was performed every 2 days from the twelve weeks of HFD. It was observed that the recombinant probiotic did not induce significant changes in relation to the group supplemented with wild type bacteria or the obese group. Overall, these results suggest that HFD-induced obese mice show increased intestinal permeability at the twelfth week, when they also decrease IL-17 secretion compared to SD-fed mice. **Keywords:** metabolic syndrome;mucosal immunology;inflammation.

DO - 214 - **ANTI-INFLAMMATORY POTENTIAL OF A NANOSTRUCTURED COMPOSITION OF AR23 COMPOUND, A RESVERATROL ANALOGUE**

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Introduction: Resveratrol (RVT) is a phytoalexin found in plants, mainly grapes, with significant anti-inflammatory and immunomodulatory effects. Despite its activities, RVT has low bioavailability in mammals and solubility problems, which drives the search for analogues capable of overcoming these barriers. In this context, our research group synthesized and evaluated an analogue, called AR23, which showed excellent anti-inflammatory and immunomodulatory activity. Although AR23 proved more effective than RVT, it still showed solubility problems that could limit its use. Thus, to optimize its biological potential, the objective of this study is to develop nanostructured compositions of the AR23 analogue or resveratrol with 2-Hydroxypropyl-β-Cyclodextrin (βCD) and to evaluate its anti-inflammatory activity. **Methods and Results:** First, the cytotoxic potential of the compositions was determined on J774A.1 cell line (MTT). After, the anti-inflammatory activity was evaluated through the impact on the nitric oxide (Griess reaction) and cytokines (IL-6, IL-12 and TNF-α - ELISA) production in the supernatant of stimulated cells. The results revealed that the nanostructured compositions presented acceptable cytotoxicity at concentrations up to 400uM (viability above 80%). Regarding anti-inflammatory activity, βCD-AR23 and βCD-RVT were very effective, reducing nitric oxide production by 70.76±4.63% and 100% respectively, at the highest concentration assessed (400uM). Similarly, βCD-AR23 and βCD-RVT also impacted cytokines production reaching a reduction of 50.02%±4.84 and 99.87±0.13 on IL-6; 36,94±9,70% and 100% on TNF-α and 76.36±2.24% and 93.47± 1.23% on IL-12, respectively. **Conclusion:** So far, the results demonstrated that the nanostructured compositions have relevant anti-inflammatory activity. Despite this, more studies are being conducted to confirm these activities and evaluate other impacts on the immune system. Financial support: FAPEMIG, CNPq and CAPES. **Keywords:** NANOSTRUCTURED;RESVERATROL; ANTI-INFLAMMATORY.

DO - 215 - **METABOLIC AND IMMUNE IMPACTS ON THE LIVER AS A RESULT TO CHANGES IN THE MICROBIOTA DUE TO PREMATURE WEANING**

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The gut microbiota is a collection of microorganisms which live within the gastrointestinal tract. Colonization of the intestine begins at birth and progressive changes occur throughout the individual's development until the establishment of a more stable microbiota. Abrupt changes take place during neonatal phase, which comprises weaning - a key event that profoundly impacts the organism and may induce changes in microbiota. The gut and liver are connected and this reciprocal interaction allows the transport of products from the gastrointestinal tract to the liver, and vice-versa. Therefore, it is to be expected that weaning and changes in the colonization of the gut, which occur concomitantly with liver development, interfere with the maturation and functions of the liver, in a specially critical period known as the “neonatal window of opportunity”. Given the importance of the postnatal period, we aimed to evaluate the influence of premature weaning on the composition of the microbiota and its impacts on the individual's immune and metabolic competence. For such study, C57BL/6 mice (WT) between 1-8 weeks of age had their faeces collected for sequencing. Mice from 3-8 weeks were divided into two groups by a different weaning protocol: the conventional weaned mice (at 21st day) and the early weaned ones (at day 14). Previous data from our group shows that full hepatic metabolic capacity is acquired during weaning period and that early weaning profoundly disturbs the expression of several hepatic metabolic pathways, which persisted into adulthood. This data matches the altered composition of the intestinal microbiota that was observed in mice from the premature weaning group. Next, it is important to evaluate the impact of early weaning on infection susceptibility and outcome. Understanding the relationship between microbiota, neonatal hepatic immune development and weaning will allow the creation of appropriate interventions for children who are weaned prematurely. **Keywords:** Microbiota;Weaning;Neonatal development.

DO - 216 - **GAS6 treatment promotes protection against Mycobacterium tuberculosis infection in mice.**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) bacilli is one of the most lethal infectious diseases in the world. Modulation of host immune responses have been proposed as promising strategies to optimize TB antibiotic treatment. Axl and MerTK receptors recognize Gas6-bound phosphatidylserine on the surface of apoptotic cells and trigger immunomodulatory intracellular signaling. We found that the expression of both Axl and MerTK were increased in response to Mtb infection mainly in lung interstitial macrophages in C57BL/6 mice and that Axl+ and MerTK+ macrophages expressed higher levels of iNOS than Axl- and MerTK- cells. In addition, Mtb infection induced increases in the concentration of GAS6, soluble Axl and MerTK in the lungs of infected mice, indicating that the presence of the ligand can trigger receptor signaling in cells at the infection site, although soluble receptors might compromise the pulmonary GAS6 availability for this end.To evaluate the role of Axl and MerTK during TB, we used Mtb-infected C57BL/6, Axl-/- and MerTK-/- mice. Although Axl-/- mice displayed higher the levels of lung IL1β and TNF expression and IFN-γ production by T cells compared to C57BL/6 and MerTK-/- animals, no difference in mortality, bacterial loads, myeloid cell infiltration and macrophage iNOS expression in the lungs was found among the groups. Although Axl and MerTK deficiency did not impact resistance to Mtb infection, we next treated or not Mtb-infected C57BL/6 mice with recombinant Gas6 intranasally from 2 to 4 weeks post infection to evaluate if increasing ligand availability could interfere with resistance to infection. Gas6 treatment resulted in reduction of pulmonary bacterial loads along with increased alveolar macrophage levels. Thus, these results indicate that Gas6 signaling through TAM receptors play a host-protective role during Mtb infection and reveal intranasal Gas6 supplementation as a potential host-directed therapy for TB. FS:FAPESP **Keywords:** GAS6;TAM Receptors;Mycobacterium tuberculosis.

DO - 217 - **MACROPHAGES PRE-EXPOSED TO IRON OXIDE NANOPARTICLES PRESENTED DECREASED PARASITE LOAD**

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The currently available treatments against tegumentary leishmaniasis (TL) can cause severe signs of toxicity. In addition, therapeutic failures can be observed. Such problems point to the need to develop new therapeutic alternatives against TL. Therefore, this project aims to evaluate the anti-*Leishmania* effect of iron oxide nanoparticles (IONPs) associated or not with Glucantime®. Firstly, THP-1 macrophages were treated with IONPs (0.06 mg/mL) and/or Glucantime® (38.8 µg/mL) and the viability was assessed by the MTT method. Subsequently, THP-1 macrophages ($3x10^5$) were infected with *L. (L.) amazonensis* promastigotes ($1.5x10^6$) and treated with IONPs and/or Glucantime® for 24h, 72h and 144h. Alternatively, macrophages were treated with IONPs and/or Glucantime® for 24h, 72h and 144h and then, infected with *Leishmania*. After the end of the incubation periods, cells were analyzed by light microscopy to assess the infection index and the initial attachment/internalization. Results showed that IONPs and/or Glucantime® were not cytotoxic. Moreover, there was a reduction in the infection index when *Leishmania*-infected cells were incubated only with IONPs or Glucantime® for 24h. Another relevant finding was that cells treated prior to *Leishmania* infection presented a decrease in the infection index at all incubation periods tested, when exposed only to IONPs, and at the incubation period of 24h and 72h, when exposed to IONPs associated with Glucantime®. It was also observed that IONPs associated or not with Glucantime®, prior to *Leishmania* infection, decreased the initial attachment/internalization at the incubation period of 144h. Thus, it was concluded that IONPs and/or Glucantime® were not toxic to macrophages. In addition, only IONPs showed the ability to keep a reduced parasite load in pre-exposed cells. Also, the association IONPs plus Glucantime® did not result in a more potent antiparasitic action than Glucantime® alone. **Keywords:** Leishmania;nanoparticles iron oxide;Glucantime®.

DO - 218 - **Nucleotide guanine exchange factor RasGEF1b regulates cell migration in macrophages through Rho GTPases and actin cytoskeleton dynamics**

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Macrophages, essential innate immune cells, require the dynamics of the actin cytoskeleton to efficiently respond to infections. This response is mediated through cell surface projections such as filopodia, lamellipodia, and dorsal ruffles, ensuring effective and timely immune responses. These projections are critical for cell migration, macropinocytosis and phagocytosis, and are regulated by Rho GTPases RhoA, Rac1 and Cdc42. We examined if RasGEF1b, a RasGEF-domain containing family member activator of small GTPases and abundantly expressed in macrophages, plays a role in actin cytoskeleton dynamics. Using bone marrow derived macrophages (BMDMs) and RAW264.7 cells deleted or silenced of RasGEF1b, respectively, we show that cells present aberrant changes in morphology, cell surface and actin cytoskeleton distribution. BMDMs labeled with Wheat Germ Agglutinin (WGA) showed reduced size and altered cellular morphology. Besides, the number of dorsal ruffles was reduced in cells lacking RasGEF1b. Confocal microscopy of actin cytoskeleton in RAW264.7 knocked-down (KD) of RasGEF1b showed cellular retraction as indicated by increased image thickness in the Z-stack plane. Transwell and wound healing assays with either RasGEF1b-cKO BMDMs and RAW264.7-KD exhibited a significant reduction in cell migration. We investigated the underlying molecular mechanisms of these observations and found that RasGEF1b is required and sufficient to regulate the activation of RhoA, and Cdc42. *In vivo* studies indicated a significant decrease in the number and percentage of F4-80+ cells in the liver of RasGEF1b-cKO mice, identified through immunofluorescence microscopy and flow cytometry. We also found that mice devoid of RasGEF1b are more susceptible to bacterial infection, albeit not statistical significance. Our results demonstrate that RasGEF1b plays a crucial role in macrophage cytoskeleton dynamics, impacting morphology, cell migration, and specialized structures for pathogen elimination. **Keywords:** macrophages;cytoskeleton;RasGEF.

DO - 219 - **ASP-2/Trans-sialidase chimeric protein induces robust protective immunity in experimental models of Chagas' disease**

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Immunization with the Amastigote Surface Protein-2 (ASP-2) and *Trans*-sialidase (TS) antigens either in the form of recombinant protein, encoded in plasmids or human adenovirus 5 (hAd5) confers robust protection against various lineages of *Trypanosoma cruzi*. Herein we generated a chimeric protein containing the most immunogenic regions for T and B cells from TS and ASP-2 (TRASP) and evaluated its immunogenicity in comparison with our standard protocol of heterologous prime-boost using plasmids and hAd5. Mice immunized with TRASP protein associated to Poly-ICLC (Hiltonol) were highly resistant to challenge with *T. cruzi*, showing a large decrease in tissue parasitism, parasitemia and no lethality. This protection lasted for at least 3 months after the last boost of immunization, being equivalent to the protection induced by DNA/hAd5 protocol. TRASP induced high levels of *T. cruzi*-specific antibodies and IFNg-producing T cells and protection was primarily mediated by $CD8^+$ T cells and IFN-g. We also evaluated the toxicity, immunogenicity and efficacy of TRASP and DNA/hAd5 formulations in dogs. Mild collateral effects were detected at the site of vaccine inoculation. While the chimeric protein associated to Poly-ICLC induced high levels of antibodies and $CD4^+$ T cell responses, the DNA/hAd5 induced no antibodies, but a strong $CD8^+$ T cell response. Immunization with either vaccine protected dogs against challenge with *T. cruzi*. Despite the similar efficacy, we conclude that moving ahead with TRASP together with Hiltonol is advantageous over the DNA/hAd5 vaccine due to pre-existing immunity to adenovirus vector, as well as the cost-benefit for development and large-scale production. **Keywords:** Chagas Disease;Trypanosoma cruzi;Vaccine.

DO - 220 - **Integrated bioinformatic analysis and validation reveals key microRNAs and their potential gene-targets for immune modulation and glioblastoma progression**

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Several microRNAs (miRNA) are important to influence the plasticity of cells in the tumor microenvironment, regulating the progression of several tumor types and affect the immune microenvironment. The present study aimed to explore the expression profile of miRNAs related to tumorigenesis and modulation of the immune response in glioblastoma (GBM) microenvironment. Additionally, evaluating the cellular impacts of inhibiting overexpressed miRNAs in GBM cell lines, considering metabolic and immunological aspects. For this purpose, bioinformatic analysis was performed to evaluate differentially expressed miRNAs (DEmiRNAs) in GBM and activated T cells. The main DEmiRNAs were validated by RT-qPCR and inhibitors of overexpressed miRNAs were transfected into GBM cells and their effects on gene expression and protein targets were analyzed by RT-qPCR and flow cytometry. Twelve miRNAs were upregulated in GBM and activated T cells. After validation in tumor tissue and GBM cell lines, the miRNAs that showed increased expression were miR-27a-3p and miR-155-5p, which mainly regulate genes associated with metabolic and immune pathways. Inhibition of these two targets in the A172 cell line reduced cell viability, but only miR-27a-3p inhibition leads to apoptosis, reduced glucose uptake and mitochondrial depolarization. Both inhibitors provided gene modulation in metabolic and immune targets, in particular the glycolysis pathway and TGF-β expression, but not in immune checkpoints. Preliminary studies of coculture of immune and tumor cells indicate that immunomodulation may be occurring by inhibition of miRNAs in tumor cells. The results of this work provide new insights into potential targets for modulations of the immune-metabolic system and their implications on tumorigenesis, highlighting new therapeutic approaches for GBM. Additional studies of immune cell profile modulation with miRNA inhibitors should be carried out to improve the understanding of tumor immunometabolism. **Keywords:** Glioblastoma;MicroRNA;Immunomodulation.

DO - 221 - **The parasite lipoprotein Antigen B: a possible link between immunoregulation and lipid metabolism**

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The larvae of *Echinococcus granulosus* s.l. parasite (hydatid) grows in the viscera of the intermediate hosts (domestic ungulates, accidentally humans), causing a chronic infection. The hydatid is well adapted to its host since it is able to control inflammation and acquire host lipids (cholesterol) that the parasite cannot synthesize. Antigen B (EgAgB) is a 230 kDa lipoprotein containing around 50% in mass of lipids (polar and neutral), resembling the protein/lipid ratio of HDL_3. EgAgB protein moiety is encoded by five genes (EgAgB1-5) belonging to a cestode-specific family of hydrophobic ligands binding proteins of unknown function. EgAgB interfered with dendritic cell (DC) activation *in vitro*, but the modulatory mechanisms involved have not been elucidated. Since HDL's ability to remove cholesterol has been related to immunomodulation effects in innate cells, we hypothesize that EgAgB uptakes host cholesterol from myeloid innate cells, including DCs, contributing to regulate inflammatory activation pathways. We found that native EgAgB bound to DCs in a TLR4 and TLR2-independent manner. When co-administered with LPS, EgAgB inhibited TLR4 dimerization and cytokine (IL6, IL12, IFNβ) secretion, but not CD86 and CD40 expression in DCs. Furthermore, EgAgB inhibited LPS binding to DCs, suggesting it might neutralize LPS in the milieu as HDL_3 does, contributing to their inhibitory effects. In addition, in a mixed lymphocyte reaction, EgAgB seemed to favor a Th2-type differentiation profile. On the other hand, EgAgB promoted cholesterol efflux from THP-1 macrophages similarly to HDL and HDL_3. An increase of ABCA1 expression (induced by an LXR agonist) or blocking ABCA1-mediated efflux (by BLT4 inhibitor) did not affect EgAgB's ability to efflux cholesterol from macrophages, suggesting the involvement of other receptors. Further studies are needed to examine a putative relation between EgAgB's ability to efflux cholesterol and to regulate the activation of innate cells. **Keywords:** Lipoprotein;Dendritic cells;Cholesterol efflux.

DO - 222 - **Description Of Human Innate Response Immunomodulation by Uropathogenic**

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Urinary tract infections are one of the main causes of seeking medical attention. That is defined as a microbial invasion of any organ of the urinary tract, which has a sterile nature, from the urethra to the kidneys. Escherichia coli, a gram negative bacterium commensal to humans, is the most common agent isolated in 75-95% of acute urinary tract infections of bacterial origin. Causing disease in these organs, they are known as UPECs. Dendritic cells (DCs) are professional antigen presenting cells (APCs), essential in the early response to infections by acting as sentinels and bridging the innate and adaptive immune response of the host. Thus, we perform experiments to understand the interaction of DCs and UPECs. To that end, DCs were obtained from peripheral blood monocytes from healthy donors cultured in the presence of IL-4 and GM-CSF and infected with the V27 and J96 strains of UPECs for 24 hours. We observed the ability of UPECs to infect DCs. Cells were then analyzed acquired by flow cytometry to evaluate the expression of surface molecules such as CD11c, CD1a, CD83, CD62L, CCR7, CD209, HLADR, CD86, CD80, CD40 and CD274. After the DCs-UPECs interaction, we found that CD11c and CD209 (DC-SIGN) decreased its expression, we observed a tendency to decrease percentage and MFI of HLA-DR and na increase of CD80 increased and decrease in MFI mean CD86 expression. Evaluating cell death we observed that V27 strain induced death by apoptosis or a later apoptosis process in DCs. On the other hand, strain J96 seems to stimulated a tendency towards death by necrosis or late apoptosis. In conclusion, UPECs negatively regulate the expression of DC-SIGN ans CD11c, and costimulatory molecules such as CD86, in addition to induce cellular death in the DCs. In this way, bacteria semms to be able to mount a possible evasion mecanism of human immune response. **Keywords:** UPECs;dendritic cells;surface molecules.

DO - 223 - **STEROID HORMONES' MODULATION OF COVID-19 OUTCOMES IN DIABETIC PATIENTS**

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Introduction: COVID-19 can affect individuals of all ages, but severe cases are more common among the elderly, unvaccinated individuals with underlying health issues, and males. Diabetes mellitus (DM) hampers immunity, and studies have linked declining testosterone levels to changes in insulin receptors. Thus, our aim was to investigate how DM and reduced testosterone levels impact COVID-19 exacerbation in men. **Methods**: Study participants were enrolled before COVID-19 vaccines were available, with samples collected during domiciliary quarantine or at hospitals in Brazil from April 2020 to January 2021 (ImmunoCOVID consortium). The study included 96 uninfected female and male non-hospitalized volunteers (controls) and 182 SARS-CoV-2 positive patients of both sexes identified through RT-PCR or serological testing (IgM+). COVID-19 cases were categorized as non-severe or severe. **Results:** When compared to non-diabetics, men with diabetes were more obese, required more respiratory support, had hypertension, used corticosteroids frequently, and had lower testosterone levels. Men with severe COVID-19 and diabetes showed higher dehydrocorticosterone levels than non-severe patients and reduced adrenosterone hormone levels compared to severely affected women. Additionally, diabetic men with non-severe COVID-19 had lower plasma cortisone levels than non-diabetic men with mild COVID-19. These findings were independent of sexual hormone binding globulin (SHBG), which remained unchanged in comorbid patients. Diabetic men with COVID-19 experienced longer hospitalization periods, needed more respiratory assistance, and used corticosteroids more than women with the same condition. **Conclusion**: diabetes negatively affects COVID-19 outcomes in a sex-dependent manner. Understanding the interplay between pre-existing conditions and viral infections is vital for targeted interventions and improved outcomes in vulnerable populations. **Keywords:** COVID-19;Testosterone;Diabetes.

DO - 224 - **Selection and synthesis of immunogenic peptides from Leishmania species capable of exerting immunomodulatory roles**

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Leishmaniasis encompasses a complex group of parasitic diseases in tropical and subtropical areas of the world, caused by different species of protozoa of the genus *Leishmania*. Although there is increasing progress in the development of molecular technologies, there is still no effective human vaccine and the therapeutic arsenal is quite limited. Despite advancements in molecular technologies, the development of an effective human vaccine and the availability of therapeutic options remain limited. Therefore, the exploration of new antigens is crucial in the search for more effective treatments and innovative disease control approaches. This study aims to select and synthesize immunogenic peptides from *Leishmania* species capable of exerting immunomodulatory effects, primarily in a murine model. The *Immune Epitope Database* is a repository that catalogs immune epitopes and their associated experimental data. The search term "Leishmania" was used to identify *Leishmania* specific epitopes , followed by the application of criteria affinity with T lymphocytes and binding to major histocompatibility complex class I molecules. A total of 138 antigenic epitopes were selected, considering factors such as antigenicity and conservation, which are crucial for eliciting a strong and specific immune response. Among the selected epitopes, ten sequences exhibiting the highest immunogenicity scores were chosen for solid-phase synthesis. The peptide sequences were found to derive from proteins ATPase 2 subunit, Kinetoplast Membrane Protein 11, and Cysteine peptidase, all located in conserved regions and demonstrating high immunogenicity in previous studies. The synthesis procedures resulted in soluble peptides with high concentrations. Moving forward, these molecules will be utilized in immunization assays and therapy in a murine model of Leishmania infections. **Keywords:** peptide;leishmania;immunogenicity.

DO - 225 - **2´-FUCOSYL-LACTOSE HUMAN MILK OLIGOSACCHARIDE ISOLATION: EVALUATION OF ITS ACTIVITY IN INFLAMMATORY PROCESS AND ITS THREE-DIMENSIONAL CONFORMATION IN SOLUTION**

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INTRODUCTION: Human milk oligosaccharides (HMOs) present high homology with the cell surface of their species. Their main described functions are: competing with the host's mucosa for microorganism interaction; promoting the growth of the neonate's microbiota; and providing systemic defense to the host. The study aiming to evaluate the anti-inflammatory activity of 2'-fucosyllactose (2'-FL). METHODOLOGY: Step 1: For isolation of oligosaccharides, a delipidation process was performed by centrifugation, the material was then subjected to ethanol precipitation (2:1,v/v) for protein removal, and the supernatant was lyophilized and fractionated using a column (70 mL) with an activated *mesh* charcoal and celite 545 (1:1) as stationary phase. A continuous gradient of H2O:EtOH, ranging from 100:0 to 40:60, was used as the mobile phase. To obtain the different components of HMOs, samples were injected in a silica column chromatography. Step 2: The anti-inflammatory capacity of 2'-FL, isolated in the previous step, was evaluated by analyzing its Toll-like receptors (TLR) modulation activity. Step 3: structural characterization and conformational studies of 2'-FL by NMR and Docking assays. RESULTS: Step 1: a significant amount of 2'-FL with high purity (80.7%) was obtained. Step 2: compared to Lipopolysaccharide (LPS) treatment (positive control), 2'-FL at a concentration of 0.02 mg/mL reduced the LPS-induced inflammatory process by 26.26%, at 0.2 mg/mL by 24.18%, and at 2 mg/mL by 31.63%. Step 3: a molecular structure similar to a fishhook for 2'-FL was observed, and possible binding sites were identified for its interaction with TLR4 receptors (step 3). CONCLUSION: (1) the anti-inflammatory capacity of 2'-FL; (2) a fishhook-like structure pattern for 2'-FL in solution; and (3) possible binding sites between 2'-FL and TLR4, confirming the potential modulating inflammatory activity by HMO 2'-FL through the interaction of its fishhook-like structure with TLR4 receptors. **Keywords:** HMO;TLR;2´-FL.

DO - 226 - **NLRP3 and NLRP1/CARD8 pathways differently contribute to pyroptosis of CD8+ T cells of ART-treated HIV patients**

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Introduction: Chronic HIV-infected patients show immune dysregulation and inflammasome activation, contributing to immunologic dysfunction. The inflammasome, a cytosolic complex responsible for producing IL-1β and IL-18 and pyroptosis, is highly activated in HIV patients' peripheral blood mononuclear cells (PBMC), suggesting their involvement in this immune dysfunction. Our recent findings demonstrate the impact of the NLRP3 inflammasome pathway on HIV leukocytes. While monocytes, B cells, and CD4+ T cells were studied, limited knowledge exists of CD8+ T lymphocytes. Therefore, in this study, we characterize inflammasome activation in CD8+ T cells, focusing on the NLRP3 and NLRP1/CARD8 pathways previously described in T cells. **Methods:** The study adhered to ethical guidelines. CD8+ T lymphocytes from non-HIV healthy donors (HD;n=15) and HIV patients (HIV;n=12) were analyzed ex vivo and stimulated in vitro using known activators of NLRP3 (CD3/CD28) and NLRP1/CARD8 (DPP9 inhibitor ValboroPro, VbP). Caspase-1 activation, cytokine release, and pyroptosis were used to evaluate inflammasome activation and immunofluorescence to detect receptor/complex assembly oligomerization. **Results:** CD8+ T cells from HIV exhibited constitutively activated caspase-1 correlating with their activation cell state, confirming the relationship between inflammasome and CD8+ T cell alterations. These cells did not produce IL-1β or IL-18 but increased pyroptosis. HIV CD8+ T cells show higher activation and NLRP1/CARD8-pyroptosis resistance than HD. On the other hand, NLRP3-pyroptosis occurs exclusively in HIV CD8+ T cells. **Conclusion:** Pyroptosis primarily occurs via NLRP3 in HIV CD8+ T lymphocytes, whereas in HD, NLRP1/CARD8 plays a significant role, suggesting dysregulation of NLRP3 inflammasome observed in other leukocytes. Inflammasome activation in CD8+ T cells predominantly manifests as pyroptosis, potentially contributing to chronic inflammation in HIV patients. **Keywords:** Inflammasome; CD8+ T lymphocyte;HIV-1 chronic inflammation.

DO - 227 - **Effects of blue LED light phototherapy in macrophage culture infected by Leishmania amazonensis**

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Leishmaniasis is a tropical neglected disease caused by a protozoa parasite from over 20 *Leishmania* species. Cutaneous leishmaniasis (CL) is the most common form of the disease and affects mostly the tegumentary system leading to severe skin lesions. Currently, there are few therapeutic strategies to eliminate parasites such as the use of pentavalent antimonials, pentamidine and amphotericin B. However, characteristics such as high toxicity and the potential for generating parasite resistance led to the investigation of new compounds or even new strategies to eliminate the protozoan that can induce the necessary T helper-1 (Th-1) response from the infected organism. The blue light phototherapy (BLP) is a promising strategy capable to eliminate bacteria, fungus, and protozoan by changing in membranes, organelles, and oxidative pathways of these microorganisms. In this present study, BLP were evaluated in mice peritoneal macrophages infected by *Leishmania amazonensis*. The immune cells were plated on a 24-well plate with RPMI and then infected and treated for 24 and 48 hours with the BLP and with amphotericin, alone or combined with BLP. The supernatant of the culture was used to quantify the concentration of gamma interferon (IFN-γ) and interleukin 4 (IL-4). Previously results suggested that BLP induces *L. amazonensis* killing detected in axenic culture, suggesting a promising treatment for CL. Besides, the macrophages treated with the BLP presented a reduced infection rate and a higher production of the Th-1 cytokine IFN-γ in the culture supernatant. Therefore, it is concluded that the BLP can reduce the infection caused by the protozoan and induce the production of IFN-γ by the infected macrophages, resulting in a Th-1 like profile immune response and promoting the killing of intracellular parasites. This research was funded by CAPES and held in the Federal University of Minas Gerais. **Keywords:** Leishmaniasis;Phototherapy;Immune profile.

DO - 228 - **Impact of aerobic exercise intensity and pattern on macrophage polarization in healthy young adults. Preliminary data.**

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Exercise is proposed to improve macrophage function and encourage polarization towards an anti-inflammatory phenotype. The anti-inflammatory macrophage phenotype can be stimulated by the systemic environment modulated by exercise, which contributes to the reduction of chronic low-grade inflammation and the development of metabolic diseases. However, little is known about the impact of exercise intensity and pattern on macrophage polarization. We determined if aerobic exercise intensity – prescribed using lactate threshold, which elicits more specific/homogenous metabolic perturbations than maximal anchors – and pattern (interval vs. continuous) when controlling for energy expenditure impacts macrophage polarization and function. Active adults (8 males and 6 females; age: 30±3 yrs, BMI: 23.2±2.2 kg/m^2; VO_2peak: 51.6±4.2 ml/kg/min) completed a no-exercise control session (CTL) and three work-matched bouts of continuous or interval cycling above (heavy continuous or interval exercise; HCE or HIE) or below (moderate continuous exercise; MCE) lactate threshold. Blood was collected immediately post-exercise for isolation and polarization of CD14+ monocytes into pro-inflammatory (50 ng/mL lipopolysaccharide + 20 ng/mL interferon-y; M(LPS+IFN-y)) or anti-inflammatory (20 ng/mL interleukin 10; M(IL-10)) macrophages. Phenotypic (CD163 and CD80 membrane expression and tumor necrosis factor-α release) and functional (inflammatory suppression capacity after challenge with LPS alone or with IL-10) changes were determined. The percent expression (relative to untreated cells) of CD80 in M(LPS+IFN-y) decreased following MCE and HIE (-58% p=0.028 and -71% p=0.031, respectively) versus CTL, with no differences between HCE (-24% p<0.05) and CTL. These findings suggest that the decrease of pro-inflammatory macrophage phenotype following acute aerobic exercise appears to be independent of intensity, as long as the exercise above the lactate threshold is performed intermittently. **Keywords:** Exercise;Macrophages;Inflammation.

DO - 229 - **Previous gastrointestinal infection leads to failure in local regulatory T cell function in gut mucosa and interfere in susceptibility to inflammatory disorders**

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Unraveling the etiology of chronic and inflammatory disorders, such as inflammatory bowel disease (IBD) and colorectal cancer (CRC), is a complex challenge to overcome. Here, we hypothesized that mucosal infection could represent a confluence point to address this question. In this context, after *Yersinia pseudotuberculosis* (YP) gastrointestinal infection, there is a permanent remodeling in immunological and lymphatic systems in the mesentery, leading to chronic inflammation that blocks canonical regulatory responses in the gut mucosa (Cell, 163:354-366, 2015). Here we analyzed whether this process would affect the progression of IBD and found that following dextran-sodium-sulfate treatment, YP-infected mice developed a severe and systemic form of IBD due to failure of local regulatory T cell function. Also, this defect in gut regulatory components impacted local and systemic immunosurveillance, preventing the azoxymethane-induced CRC but promoting lung tumors. Therefore, our data show that mucosal infections can shape tissue immunosurveillance and define the outcome of complex chronic disorders. **Keywords:** Microbiota;Inflammatory Bowel Disease;Colorectal Cancer.

DO - 230 - **Chronic inflammation driven by high dose of Ascaris suum eggs inhibits primary tumor growth and lung metastases in an experimental model of murine breast carcinoma**

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The inflammatory microenvironment can promote or inhibit the evolution of some types of cancer. *Ascaris* sp. for, exemple, is a helminth capable of modulating the immune response in a host. Breast cancer is the most prevalent type of tumor in women and it is a public health problem, just like ascariasis. The relationship between these diseases has never been explored. Thus, we aim to investigate the relationship between the chronic inflammation promoted by Ascaris suum infection with the progression of breast cancer and lung metastases in experimental model. Female BALB/c mice were infected with 2500 embryonated A. suum eggs. After 14 days, we induced cancer with $1x10^6$ cells from the 4T1 cells line. Through scintigraphic images with radiopharmaceuticals, Ex vivo experiments using radiation and histopathology analysis, we showed that the inflammation promoted by high doses of *A. suum* eggs were able to reduce the size of the primary tumor and decrease the number of metastatic foci in the lung. Furthermore, histopathological analysis showed increased areas of inflammatory infiltrate, NAG, EPO and MPO levels in the lung. Cytokines were measured by CBA and IL-10, IL-17, TNF and IL-5 were the main increases. Due to the eosinophilia induced by A. suum infection, we decided to conduct a pilot study to evaluate survival between a wild type (WT) group and $GATA1^{-/-}$ both with cancer and without infection. Our results revealed that the absence of GATA1 promoted a decrease in animal survival ($p<0.05$). In summary, high doses of *A. suum* infection contributed to reduction of metastatic foci in animal's lung without worsening its clinical condition in general. Furthermore, we observed that eosinophils driven growth control of metastatic foci due to enrichment in the lung microenvironment, which will lead us to further investigations in the future to better understand its role in breast cancer and in association with helminth infection. **Keywords:** Breast cancer;parasitic diseases;Risk factor.

DO - 231 - **Interleukin 33 derived from sciatic nerve mesenchymal cells helps the differentiation of monocytes-derived dendritic cells leading to the maintenance of nerve degeneration**

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Wallerian degeneration (WD) is a process of degradation of myelin remnants and axons after damage to the peripheral nervous system. The IL-33 cytokine belongs to the IL-1 superfamily and has been described as important for activating immune cells via the ST2 receptor. We aimed to investigate the role of IL-33 in the development of WD. We integrated scRNA-seq data from the sciatic nerve of mice and evaluated the presence of IL-33, we observed that IL-33 is expressed by mesenchymal cells. We isolated $CD45^+$ cells from the sciatic nerve of WT and *Il33*$^{-/-}$ mice 3 days after PNI and performed scRNA sequencing. We observed different clusters of monocyte-derived cells, in which the frequency of these cells was decreased in *Il33*$^{-/-}$ animals. We observed ST2 expression mainly in monocyte-derived dendritic cells (MoDC) and confirmed by flow cytometry that these cells are the most positive for $ST2^+$ when compared to other populations. In addition, we performed flow cytometry on days 1, 3, 5 and 7 after PNI and observed a decrease in monocyte maturation $CCR2^+Ly6C^{int}$ and $CCR2^+Ly6C^-$ cells from the 3rd day on, as well as in MoDC. To confirm that IL-33 is important in MoDC differentiation, we performed a pseudo-time in our scRNA-seq data and observed that MoDC from WT was at a higher stage of differentiation than from *Il33*$^{-/-}$. Furthermore, we isolated bone marrow monocytes and cultured them for seven days in the presence of GM-CSF and IL-4, and added IL-33. Thus, we observed that cells in the presence of IL-33 increased the frequency of $CD11b^+CD11c^+$, as well as the expression of MHCII. At the very least, we bred ST2-deficient animals in cells expressing Lyz2 (*Lyz2*^{Cre}*-Il1rl1*^{Flox}) and observed a reduction in the number of MoDC and maturing monocyte cells on the 3rd day. We conclude that IL-33 released during WD acts directly on the differentiation, activation and specialization of monocyte-derived cells. Becoming important for the inflammatory and degenerative state of the sciatic nerve. **Keywords:** Interleukin 33;Monocyte-derived cells;Inflammation.

DO - 232 - **DENDRITIC CELLS IN RECOGNITION AND DEVELOPMENT OF LEISHMANIA INFECTION IN VITRO TO DC-SIGN MOLECULE (CD209)**

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The Leishmaniasis is a neglected tropical disease. In Brazil the species *Leishmania infantum* (Li) and *Leishmania braziliensis* (Lb) are responsible for the Visceral Leishmaniasis and Cutaneous Leishmaniasis froms, respectively. In the human host, they can be recognized by dendritic cells (DC) for DC-SIGN (CD209) and *Leishmania* has several mechanisms for evading the DC response as modulation of surface molecules. So, the objective of this study was to analyze the interaction of DC in co-culture with Li or Lb, after of blocking the DC-SIGN molecule in DC. For that, DC derived from monocytes of human peripheral blood with IL-4 and GMCSF, after 7 days the DC were incubated with Li or Lb, previously marked with CFSE, after 24h the surface molecules of DC were marked, and the cells were acquired by flow cytometry. The results showed the increased expression of CD83 (CN: 5,200%+/-4,616; Lb: 38,20%+/-12,74; Li: 64,90%+/- 16,00; Lb+anti-DC-SIGN: 27,40%+/- 14,85; Li+anti-DC-SIGN: 63,30%+/-14,23) and CCR7 (CN: 0,2800%+/-0,2341; Lb: 19,60%+/-10,06; Li: 38,50%+/-14,66; Lb+anti-DC-SIGN: 9,110%+/-13,57; Li+anti-DC-SIGN: 48,30%+/-13,68), the maturation process and targeting the lymph node for in infected DC. On the other hand there the lymphocyte activation mechanism showed no significant differences as HLA-DR (CN: 99,00%+/-0,4212; Lb: 99,60%+/-0,4301); Li: 99,60+/-0,3487; Lb+anti-DC-SIGN: 98,50%+/-0,6120; Li+anti-DC-SIGN: 98,40%+/-0,6071), and CD86 (CN: 93,10%+/- 4,742; Lb: 97,00%+/- 4,456; Li: 79,50+/-11,35; Li+anti-DC-SIGN: 89,30%+/- 5,520; Lb+anti-DC-SIGN: 91,60%+/- 4,005). In view of the results was observed the modulation of molecules of lymphocyte exhaustion PD-L1 (NC: 47,90%+/- 17,40; Lb: 57,20%+/- 16,95; Li: 74,00%+/- 15,32; Lb+anti-DC-SIGN: 69,80%+/- 17,73; Li+anti-DC-SIGN: 50,20%+/- 17,07). Furthermore, the blocking of DC-SIGN has not been shown to prevent infection in DC and has not altered the expression of the profile of molecules compared to unblocked DC. **Keywords:** Dendritic cells;DC-SIGN;Leishmania.

DO - 233 - **Evaluation of CD8-mediated non-specific T cell damage in the lung of patients with COVID-19**

LOPES, P.D.O.[1]; DANIEL, C.B.[2]; SARMENTO, I.V.[1]; DE MOURA, R.G.[1]; BARBOZA, B.D.A.[1]; DOLHNIKOFF, M.[3]; MAUAD, T.[3]; ZEIDLER, S.L.V.V.[2]; AKBAR, A.[4]; GOMES, D.C.D.O.[1]. 1. NÚCLEO DE DOENÇAS INFECCIOSAS, UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. DEPARTAMENTO DE PATOLOGIA, UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 3. DEPARTAMENTO DE PATOLOGIA, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. DIVISION OF MEDICINE, UNIVERSITY COLLEGE LONDON, LONDRES - REINO UNIDO.

Patients who develop more severe symptoms of COVID-19 often present an acute inflammatory lung injury characterized by the presence of acute respiratory distress syndrome (ARDS). Senescent cells accumulate in tissues during ageing, chronic stimulatory processes and infections. These cells present phenotypic and functional disorders, resulting in the inability to control pathogens, as well as tissue pathogenesis mediated through intense inflammatory activity. The latter favours the acquisition of natural killer cell receptors (NKRs) on CD8 T cells, and their ligands (NKRL) in other cell populations. In this work, using immunohistochemistry and immunofluorescence techniques, we evaluated the presence of senescent cells in the lung injury of patients with fatal COVID-19 and the expression of the NK cell receptor (NKG2D), and his ligand MICA/B. Our analyses demonstrate diffuse alveolar damage (DAD) in patient's lung showing intense inflammatory exudate in the intra-alveolar and interseptal regions. Compared to healthy controls, patients with COVID-19 showed an intense cellular inflammatory infiltrate, with an accumulation of CD8 T cells and senescent alveolar macrophages (CD68+). In addition, analyses revealed that the presence of cells expressing MICA/B, is significantly more closely associated with macrophages. Interestingly, no differential expression was seen in lung epithelial cells (AE1AE3). The presence of cells expressing NKG2D was determined in the lung lesion, however, compared to healthy controls, we did not find its expression associated with infiltrating CD8 T lymphocytes. It's possible that damage mediated by non-specific antigen activation of CD8 T cells could be led by other NK cell ligands. The results of this work contribute to a better understanding of the participation of senescence and the activity of NKRs expressed by senescent T cells in the immunopathogenesis of COVID-19. **Keywords:** Immunophatology;Immunosenescence;COVID-19.

DO - 234 - **Combinatory immune checkpoint blockage potentially compromising selection of B cells in tDLNs in murine breast cancer model**

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Despite their homeostatic role in balancing the immune response, it is well recognized that tumors cells can hijack the mechanisms of immune checkpoint (IC) molecules (CTLA-4, PD-1/PD-L1), by promoting their sustained overexpression in the tumor microenvironment, and ultimately contributing to tumor expansion (Nat. Rev. Cancer 12:252-264, 2012). In the last decades, immune checkpoint blockade (ICB) has brought major advance to the treatment of solid tumors. Although studies have been primarily focused on understanding the effect of ICB in tumor-infiltrating T cells, emerging evidence shows that B cells may also be affected, yet little is known. Considering the higher and co-expression of PD-1 and LAG-3 in triple-negative breast tumors, and recent studies demonstrating reestablishment of the immune response in the tumor microenvironment, we hypothesized that the therapy could also affect B-TILs. Here, using a syngeneic murine breast model we demonstrated that combinatory anti-PD-1/LAG-3 caused major abrogation in E0771 murine tumors compared to untreated and isotype. Interestingly, despite causing elevation of infiltrating TCD4 and TCD8 cells in a dose-dependent manner, B-TILs were not affected, suggesting that the effect of ICB is mostly restricted to T cells. Though no major changes were seen for B-TILs, we observed a disrupted elevation in the germinal center B cell compartment in both non-draining (nDLN) and tumor-draining (tDLN) lymph nodes of treated mice. Interestingly, despite affecting the GC B cell compartment in either LNs, only tDLN presented an expressive elevation of plasma cells. Although treated mice presented a skewed elevation of serum IgG1 antibodies, we couldn’t detected higher antigen recognition when we assayed GC-produced antibodies against E0771 proteins. Though treatment enhanced cellular immune response in the TME, combinatory anti-PD-1 + anti-LAG-3 could not cause changes in B-TILs. **Keywords:** Immune checkpoint;Breast cancer;B cell.

DO - 235 - **Durability of cellular and humoral responses to booster vaccination against Covid-19: a longitudinal study.**

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Vaccination against SARS-CoV-2 has been effective in reducing new infections and morbidity by Covid-19 worldwide. However, as new variants of concern (VOCs) arise, questions regarding the persistence of the immune response generated by vaccines and whether it can protect against emerging VOCs are still of primordial relevance. This study aimed to longitudinally quantify cellular and humoral responses after homologous or heterologous Covid-19 booster vaccine dose. Anti-RBD IgG response was measured by ELISA and expressed as BAU/ml, and Anti-spike CD4 and CD8 T cell response was measured by IFN-gamma ELISpot and expressed as SFU/10^6 cells. Individuals (n=694) were recruited from an ongoing DETECTCoV cohort at 120 days maximum before their first booster dose and followed up to 15 months after the second booster dose. Before receiving booster vaccination, individuals immunized with the inactivated vaccine had significantly lower Spike-specific spot-forming units (SFUs) counts and anti-Spike IgG BAU/mL levels compared to adenovirus vectored and mRNA vaccines. Also, individuals with previous COVID-19 infection had elevated anti-SARS-CoV-2 humoral and cellular response. However, homologous or heterologous booster vaccine dose increased peripheral humoral response among all vaccine platforms. B and T cell response declined to below the detection limit in almost all individuals by 6 months. In contrast, booster vaccination induced antigen-specific memory B and T cells that persisted and remained stable for at least 9 months post second booster. The durability of serum antibody responses improves only marginally following homologous booster immunizations compared to heterologous booster. These findings describe the durability and magnitude of immunological responses elicited by different Covid-19 vaccines over time, but functional assays are needed to further correlate it with protection against SARS-CoV-2. **Keywords:** Covid-19;T cell;antibody.

DO - 236 - **Presence of viral particles and cellular origin of extracellular vesicles in patients with COVID-19**

DE CARVALHO, J.F.N.[1]; BARBOSA, G.R.[1]; SILVESTRINI, M.M.A.[2]; SANTOS, S.S.[1]; DE FREITAS, F.G.R.[3]; BELLEI, N.[1]; DE CARVALHO, A.T.[2]; TORRECILHAS, A.C.T.[4]; SALOMÃO, R.[1]. 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. INSTITUTO RENÉ RACHOU (FIOCRUZ MINAS), BELO HORIZONTE - MG - BRASIL; 3. HOSPITAL SEPACO, SÃO PAULO - SP - BRASIL; 4. UNIFESP, DIADEMA - SP - BRASIL.

Objectives: To analyze and characterize by flow cytometry the Extracellular vesicles (EVs) isolated from plasma samples of patients with sepsis with COVID-19 and to verify the presence of viral particles, as well as variants of SARS-CoV-2. **Methods:** Plasma samples were collected from patients with COVID-19 and sepsis (N=42) hospitalized between 03/2021 and 08/2021 at Hospital Sepaco, São Paulo, and from healthy individuals (N=09). Here, we selected 20 patients with the highest EVs concentrations (particles/mL) by Nanoparticle Tracking Analysis (NTA). The presence of SARS-CoV-2 viral particles was verified in EVs using the RT-qPCR kit directed at the RdRp, Envelope (E) and Nucleocapsid (N) genes. Flow cytometry (CytoFlexS) was used to characterize EVs, phosphatidylserine (Annexin V) and tetraspanins (CD9 and CD81), and the cellular origin of platelets (CD42a), endothelial cells (CD144), T lymphocytes (CD3) and neutrophils (CD66b). **Results**: RT-qPCR showed gene amplification in 14 EVs samples from patients and none of the controls. In two samples a Gamma variant was confirmed. Levels between EVs and cellular origin markers were quantified by flow cytometry, with no significant differences between controls and patients. Among patients, the mean percent expression of Annexin V-labelled EVs with markers of platelets origin CD42a (18.84% ±9,409) followed by CD3 T lymphocytes (15.55% ±4,647), CD66b neutrophils (10.39% ±3,756), CD144 endothelial cell (7,88% ±1,983). In the analyzes of the cellular origin of EVs comparing the differences in the clinical outcome of the patients such as days of hospitalization, hospital discharge vs death there were no significant differences for each marker.**Conclusion:** EVs showed potential carriers of SARS-CoV-2 viral particles, contributing to its propagation and making healthy cells more susceptible to infection. In the flow cytometry evaluating cellular origin revealed higher proportion of platelets origin. **Keywords:** Extracellular Vesicles;COVID-19;Sepsis.

DO - 237 - **Construction and production of chimeric antigens for use in vaccine formulations against the cattle tick Rhipicephalus microplus**

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Background Ticks are hematophagous ectoparasites that transmit diseases to humans and animals. Among them, the tick *Rhipicephalus microplus* is considered one of the most harmful parasites for cattle in tropical and subtropical regions, posing a significant challenge to cattle farming productivity. Control of *R. microplus* is primarily achieved through the use of chemical acaricides. However, the extensive use of acaricides has led to environmental poisoning, contamination of animal products, and the development of acaricide-resistant tick populations. Therefore, there is a need for more sustainable and efficient methods, such as vaccines, to control bovine ticks. Our research group has conducted experimental immunizations of dairy cattle using a combination of nine recombinant antigens derived from *R. microplus* saliva. Our formulation exhibited a protective response with an efficacy of 78%. However, a challenge in the production of this vaccine is the expression of multiple proteins in sufficient and equivalent amounts to effectively immunize cattle. **Objective** To simplify production, we designed three polycistronic inserts, each containing three tested antigens. **Methods and Results** We performed computational analysis, including structural predictions of nine chimeric proteins, using the Robetta server and RoseTTAFold analysis based on homology modeling. Pet 17b plasmids containing the designed sequences, with different spacers between each of the three proteins were acquired. Chimeras were produced by *Escherichia coli* strains at varying temperatures. The chimeras Q2 and Q5, containing helical rigid linkers, and Q7, containing semi-rigid linkers, showed the highest yield. Q2 and Q5 were successfully produced in *E. coli* Rosetta at 37°C, while Q7 was produced in *E. coli* Artic at 30°C. Currently, we have a minimum of 10 mg of each chimera, which will be used in immunogenicity assays in mice and a randomized clinical trial in dairy cattle. **Keywords:** veterinary vaccinology;chimeric antigens;chimeric linkers.

DO - 238 - **Unraveling Conformational B-cell Epitopes of Vaccine Antigens from Rhipicephalus microplus Tick Salivary glands using Next Generation Phage Display and Bioinformatics**

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Rhipicephalus microplus is a main problem in livestock activity worldwide. Current methods to control ticks have limited efficacy and poison the environment. Therefore, the development of vaccines is required as a sustainable alternative. Our group has developed a multi-antigen anti-tick vaccine that was ~78% protective against the cattle tick. Nowadays, we are developing new chimeric vaccines based on epitopes from the protective formulation. In this study, we screened the conformational B-cell epitopes of different protective antigens by Next-Generation Phage Display (NGPD) and bioinformatics approaches. First, IgG purified from the sera of protected and non-protected bovines were separately subjected to biopanning with phages displaying linear or constrained random peptides. Second, the phages selected by the IgG were recovered by competitive elution with the different antigens, and the peptide-coding region was amplified by PCR and submitted to Next-Generation Sequencing (NGS). Third, the NGS data were analyzed using a bioinformatics pipeline to: (1) identify the enriched peptides (Z-score>4) by comparing the peptides selected by sera from tick-protected and non-protected bovines; (2) identify exclusive peptides for each antigen; and (3) track the potential protective epitopes in the three-dimensional (3D) structure of each antigen using PepSurf on models predicted by RoseTTAFold. Data showed that 2,444 to 44,085 different peptides were selected by the bovine IgG, of which 4 to 8,866 (0.2 – 22.7%) were found to be exclusive to each antigen. Finally, we determined the position of the amino acids that compose the potential B-cell epitope on the surface of the 3D structure. In conclusion, our study has shown utility of NGPD and bioinformatics approaches for mapping potential B-cell epitopes of tick antigens. We have also identified epitopes to design new anti-tick vaccines for bovines. Funding: FAPESP 2015/09683-9; 2022/07400-3. **Keywords:** Phage display;Next-Generation Sequencing;Anti-tick vaccine.

DO - 239 - **Patients with cutaneous leishmaniasis accumulate T cells with senescence-associated features in the skin**

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Leishmaniasis is a group of diseases characterized by a complex interface between innate and adaptive immunity, which are responsible for the host resistance or pathogenesis of the infection. In the cutaneous forms, ulcers are formed due to an exacerbated immune response, generated by a complex interaction of inflammatory and regulatory processes. Recently, we have demonstrated that cellular senescence plays a role in cutaneous leishmaniasis (CL) immunopathogenesis, as patients accumulate cytotoxic senescent T cells in the blood and skin, and are associated with an highly inflammatory and cytotoxic environment, features that are known to induce cellular senescence. In this work we extended these findings by further analyzing the skin of CL patients for core features present in senescent cells. Immunofluorescence data shows patients with CL accumulate $CD4^+$ and $CD8^+$ cells in the lesions. Additionally, we demonstrate that the senescence markers CD57 and KLRG1 are highly expressed in skin lesions when compared to the skin of healthy individuals. Furthermore, we examined the expression of the senescence markers p16, ATF2 and y-H2Ax, which are associated with cell cycle arrest, cellular stress pathways and DNA damage, respectively. Compared to healthy skin, $ATF2^+$, $p16^+$ and $y\text{-}H2Ax^+$ T cells accumulate in the lesions. Collectively, these data indicate that T cells with senescence phenotype accumulate in the lesions during CL. This accumulation could be associated with progression of the disease, as senescent T cells are known for their highly cytotoxic features, making them a potential target for future immunotherapies. **Keywords:** Leishmaniasis;Senescence;Skin immunology.

DO - 240 - **Unveiling the immune response of oncologic patients against SARS-CoV-2 infection: An in-depth analysis of soluble and cellular factors leading to recovery or death.**

OSHIRO, F.P.[1]; FIGUEIREDO, A.B.[2]; GALDINO, N.A.D.L.[2]; CAVALCANTI, C.M.[2]; MORAIS, K.L.P.[2]; DUTRA, W.[3]; SILVA, B.G.D.M.[1]; FERIANI, D.[1]; ABRANTES, F.D.A.[1]; FRANÇA, I.L.[1]; SCHMIDT FILHO, J.[1]; FRAMIL, J.V.D.S.[1]; CESCA, M.G.[1]; BATISTA, M.V.[1]; GOLLOB, K.J.[2]. 1. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

In 2019 an outbreak of unknown pneumonia was identified as the Coronavirus Disease 2019 (COVID-19). The illness, caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has impacted many aspects of human life ever since. Despite the efforts to better understand the pathology, studies conducted on specific populations were lacking. In case of oncologic patients broad recommendations were made as a way to provide protection, but with little information about the immune response of individuals with different types of cancer. In this study we evaluated the systemic immune response of oncologic patients through the analysis of soluble and cellular factors present in blood collected within the early days of COVID-19 diagnosis. Our analysis shows that, among individuals with hematologic malignancies that progressed to death, there was an overall lack of T cell populations, as well as an exacerbated production of multiple inflammatory factors. Conversely, patients with higher frequencies of lymphocytes, activated B cells, and increased production of antibodies had only mild COVID-19. Among the solid tumors cohort we observed a less pronounced impact of soluble immune factors. Patients with a more regulated immune response had better outcomes, whereas individuals that progressed to death presented multiple exhausted T and B cell populations, and lacked a clear regulatory T cell population. Curiously, while exhaustion of T cells seems impactful for oncologic patients in general, the loss of specific lymphocyte subpopulations and possible cytokine-release syndrome observed among hematologic patients could be the most important factor leading to death, whereas the lack of immune regulation and broad exhaustion profile probably had a greater impact for patients with solid tumors. Our work helps to shed light into the mechanisms associated with death and recovery among oncologic patients, highlighting the impact of the immune system in the outcome of COVID-19. **Keywords:** SARS-CoV-2;COVID-19 in oncologic patients;Systemic immune profile.

DO - 241 - **The impact of short-term fasting and STING activation on metabolic-functional changes of T lymphocytes and antitumoral therapeutic efficacy**

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Cutaneous melanoma is the leading cause of death from skin cancer worldwide and shows relatively high resistance to treatment with radiotherapy and chemotherapy. The partial success achieved with the use of immunotherapy, especially checkpoint inhibitors, suggests that immunological interventions are the way to go. The signaling of the STING-dependent DNA recognition pathway in tumor cells, endothelial cells, and dendritic cells is important for inducing an antitumor immune response. In particular, the activation of dendritic cells by capturing DNA from tumor cells undergoing apoptosis and the production of type I IFN (interferon) are key factors for the activation of CD8+ T lymphocytes and tumor control. Although highly effective in experimental models, STING activation-based therapies still need improvement in the clinical context. Short-term fasting (STF) has been considered as an adjunct strategy to anticancer therapy because it induces the effect of differential stress resistance, in which tumor cells are more affected than normal cells due to low energy levels during cancer treatment, thereby increasing its efficiency. In this project, we hypothesize that short-term fasting and STING activation lead to metabolic-functional changes in T lymphocytes that promote an effective immune response against tumors. Our results showed that short-term fasting had less success in controlling tumor growth in STING knockout animals, suggesting the importance of this signaling pathway in the antitumor response in the context of metabolic stress caused by fasting. **Keywords:** STING;short term fasting;melanoma.

DO - 242 - **Non-canonical autophagy regulates the expression of IL4-Rα in response to apoptotic cells**

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Multiple insults induce inflammation that can lead to tissue damage. Once the insult is eliminated, the induction of resolution dampens the inflammation and promotes tissue repair to reestablish homeostasis (Immunity. 44:450-462, 2016). Macrophages (MOs) play an essential role in the resolution phase of the immune response. Of note, the phagocytosis of dead cells, known as efferocytosis, is coupled to signaling by the cytokines IL-4 or IL-13 through IL4-Rα to promote the genetic wiring of MOs for efficient tissue repair (Science. 356:1072-1076, 2017). The degradation of apoptotic cells (ACs) by MOs relies on the lipidation of the protein LC3 in the phagosomal membrane by non-canonical autophagy (Cell. 175:429-441, 2018). Based on these evidences, our hypothesis is that non-canonical autophagy shapes the anti-inflammatory and tissue repair genetic reprogramming of MOs in response to efferocytosis in the context of resolution. Corroborating our hypothesis, our preliminary data evidence a reduction of the expression of IL4-Rα on the surface of bone marrow-derived macrophages (BMDM) deficient in Rubicon (RUBCN), a component of non-canonical autophagy pathway, in response to ACs. This reduction also occurs at the gene expression level by qPCR analysis. Functionally, IL-4Rα reduction in BMDM Rubcn$^{-/-}$ in response to ACs was associated with impaired expression of anti-inflammatory markers, such as CD206. To analyze this response in situ, we evaluated the role of RUBCN in MOs using a conditional deletion model and induction of peritonitis. We observed reduced IL4-Rα expression on the surface of peritoneal MOs from LysM-Cre^{+} Rubcn$^{fl/fl}$ mice compared to WT littermate controls. Taken together, these data indicate that non-canonical autophagy regulates the expression of IL4-Rα in MOs in response to ACs, which suggest a mechanism that licenses MOs to its anti-inflammatory function in the context of resolution. **Funding: FAPESP (18/25559-4), CAPES (88887.825497/2023-00). Keywords:** tissue repair;non-canonical autophagy;efferocytosis.

DO - 243 - **Natalizumab induces regulatory B cells in the peripheral blood of multiple sclerosis patients**

FERRARI, B.B.[1]; LIMA, A.D.D.R.[1]; PRADELLA, F.[1]; DE CARVALHO, R.M.[1]; RIVERO, S.L.S.[1]; AMORIM, L.D.S.[2]; ALVES, N.M.[1]; DE OLIVEIRA, E.C.[3]; DAMASCENO, A.[1]; STELLA, C.R.A.V.[1]; DE OLIVEIRA, E.M.L.[2]; DOS SANTOS, L.M.B.[1]. 1. UNIVERSITY OF CAMPINAS - UNICAMP, CAMPINAS - SP - BRASIL; 2. PAULISTA SCHOOL OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO - UNIFESP, SÃO PAULO - SP - BRASIL; 3. FACULTY OF SOROCABA - PAULA SOUZA CENTER OF TECHNOLOGICAL EDUCATION, BIOMEDICAL SYSTEMS - FATEC, SOROCABA - SP - BRASIL.

Background and aim: Multiple sclerosis (MS) is an autoimmune disease that causes inflammation in the central nervous system. Natalizumab (NTZ) is a monoclonal antibody that blocks the α4 domain of the VLA4 integrin of leukocytes, maintaining these cells in the periphery. NTZ is used for treating the relapsing/remitting form of MS. Studies show no increase in regulatory T cells with this treatment; however, little is known about the regulatory function of B cells. Thus, we aim to evaluate the potential role of B cells in downregulating the self-reactive T cells that remain in the peripheral blood during the treatment with natalizumab. **Methods:** The project was approved by the ethics committee under number CAAE: 99798918.0.0000.5404. Peripheral blood mononuclear cells (PBMC) of healthy donors (49) and MS patients treated with NTZ (37) and dimethyl fumarate/DMF (34) were isolated and labeled for analysis by flow cytometry (FACSymphony A5 BD). We investigated the B cell phenotype and the production of anti-inflammatory cytokines. CD19+ cells were purified from PBMC to perform RT-PCR and stimulated with IL-21, anti-BCR, and ODN-CpG. TGF-β was measured by ELISA in the supernatant. **Results:** B cells from NTZ-treated MS patients are significantly increased in the peripheral blood. A significantly higher expression of anti-inflammatory cytokines such as TGFb-1 and IL-27/IL-35 compared to healthy controls and MS-DMF groups was demonstrated in CD20+CD5+ B cells. Moreover, a significantly increased production of TGFb was demonstrated in the supernatant of purified MS-NTZ B cells stimulated in culture. **Conclusions:** Our results indicate that NTZ treatment may induce B cells with regulatory profiles expressing anti-inflammatory molecules, which downregulate the inflammatory response of autoreactive T cells that remain in peripheral blood during the treatment.
Financial support: FAPESP, CAPES, CNPq (INCT-NIM), and Biogen Idec. **Keywords:** Regulatory B cells;Anti-inflammatory cytokines;Relapse-remitting multiple sclerosis .

DO - 244 - **Investigation of the role of neutrophil extracellular traps in the pathophysiology of inflammatory bowel disease**

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are complex and chronic inflammatory disorders characterized by inflammation in the intestines. Recent evidence has shown the presence of neutrophil extracellular traps (NETs) in intestinal samples from patients with Crohn's disease and ulcerative colitis. However, the biological mechanisms through which NETs may contribute to the progression of the disease have not yet been investigated. Therefore, the objective of this study is to evaluate the role of NETs in experimental IBD and investigate the mechanisms by which NETs contribute to the development of colitis. In this study, female C57BL/6 mice were exposed to a 2.5% DSS solution in their drinking water to induce acute colitis. The weight of the mice and clinical signs of the disease were monitored to assess the disease's clinical score. After euthanasia, the length of the colon was measured, and colon samples were processed to obtain tissue sections for histological analysis. Immunofluorescence was used to detect NETs, and the levels of NETs and cytokines in the colon homogenate were measured using the ELISA assay. The study was approved by the Ethics Committee for the use of animals under protocol number 079/2021. Mice exposed to 2.5% DSS exhibited significant weight loss, colon shortening, and an increased clinical disease score. Furthermore, the levels of NETs were significantly higher in the colon samples of DSS-exposed mice. Mice treated with GSK-484, an inhibitor of NET formation, showed less weight loss, lower clinical scores, reduced colon shortening, and decreased production of IL-6 in colon homogenate samples. In summary, our study demonstrated that NETs increase during DSS-induced acute colitis and appear to actively contribute to the pathogenesis of intestinal inflammation. As a future perspective, this study will investigate whether NETs can alter the populations of gamma delta lymphocytes, Th1, and Th17 subsets. **Keywords:** Neutrophils;intestinal inflammation;NETs.

DO - 245 - **Selection of potential repurposed drugs through High Content Screening and In Vivo Evaluation in IFNAR-/- Mice Model in Oropouche orthobunyavirus infection**

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Drug discovery is a complex and resource-intensive process, that requires a huge expenditure of investment and time. In the context of tropical diseases, drug repurposing emerges as a promising alternative for identifying potential treatments. This alternative consists in using a drug that is already approved for human use, to treat another disease. Oropouche fever is a dengue-like disease caused by *Oropouche orthobunyavirus (OROV),* a virus transmitted by mosquitoes mostly in the Amazon region and is now spreading through Latin America. Additionally, there is no specific antiviral treatment nor vaccine. For that reason, in this study we have screened a library of compounds approved for human use, using drug repurposing to identify possible treatment candidates. Firstly, we've done a high content screening using VERO cells treated with a library to check the cytoprotective effect of those compounds. After that, we performed another assay with selected compounds in a human cell lineage of hepatocytes (HuH 7.0) to validate one promising candidate. Additionally, we used a HuH 7.0 lineage knock-out for RIG-I cells (HuH 7.5) to explore the importance of this immune pathway in this context, but there were slightly differences in the reduction of viral load observed between these cell types, which suggest that our compound does not stimulate RIG-I response. To assess the compound's efficacy in vivo, we aimed to develop a mice model of infection with OROV. Initially, we attempted to establish a young wild-type mice model using C57BL/6 mice, but we found that even with three weeks, the wild-type mice did not replicate the virus. As a result, we used a IFNAR knock-out six-week-old-mice model to study the viral load in that context and evaluate the efficacy of the drug *in vivo*. In conclusion, we found a compound that is cytoprotective and reduces OROV viral load in different cell lines and established a mice model to test the efficacy of the compound *in vivo*. **Keywords:** antiviral;arbovirus;orthobunyavirus.

DO - 246 - **The use of D81 and DETC association in the treatment of leishmaniasis in vitro**

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Current treatment for leishmaniasis relies on highly toxic drugs that cause undesirable side effects. Therefore, the search for new drugs with high leishmanicidal activity and reduced toxicity is crucial. This study assesses the *in vitro* anti-leishmanial activity of Diethyldithiocarbamate (DETC), a copper chelator, and D81, an inorganic compound with an amine group. The combination of these drugs targets *Leishmania (L.) braziliensis, L. amazonensis, and L. infantum* by inhibiting SOD1 and increasing superoxide and nitrite peroxide production. Toxicity tests were conducted on the promastigotes and amastigotes of the mentioned *Leishmania* species, and murine macrophages derived from BALB/C mice. The 50% inhibition index was 33.03µM for D81, 0.247µM for DETC, and 1.676µM for the combination (1:2.5), with similar results for all three species. The inhibition index for murine macrophages was 237µM for D81, 11.47µM for DETC, and 67.16µM for the combination (1:2.5). The combined use of 20µM DETC and 500µM D81 demonstrated an antagonistic action, resulting in no inhibition of cell viability in murine macrophages, indicating less toxicity than the individual drugs. Conversely, the combination resulted in a synergistic effect and complete inhibition of cell viability in *Leishmania* species. The combination of DETC and D81 reduced the infection rate by over 44% and the number of amastigotes per 100 cells by more than 77% in murine macrophages infected by *L. braziliensis*, *L. amazonensis*, and *L. infantum*, compared to the control group. Preliminary results also showed a similar reduction in human macrophages infected with *L. infantum*. In conclusion, the association of D81 with DETC demonstrated potent antiproliferative effects on promastigotes and amastigotes of *L. braziliensis*, *L. amazonensis*, and *L. infantum*, with reduced toxicity in murine macrophages. Further studies are needed to investigate its effect on human macrophages infected by *L. braziliensis* and *L. amazonensis*. **Keywords:** Leishmania;Treatment;Superoxide.

DO - 247 - **Using Swab Sampling for Longitudinal Evaluation of Immune Response in Localized Cutaneous Leishmaniasis (LCL) Patients**

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Leishmaniasis is a complex disease that depends on both the parasite and the host's immune response, but there is not yet an established immunological profile pre-treatment for disease cure. Evaluating a patient's immune status is critical for determining therapy success and discovering new treatments and vaccines. However, the tools used to monitor the immune response are invasive and cross-sectional. The swab, a tool widely used for diagnosing and tracking different viral and bacterial infections, could be a promising alternative. It is easy to collect, non-invasive, low-cost, and allows for longitudinal studies to assess the in-situ immune response in individuals infected with leishmaniasis. In this context, this study aimed to evaluate the efficiency of the swab as a tool for longitudinal assessment of the immune response and healing of the leishmaniasis lesion. For this, LCL patients (n=8) were recruited from a referral clinic in Jiquiriçá, an endemic area of Bahia. The study subjects had not started treatment and had a single lesion. Swabs of the lesions were collected from all patients upon admission and weekly until total healing. The first sample from each patient was taken before treatment. To assess the gene expression of chemokines and cytokines in the lesion swab samples, RT qPCR assays were performed. Results showed an initial immune activation in the LCL patients' lesions, evidenced by increased expression of CCL2, CXCL10, IL-6, TNF, IL-23A, IL-1β, and TINP1, which was modulated longitudinally and reduced during lesion resolution. These results suggest that the swab could be a promising tool to assess the in-situ immune response in LCL patients, allowing for longitudinal monitoring of disease progression and host immune response. **Keywords:** Leishmania;Cytokines;Gene Expression.

DO - 248 - **A new experimental model to study shrimp allergy**

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Shrimp allergy is a significant concern due to its high allergenicity and association with anaphylactic reactions. However, the current understanding of this condition is limited, and novel therapeutic strategies are required. In this study, we aimed to establish a new experimental model of shrimp allergy to facilitate the evaluation of prophylactic and therapeutic approaches. BALB/c mice were subcutaneously sensitized with 100 µg of Litopenaeus vannamei shrimp proteins adsorbed in 1 mg of aluminum hydroxide on day 0, followed by a booster (100 µg of shrimp proteins only) on day 14. For oral challenge, 5 mg/ml of shrimp proteins were administered in the water from day 21 to day 35. The shrimp extract analysis revealed the presence of at least 4 major allergens reported in L. vannamei. Allergic mice exhibited a significant increase in IL-4 and IL-10 production in restimulated cervical draining lymph node cells following sensitization. High levels of serum anti-shrimp IgE and IgG1 antibodies indicated the development of shrimp allergies, and the Passive Cutaneous Anaphylaxis assay confirmed an IgE-mediated response. Immunoblotting analysis further demonstrated the presence of antibodies against multiple shrimp extract antigens. These findings were corroborated by detecting anti-shrimp IgA production in intestinal lavage samples and morphometric changes in the intestinal mucosa. Thus, this new experimental protocol provides a valuable tool for the assessment of prophylactic and therapeutic approaches in shrimp allergy research for potential clinical applications at an international level in the field of immunology. **Keywords:** Food allergy;Intestinal mucosa;Shrimp allergy.

DO - 249 - **IMMUNOREGULATORY ROLE OF THE B2-ADRENERGIC RECEPTOR IN POPULATIONS OF INNATE LYMPHOID CELLS (ILCs) DURING INFLUENZA VIRUS INFECTION IN MICE**

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The Influenza A virus (IAV) is a critical pathogen that can cause human infections, leading to mild and severe cases of the disease, with approximately 650,000 deaths annually in the world, impacting the economy and the health system. The innate immune response plays a vital role in protecting the organism, forming a resident niche in lung tissues that orchestrate inflammatory responses and tissue repair. One of the critical mechanisms that coordinate the actions of the immune response is the Sympathetic Nervous System. Our study aimed to analyze the role of the beta-2 adrenergic receptor (B2AR) during IAV infection. For this, wild-type (WT) and B2AR receptor knockout mice (B2ARKO) were infected intranasally with 250 PFU of the A/PR/8/34 virus and followed for 7 days. We evaluated leukocyte subpopulations in bronchoalveolar lavage by FACS, mice survival, histopathological changes, inflammatory mediators by CBA array, and humoral immune response by ELISA. B2ARKO mice showed a higher number of eosinophils, neutrophils, monocytes, macrophages, and dendritic cells when compared to WT mice. In addition, they presented a deregulated ILCs response, with an increase in the populations of ILC1, ILC2, ILC3, and ILCreg, differently of the WT mice that mounted a mixed response, with the expansion of ILC1 and ILC2. B2ARKO mice showed severe histopathological changes characterized by septal and alveolar neutrophilic inflammatory infiltrate, edema, and congestion. Consequently, causing a greater impairment of ventilatory functions compared to WT mice. In addition, increased IFN-g, IL-6, and TNF levels were observed in B2ARKO mice. B2ARKO mice had higher specific IgA titers compared to WT mice, however, no differences in pulmonary viral load, but the B2ARKO succumbed to the infection early and had higher mortality. Thus, this finding indicates the importance of the B2AR receptor during the inflammatory response during infection by the influenza A virus. **Keywords:** Innate lymphoid cells;Influenza;ß2-adrenergic receptor.

DO - 250 - **SPATIAL CHARACTERIZATION OF MACROPHAGE SUBPOPULATIONS IN COLORECTAL CANCER**

QUAGLIATO, E.N.[1]; FILGUEIRAS, I.S.[2]; DA SILVA, E.M.[3]; CABRAL-MARQUES, O.[4]; CAMARA, N.O.S.[4]; ANDRADE-OLIVEIRA, V.[5]; GALON, J.[6]; RAMOS, R.N.[2]. 1. IDOR, SÃO PAULO - SP - BRASIL; 2. D'OR INSTITUTE FOR RESEARCH AND EDUCATION, IDOR, SÃO PAULO, BRAZIL, SÃO PAULO - SP - BRASIL; 3. PAULISTA SCHOOL OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO (UNIFESP), SÃO PAULO - SP - BRASIL; 4. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 5. CENTER FOR NATURAL AND HUMAN SCIENCES, FEDERAL UNIVERSITY OF ABC (UFABC), SANTO ANDRÉ - SP - BRASIL; 6. INSERM, SORBONNE UNIVERSITÉ, UNIVERSITÉ DE PARIS, EQUIPE LABELLISÉE LIGUE CONTRE LE CANCER, PARIS - FRANÇA.

Introduction: Colorectal cancer (CRC) is the second most fatal type of cancer worldwide, and the younger population has been the most affected lately. This highlight the urgent need for understanding early diagnosis, the initial processes, and disease progression. The role of macrophages (Mac) has been controversal in CRC, due to their different locations, densities, and subpopulations. Our recent study has revealed FOLR2+ Mac as tissue-resident macrophages (TRM) and TREM2+CADM1+ Mac as monocyte-derived tumor-associated macrophages (TAMs), but their cellular density, sub-group diversity and spatial distribution in CRC remains unexplored. **Methods and Results:** Using multiparametric flow cytometry in CRC patients and in a murine colitis-induced model, we found that the proportion of TAMs is elevated in tumors compared to non-tumor tissues, while the frequency of TRM shows the opposite phenomenon. Next, using multispectral immunohistochemistry, we analyzed 61 CRC patients for Mac subpopulations in four distinct zones: non-tumor tissue (NTT), stroma (ST), tumor margin (TM), and tumor center (TC). We revealed seven distinct Mac/monocyte subsets including: CD14+, FOLR2+, TREM2+, CD14+TREM2+, CD14+FOLR2+, FOLR2+TREM2+ and CD14+FOLR2+TREM2+. We found a significant increasing of CD14+FOLR2+ and FOLR2+ subsets in NTT and ST, while TREM2+ and CD14+ cells were increased in the TC. Interestingly, the TM has the most variable profile of Mac infiltration among patients, but with an increased proportion of TREM2+ cells. In addition, CRC patients presenting high CD3/CD8 immunoscore density (top 25%) in the TM show CXCL10 and CXCL11 genes positively correlated with TREM2+ Mac but negatively correlated with FOLR2+ Mac. **Conclusion:** Our data suggest a distinct spatial distribution and oposite role of Mac subsets on patients immune responses. By using bulk RNA-seq and bioinformatic tools we will futher clarify the contribution of each Mac subset and their impact on patients survival. **Keywords:** Colorectal cancer;Macrophages;Tumor Immunology.

DO - 251 - **Acute Lung Injury is induced by SARS-CoV-2 structural proteins in mice**

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Acute lung injury (ALI) is a lung disorder that leads to the alveolar epithelial and endothelial barrier disruption, compromising alveolar-capillary membrane integrity. ALI causes the release of pro-inflammatory mediators such as IL-6, IL-8, and TNF by damaging epithelial cells and a subsequent increase in endothelial permeability and trans-epithelial neutrophil influx into the airways. The source of pro-inflammatory cytokines is associated with sustained lung tissue injury and dysfunction. Neutrophil predominance is the hallmark of ALI, mainly occurring in the first 24 hours after damage or infection, being gradually replaced by macrophages and lymphocytes. Various illnesses, including sepsis, bacterial infection, and COVID-19, induce ALI. Thus, this project investigated the role of SARS-CoV-2 recombinant proteins RBD and N (nucleocapsid) as potential ALI inducers, compared with the classic model of ALI induced by LPS. C57BL/6 with 8-10 weeks old male mice were anesthetized with ketamine and xylazine and then were instilled with the proteins (LPS, RBD, and N) suspended in 40 uL of sterile PBS. After 24 hours of the protein instillation, mice were euthanized, and the bronchoalveolar lavage (BAL) and lungs were obtained to be analyzed. Our results showed that RBD and N induce ALI in mice, marked by tissue inflammation and mechanical dysfunction similar to LPS. The RBD instillation induced mixed neutrophilic and myeloid cell (alveolar and inflammatory macrophages) influx into the airways. However, N instillation is a mixed myeloid (dendritic cells) and lymphoid cell influx. RBD instillation recruits CD4+ and CD8+ lymphocytes, but not the N instillation, which showed an increased influx of gd and NKT lymphocytes. LPS instillation increases the BAL levels of IFN-g, IL-6, IL-10, and IL-17A. Meanwhile, RBD protein induces IL-6, and N protein induces IL-17A. In conclusion, RBD and N proteins induce ALI but with different patterns of pulmonary inflammation in mice. **Keywords:** Acute Lung Injury;Inflammation;mechanical dysfunction.

DO - 252 - **Sickle cell disease patients treated with hydroxyurea have a higher percentage of non-classical monocytes and PD-L1+ monocytes**

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Monocytes have been highlighted in vasoocclusion studies as potential activators of the endothelium in Sickle cell disease (SCD) patients. Moreover, standard treatment protocols may modulate the profile of circulating human monocyte subsets (classic, intermediate and non-classical monocytes), inflammation and endothelial activation. Therefore, this study compared monocyte subsets from SCD patients with no specific treatment (NTP) (n=5), treated with hydroxyurea (HU) (n=10) and chronic exchange transfusion (TP) (n=8) with healthy subjects (CTL) (n=3). HCFM/USP patients over 18 years of age, both gender and without acute complications in the prior three months of blood collection were included. PBMC were stained with CD14, CD16, CD86, CD163 and PD-L1 mAb and analyzed by flow cytometry. Group comparison was performed using ANOVA and Tukey's test. Monocyte subset analysis showed a lower percentage of classical monocytes (CD14++CD16-) in the PBMC of TP (2.00 ± 1.78%, p 0.0009) and HU (2.46 ± 1.58%, p 0.0047) compared to CTL (7.64 ± 1.23%); and a lower percentage in TP when compared to NTP (5.43 ± 1.26%, p 0.0113). Non-classical monocytes (CD14dimCD16+) percentage was higher in HU (0.25 ± 0.1%, p 0.0484) compared to TP (0.10 ± 0.08%) and in CTL (0.34 ± 0.07%, p 0.0260) vs. TP. The percentage of PD-L1+ monocytes was higher in classical monocytes of HU (98.95 ± 17.65%) when compared with NST (47.6 ± 34.12%, p 0.0476) and CLT (32.1 ± 8.70%, p 0.0352). HU (99.75 ± 10.27%) showed a higher percentage of PD-L1+ intermediate monocytes compared with CLT (51.8 ± 8.00%, p 0.0444). When compared to CLT (40.2 ± 3.46%), HU (96.2 ± 23.16%, p 0.0283) and TP (87.65 ± 20.82%, p 0.0459) showed higher percentage of PD-L1+ non-classical monocytes. Collectively, our data suggest that HU treatment confers to monocytes an anti-inflammatory profile, with higher percentage of non-classical monocytes and of PD-L1+ monocytes. FAPESP#2022/07503-7, CNPQ#442676/2020-4 and PRONON#25000.027785/2021-21. **Keywords:** Monocytes;Sickle Cell Disease;Hydroxyurea.

DO - 253 - **ATP-citrate lyase expression in macrophages during Leishmania amazonensis infection**

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Leishmania are obligatory intracellular parasites transmitted to vertebrate hosts by sandflies. These parasites can cause a range of diseases, including the cutaneous and the visceral form. Despite approximately one billion individuals being at risk of contracting leishmaniases, the disease remains neglected, and no vaccine is currently available. The available treatment options have problems such as adverse effects and high toxicity. Plus, there has been a rise in treatment failure and reports of drug-resistant parasites. Thus, it is crucial to continue investigating the parasite-host interaction to improve the treatment and develop vaccines. Once inside the macrophages, parasites evade microbicidal mechanisms, replicate and infect other cells. Recent studies show that *Leishmania* induces metabolic reprogramming in macrophages, and they enhance lipid droplets (LDs) in these cells (1,2). Based on these findings, we investigate how the parasite modulates the host's lipid metabolism. We hypothesized that during the infection, *L. amazonensis* induces an increase in lipids in macrophages and that this is due to the modulation of the enzyme ATP-citrate lyase (ACLY). ACLY cleaves citrate in oxaloacetate and acetyl-CoA, and these two products have the potential to trigger the production of inflammatory mediators and cause epigenetic alterations. We quantified lipids in macrophages infected with *L. amazonensis* and observed an enhanced lipid expression upon infection. When pre-treated with ACLY inhibitor BMS-303141, there is a decrease in ROS production in the early stages of the infection. ACLY inhibition did not modulate NO production but diminished 41% of parasite survival. BMS-303141 did not affect parasite viability, suggesting that the effect was due to modulation of macrophage response. These results suggest that the ACLY modulates the macrophage response to *L. amazonensis*. (1)Parasite Immunol. 39:e12443, 2017. (2) PLos One, 14, 14 (12): e0225588, 2019. **Keywords:** Leishmania amazonensis;macrophages;ATP citrate lyase.

DO - 254 - **Cell Phenotypic Markers of Dogs with Visceral Leishmaniasis**

LOPES, G.D.S.; RODRIGUES, L.F.C.; MACIEL, E.P.; BERNADES, M.; MENDONÇA, L.; FALCÃO, S.; FAVALI, C.B.F.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Leishmaniasis is a vector-borne zoonosis, belonging to the group of neglected tropical diseases. Visceral Leishmaniasis (VL) is the most dangerous form of Leishmaniasis. VL affects humans and other mammal species, being the infected dogs the main domestic reservoir of the parasite. In Brazil, Canine Visceral Leishmaniasis (LVC) is generally caused by the species *L. infantum*, and manifests a broad clinical spectrum. For the clinical course of the disease, the cellular immune response has an important role, however, there are few studies characterizing in detail the expression of surface molecules in canine leukocytes, mainly differentiating symptomatic from asymptomatic. Therefore, the aim of this study was to characterize the percentage and phenotype of monocytes and lymphocytes of dogs with LVC, symptomatic and asymptomatic. In this study, 25 dogs from the Distrito Federal (Brazil), naturally infected, were distributed in three groups: uninfected dogs (NI), asymptomatic infected dogs (IAS) and symptomatic infected dogs (IS). Cellular phenotypes of monocytes (CD14+, MHC-II+) T lymphocytes (CD3+, CD4+, CD25+, CD8+), B lymphocytes (CD19+, CD25+) and the cell viability, of PBMCs, were analyzed in *ex vivo*. The results indicate a lower frequency of circulating monocytes (CD14+, MHC-II+), as well as low expression of CD44, in the IS group, suggesting that the function of antigen presentation in monocyte was compromised. Also, the higher expression of CD4 in lymphocytes of IS dogs compared to IAS dogs is a result that should be better studied to understand its impact on the cellular immune response of IS. In the evaluation of cell death, its modulation by the parasite was demonstrated in dogs with LVC, this strategy was used to evade the immune system. Some biomarkers based on the expression of surface molecules and percentage of monocytes and lymphocytes, described in this study, can be used to identify the prognostic and the host's susceptibility or resistance profile. **Keywords:** CANINE VISCERAL LEISHMANIASIS;CANINE LEUKOCYTES;SURFACE MOLECULES.

DO - 255 - **Identification of Plasmodium spp. antigens from infected red blood cell surface and recognized by antibodies**

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The prospection of novel Plasmodium antigens for malaria prophylaxis and treatment is driven by the understanding of immunological mechanisms activated in the different phases of infection. During the blood-stage, infected red blood cells (iRBC) are opsonized by circulating antibodies, which lead to phagocytosis by antigen-presenting cells and cytotoxic degranulation. Considering the immunogenic potential of parasite antigens externalized on the cell surface, here we describe the identification of protein antigens from Plasmodium falciparum W2 exposed on the iRBC membrane and recognized by opsonizing antibodies. We utilized a chemical crosslinking and immunoprecipitation approach to isolate immune complexes from iRBCs. Peptides were identified by tryptic digestion followed by mass spectrometry. The data obtained were analyzed against proteomic databases from H.sapiens and P. falciparum. We compared the performance of two crosslinkers, DSP and DTSSP, in enrichment of parasite proteins. In summary, we identified 130 parasite proteins derived from DTSSP protocol and 36 proteins from DSP. This difference should be related to the higher hydrophilicity of DTSSP compared to DSP, which results in lower membrane permeation and an optimized reaction with cell membrane proteins. Parasite proteins identified were mainly associated with enzymatic function, protein binding, ribosomal components, and some with unknown functions were enriched in both approaches. Surprisingly, ribosomal proteins were identified with a high confidence, corroborating studies that also have identified ribosomal proteins on cell membrane of parasite-infected, this finding needs to be validated, but could point to a role of these housekeeping proteins in control of infection. Concisely, the proposed method is shown to be suitable for enrichment of parasite proteins exposed on the cell surface. These proteins upon assessment could be investigated as potential immunogenic targets in malaria. **Keywords:** Malaria;Proteomics;Immune complex.

DO - 256 - **Study of the mechanisms of tolerance induction during infections**

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Surviving an infection relies on both in the capacity to detect and eliminate pathogens (resistance) and in the host adaptation to withstand the damaged caused by the virulence of the invading organism and by the host immune cells (tolerance or resilience). Tolerance induction is a multifaceted process that is still loosely defined, but that promotes maintenance of tissue barrier, downregulates proinflammatory signaling and induces anti-inflammatory cytokine secretion, initiates tissue repair and remodeling, regulates metabolic homeostasis and stimulates microbiome (re)composition. Individual genes that contribute to tolerance have already been identified in several infection models, but how these different plays converge into efficient tolerogenic responses is not well understood. The aim of this work is to investigate what drives tolerance to pathogen load during infection. Models of coinfection highlight how loss of tolerance causes susceptibility to infection is not a simple question. The interplay of the different scenarios that can lead to high morbidity and lethality suggest that different components of a general tolerance program may not have overlapping functions: tissue repair mechanisms independently of inflammatory responses may prevail locally in certain infections, whereas in other situation tolerance might be more significant systemically due to suppression of hyperinflammation. Understanding when these different mechanisms of tolerance are at play and how they are regulated in different pathologies is fundamental to direct effective therapeutic intervention approaches during life threatening occurrences. While part of normal tissue homeostasis, cell death is also a hallmark of infectious diseases, either induced as a pathogen virulence strategy or as a mechanism of host defense. Our main hypothesis is that the innate immune system relies on efferocytosis as a main signal to promote tolerance during infection. **Keywords:** Innate immunity;Tolerance;Efferocytosis.

DO - 257 - **Neutrophils restrict SARS-CoV-2 virus infection by NETs release**

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COVID-19 is a disease associated with a marked lung injury and a high presence of neutrophils and neutrophil extracellular traps (NETs), which seem to mediate the host tissue damage. However, it isn't known whether NETs could also be involved in the inhibition of SARS-CoV-2 virus. Here, neutrophils from healthy individuals ($1x10^6$) were infected *in vitro* with SARS-CoV-2 (MOI 1.0) for 4h and compared to neutrophils from COVID-19 patients by immunofluorescence (IF). Neutrophils were also, pre-treated or not with PAD4 inhibitor (100 µM), and infected *in vitro* with SARS-CoV-2 for 4h to analyze NETs formation and viral load by picogreen and RT-qPCR, respectively. To analyze the role of NETs in viral restriction in other cell types, Caco2 cells ($1x10^5$) were infected *in vitro* with SARS-CoV-2 (MOI 1.0) in the presence of purified NETs for 24h. Viral replication was analysed by RT-qPCR and IF, and cell viability by FACS. NETs were also treated with NE, MPO and histone inhibitors to assess the involvement of these molecules in viral inactivation. Lastly, K18 hACE2 transgenic mice were infected and, treated or not, with DNase I (0.5 mg/mL) to assess the role of NETs in viral control in the first 48h p.i. Our results showed that viral particles colocalize with NETs in neutrophils isolated from COVID-19 patients or isolated from healthy individuals that were infected *in vitro* with SARS-CoV-2. Also, the inhibition of NETs production increased virus replication in neutrophils. In parallel, NETs inhibited virus ability to infect and replicate in Caco2 cells. The virucidal effect of NETs seemed to be dependent on the activity of histones. In a mouse model, we observed higher viral load in animals treated with DNase I. Given the above, the results indicate that NETs act to control SARS-CoV-2 infection. Thus, our results provide evidence of the role of NETosis as a mechanism of SARS-CoV-2 viral capture and inhibition, revealing new innate mechanisms in the context of COVID-19. **Keywords:** neutrophils;neutrophil extracellular traps;COVID-19.

DO - 258 - **Effects of in vitro cell senescence induced by oxidative stress in murine thymic epithelial cells**

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The thymic epithelial cells (TECs) are responsible for producing growth factors necessary for thymocyte differentiation and survival, in addition to presenting antigens during positive and negative selections. During aging, the thymus undergoes atrophy (thymic involution), and evidence shows that TECs are one of the most affected thymic cells. The oxidative stress can cause irreversible damage to TECs, inducing cellular senescence, favoring thymic involution, however, the mechanisms responsible for this process still need to be elucidated. In this study, we aimed to establish an ***in vitro*** model of cellular senescence induced by oxidative stress in TECs. For this, the murine TEC cell lines 2BH4, 1-4C18 (cortical TEC), and 1C6 (medullary TEC) were treated with different concentrations of D-galactose (D-Gal) for 24 and 48h, to induce oxidative stress-related cell senescence. Then, viability and proliferation assays, cell counting, morphological analysis, reactive oxygen species (ROS) production, apoptosis assay and evaluation of cell senescence-related gene expression and molecules were performed. Treatment with D-gal was able to induce senescence in TECs, evidenced by a reduction in the number of cells after 24 and 48h of treatment, increased production of ROS, accompanied by changes in cell morphology, although no increase was seen in apoptosis. Also, there was an increase in p21 and p16 inhibitors, and in IL-6 cytokine. In conclusion, D-Gal-produced oxidative stress induced senescence in TECs. This model will be useful to study thymic involution mechanisms as well as pharmacological therapies to prevent or reverse this phenomenon. Funding support: CNPq, INCT-NIM and FAPEAL. **Keywords:** Thymic Epithelial Cells;Cell Senescence;Oxidative stress.

DO - 259 - **Plasma Sphingosine-1-Phosphate (S1P) in COVID-19: Implications for Immune Dynamics and Disease Severity.**

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COVID-19 exhibits diverse disease patterns with profound changes in host immune responses. Severe cases manifest significant pulmonary inflammation and systemic effects. Sphingolipids, particularly Sphingosine-1-phosphate (S1P), play crucial roles in cellular and immunological processes, controlling lymphocyte motility, cellular invasion, and immune modulation. However, S1P's role in inflammation remains controversial, as it can activate NF-κB, a key mediator of inflammatory responses. Additionally, S1P promotes M1 to M2 macrophage polarization. We employed UHPLC-MS/MS to investigate S1P production in plasma samples obtained from healthy control (n=55) and patients (n=204). We used RNAseq analysis to assess the gene expression of the S1P pathways. Our study has revealed a significant elevation in the serum concentration of S1P (18:1) among critically ill patients compared to control group (p=0.001). Interestingly, patients admitted to the Intensive Care Unit displayed lower levels of S1P when compared to those admitted to general wards. S1P levels were correlated with clinical and inflammatory parameters. We observed positive correlations (weak but significant) between S1P (18:1) and age (p=0.0001), days of infection and hospitalization (p=0.007), clinical score (p<0.001), Neutrophils (p=0.007), RNL (p<0.001), glucose (p=0.038), IL-6 (p=0.001), and IL-8 (p=0.001). In contrast, S1P (18:1) exhibited a weak negative correlation with the lymphocyte count (p=0.002). Furthermore, we observed the decreased expression of key enzymes involved in the S1P pathway and its receptor S1P receptor 1 (S1PR1) in blood cells from critically ill patients with COVID-19. Our investigations have unveiled the integration of S1P production into the immunological repertoire associated with COVID-19. Moreover, its synthesis may be intricately involved in the organism's endeavor to augment the population of lymphocytes, which play a major role in sustaining an efficient antiviral response. **Keywords:** Sphingosine-1-phosphate (S1P);Inflammation;COVID-19.

DO - 260 - **ANNEXIN A1 IS CRUCIAL DURING TOXOPLASMA GONDII INFECTION PROMOTING MODULATION OF INFLAMMATION AND MICROBIOTA COMPOSITION**

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Toxoplasma gondii (*Tg*) is arguably the most successful parasite because, in part, of its ability to infect and persist, in most warm-blooded animals, mainly in the central nervous system. *Tg* infection promotes a robust inflammatory response in the gastrointestinal tract and the immune system is fundamental controlling the toxoplasmosis pathophysiology. Annexin (ANXA) A1, a pro-resolving and anti-inflammatory protein is induced by glucocorticoid hormones. Herein, the aim was to evaluate the role of ANXA1 A1 during *Tg* infection. Peritoneal macrophages (MOs) and glial cells from Balb/c (WT) and ANXA1 knockout (KO) mice were infected with tachyzoites forms of *Tg* RH strain *in vitro*. WT and ANXA1 KO were infected or not, via intraperitonially, with 20 cysts of ME49 strain. During Tg infection, in the brain of WT mice, was observed the increased expression of intact ANXA1 at 5 dpi, followed by its decreasing at 25 dpi, that was associated with increased ANXA1cleaved levels. The deficiency of ANXA1 resulted in increased susceptibility to *Tg* infection with higher number of cysts in the brain and more severe lesion in the cortex and meninge when compared to WT mice. Notably, in the brain was observed an increased levels of N-Acetylglucosamine enzyme at 15 and 25 dpi associated with increased IBA-1 positive cells, indicating an increased presence of macrophages and microglias in the tissue. Moreover, at 15 and 25 dpi the absence of ANXA1 also elevated the gut inflammation increasing permeability that results and translocation of the enterococcus bacteria from the gut to liver. Collectively, our data demonstrated that ANXA1 is critical regulating the development of pathogeneses during *Tg* infection unvain a possible therapeutic target for treatment of this disease. **Financial support:** CNPq; Capes e FAPEMIG. **Keywords:** Toxoplasma gondii;annexin A1;macrophage.

DO - 261 - **Injection of tolerated protein as a new treatment for bone lesions**

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Bone repair involves distinct stages, starting with inflammation. During this stage, immune cells like neutrophils, macrophages, and lymphocytes migrate to the injury site. Altering the inflammatory cellular elements can impact the repair outcome, positively or negatively. "Oral tolerance" is a known phenomenon that modulates inflammation. In this process, the immune system recognizes ingested proteins in the gastrointestinal tract and become tolerated, avoiding hypersensitivity reactions upon absorption (J. Immunol. 126: 354-362, 2009). Administering a previously tolerated protein via a parenteral route reduces specific antibody levels and increases T lymphocytes. In experiments with mice using skin injury models, parenteral zein injection (a previously tolerated protein) before injury improved wound healing and reduced scarring (J. Immunol. 151: 314–323, 2017). The study aimed to observe the impact of parenteral zein injection on bone repair. Wistar rats were pre-treated with zein tolerance, and a 2 mm bone defect was created in their tibiae. Euthanization occurred at 7, 14, and 28 days post-injury. Hematoxylin and eosin staining showed significative better deposition of neoformed bone matrix in zein-treated animals. Biomechanical tests and DMO (Bone mineral density) indicated higher mechanical resistance and improved local calcification. Computed tomography revealed lower trabeculae levels in the medullary region compared to the control. These results suggest zein injection can enhance bone repair through modulation of the inflammatory process. **Keywords:** Oral tolerance;Bone injury;Inflammation.

DO - 262 - **TRANSCRIPTIONAL META-ANALYSIS IDENTIFIES METABOLIC PATHWAYS ASSOCIATED WITH HIV ELITE CONTROLLER PHENOTYPE**

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About 0.1-1% of HIV infected individuals can spontaneously control the infection, exhibiting CD4+ T cells maintenance with viremia suppression and the absence of symptoms for 10 years or more. Although some characteristics of these individuals, known as Elite Controllers (EC), have been described, a differential mechanism of them has not yet been identified. Therefore, the use of integrative data methods - such as meta-analyses - can help to identify signature patterns associated with individuals included in different cohorts. Thus, the aim of this study was to identify a transcriptional signature of EC compared to healthy donors (HD), untreated viremic (VR) and successfully treated (ST) individuals. We found 12 public transcriptomic datasets obtained from whole blood, CD4+ and CD8+ T cells samples. We identified 154 differently expressed genes (DEGs) in Elite Controllers (73 up and 81 downregulated). Pathway enrichment analysis showed that CD4+ T cells from EC showed alterations in genes related to the cell cycle, autophagy and glycolysis and gluconeogenesis, which may be involved in the reservoir maintenance. CD8+ T cells demonstrated differences involving cell cycle, mRNA surveillance, RNA transport and degradation, which may be related to alterations in CD8+ T cells proliferative capacity. Most of the differences in whole blood samples involved metabolic pathways, such as Pyruvate and Sulfur, citrate cycle and glycolysis and gluconeogenesis. These results suggest that EC display differential activity of diverse pathways that depend on the cell type and function. **Keywords:** HIV;Meta-analysis;Elite Controller.

DO - 263 - **Airway delivery of Lactobacillus delbrueckii UFV-H2b20 improves immune regulation to maintain lung homeostasis in asthma**

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The interaction of microbiota with the host immune system can modulate immune responses in many ways. Those immunomodulatory effects are also seen with some probiotic species, and their protective effect has been demonstrated in inflammatory and allergic conditions, such as asthma. Asthma is characterized by an exacerbated Th2 response, mediated by IgE, with extensive eosinophilic infiltrate, IL-4, IL5 and IL-13 activity, mucus overproduction and airway hyperresponsiveness (AHR). The significance of the lung microbiota and its importance in respiratory health is the basis of this work, which aims to investigate the effects of intranasal supplementation of LAC in respiratory health. Our results show that intranasal supplementation with LAC controls the induced allergic inflammatory response, reduces inflammatory infiltrate in lungs, especially eosinophils. This lessened inflammatory state is confirmed by decreased levels of IL-5 and IL-13. LAC also helped normalize respiratory parameters like AHR and Forced Expired Volume. Induction of regulatory mechanisms is responsible for these effects, as the treatment with LAC induces multiple regulatory cell types, such as alveolar macrophages IL-10^{+} and TGF-β^{+}, tolerogenic dendritic cells (DC CD103^{+}) and Tregs (FoxP3^{+}), with higher levels of secreted IL-10 and TGF-β. Histology showed that the treatment controls mucus overproduction and induces the formation of iBALT. In treated mice, this iBALT leads to an increased presence of cells expressing CCR7, indicative of an environment with homing characteristics. The CCR7^{+} subset in lungs of treated mice is mainly composed by naive TCD4 lymphocytes, with a lower presence of GATA3^{+} LTs. DCs CCR7^{+}CD103^{+}, are also increased in the treated group and may be an important player in inducing tolerance and controlling allergic inflammation. These results show that airway supplementation with LAC exerts immunomodulatory effects and attenuates the general pathology seen in asthma. **Keywords:** Immuneregulation;Microbiota;Lungs.

DO - 264 - **Microglia-derived soluble molecules modulate astrocyte expression of glutamate receptors. Implications for neurodegenerative disease?**

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Astrocytes are important glial cells that play a key role in maintaining CNS (central nervous system) homeostasis. It is known that the metabolism of glutamate can be affected by inflammatory conditions, such as the one observed during neurodegenerative diseases. Tight control of glutamate in the pre-synaptic cleft is pivotal to avoid exacerbated excitability of glutamatergic neurons, which causes neuronal loss mediated by apoptosis. In this sense, impaired glutamate homeostasis could aggravate neuro-inflammation, worsening disease progression. This work aims to investigate the effects of inflammatory conditions in glutamate homeostasis, as well as understand whether β2-adrenergic receptor (β2AR) signaling can interfere with it. Primary cultures of mouse astrocytes and microglia were obtained and purified by cell sorting. By stimulating microglia cultures with LPS or not, we obtained LPS-activated microglia conditioned medium (MCM-LPS) or MCM, that was used to mimic the environment encountered during neuro-inflammation. We sought to evaluate the dynamics of glutamate transporters and glutamine synthetase (GS) expression in astrocytes upon MCM-LPS exposure or purified TNFα stimulation. We observed that culture of astrocytes with MCM-LPS induces the downregulation of GLAST (EAAT-1) and GS expression, whereas GLT-1 (EAAT-2) expression is increased. Preliminary data indicate that purified TNFα is not able to modulate the expression of the glutamate transporter EAAT1 or GS, however, the expression of EAAT2 is downregulated. Moreover, we observed that β2AR signaling does not have effects in the results described above. In conclusion, it is possible that upon exposure to inflammatory stimuli, astrocytes ability to reuptake glutamate is hampered. Moreover, we described that b2AR signaling is not able to prevent it. Our data also indicate that soluble molecules other than TNFa derived from microglia are likely causing the effects elicited by MCM. **Keywords:** Neuroinflammation;Astrocytes reactive;Multiple sclerosis.

DO - 265 - **INTERPLAY BETWEEN INTESTINAL MICROBIOTA AND DEVELOPMENT OF SAINT LOUIS ENCEPHALITIS**

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St. Louis encephalitis virus (SLEV), a *Flavivirus* transmitted by *Culex* mosquitoes, causes severe neurological disease with high mortality rates. Recent studies on other *Flavivirus* have emphasized the influence of gut microbiota on viral infections, but the specific impact of the intestinal microbiota on SLEV susceptibility remains poorly understood. In this study, we investigated the hypothesis that the depletion of intestinal microbiota would enhance the susceptibility of mice to SLEV infection by impairing the development of the immune response. C57BL/6 mice were treated with antibiotics to deplete their microbiota, while an untreated control group was exposed to SLEV. Successful microbiota depletion was confirmed by a reduction in microbial gene expression and bacterial density in infected animals. Our findings revealed that mice with depleted microbiota and those infected with SLEV displayed increased susceptibility to disease development, as evidenced by higher mortality rates (83%) compared to mice with intact microbiota (50%). Microbiota depletion didn't significantly affect the viral load in infected animals but altered antiviral cytokines, with decreased INF-γ in depleted mice. Additionally, animals treated with antibiotics exhibited notable differences at the phylum level when compared to untreated animals. Specifically, a decrease in the *phylum Firmicutes* and *Bacteroidota*, known for their fermentative characteristics and immune-modulatory abilities, was observed, along with an increase in *Proteobacteria*. Our study highlights the importance of the intestinal microbiota in SLEV susceptibility, influencing disease severity and immune responses. These findings underscore the significance of investigating the intricate interplay between microbiota and viral infections, particularly those affecting the central nervous system. Understanding this interplay can lead to targeted interventions against St. Louis encephalitis and related viral diseases. **Keywords:** Viral response;Saint Louis encephalitis virus;Microbiota.

DO - 266 - **NANOPARTICLES FOR THERAPEUTIC DELIVERY OF EXTRACELLULAR VESICLES: A NEW APPROACH TO IMMUNOTHERAPY AGAINST GLIOBLASTOMA**

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Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. Median survival is low and even with several studies and intense investigation in the search for new therapeutic strategies, little benefit has been achieved in patient survival. Recently, immunotherapy and nanomedicine approaches have been added to the tools for the treatment of GBM where they have shown great potential. There is a lot of evidence that the use of Extracellular Vesicles (EV) can be an interesting approach in immunotherapy, since they have roles in innate and adaptive immunity, in addition to participating in the physiological processes of various diseases. We know that dendritic cells (DC) actively produce EV to modulate and/or enhance the immune response, and also that GBM, like other tumors, release EV that are detectable in the plasma and that can be used in the disease monitoring. Thus, this project intends to explore DC and GBM-derived EV as tools for modulating the immune response, especially in DC-based immunotherapy strategies. Specifically, we sought to develop a targeted EV delivery strategy, mediated by polymeric nanoparticles, as a way to increase its immunomodulatory activity and therefore its clinical translation potential. For this, we carried out the physical-chemical characterization of the EV derived from DC and the EV derived from GBM in order to verify the yield, the size, concentration and release of EV subpopulations. We also investigated, by flow cytometry, the phenotypic profile of DC after the incorporation of EV DC and EV GBM, evaluating the presence of molecules known to be involved in DC functions, in addition to studying the capacity of these cells to induce the proliferation of $CD4^+$ T lymphocytes and allogeneic $CD8^+$. With these data, we design and synthesize polymeric nanoparticles for targeted delivery of EV to DC, seeking to obtain the best strategy based on EV to obtain a nanovaccine against GBM. **Keywords:** Extracellular vesicles;Nanovaccine;Cancer immunotherapy.

DO - 267 - **Platelet activation and platelet-monocyte interaction support monocyte TF expression in obesity**

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Obesity is a global health problem associated with chronic low-grade inflammation that supports high cardiometabolic risk. Activated platelets secrete inflammatory mediators and interact with leukocytes modulating crucial leukocyte responses. Nonetheless, the mechanisms and functional responses of platelet-leukocyte aggregates in obesity remain poorly understood. We hypothesized that platelet activation contributes to immunoregulation in obesity, participating in mechanisms of inflammation and cardiometabolic risk. The main goal is to investigate the role of platelet activation and platelet-leukocyte interaction in immunoregulation in obesity. We evaluated platelet activation by flow cytometry and ELISA and characterized the phenotype of circulating leukocytes and platelet-leukocyte aggregates by whole blood flow cytometry. Obese patients exhibited elevated platelet activation with higher sCD62P and CCL5 secretion. In addition, we observed reduced platelet-B cell aggregate and increased platelet-monocyte aggregate formation, especially among $CD16^+$ monocytes. We did not observe platelet aggregation with neutrophils, T, NK and NKT cells. We also investigated expression of TF (tissue factor) , the main trigger of the extrinsic pathway of coagulation. We showed a trend towards increased TF expression in monocytes from obese patients, which occurred preferentially, but not exclusively, on monocytes attached to platelets. We then investigated whether platelet-monocyte interaction modulate TF expression using a co-culture model. Monocytes from obese patients, but not from eutrophic individuals, respond to the interaction with heterologous platelets by increased TF expression. These data highlight increased platelet-monocyte aggregates in obesity, which was associated with $CD16^+$ monocytes and TF expression. All these cellular interaction events are potentially involved in mechanisms of thromboinflammation in obesity. **Funding:** CNPq, Ebserh, CAPES. **Keywords:** Platelet-leukocyte interaction;Tissue factor expression;Obesity.

DO - 268 - **Cissampelos sympodialis extract attenuates lung inflammation cigarette smoke-induced acute lung injury in mice.**

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Introduction: Cigarette smoke (CS) is able to induce acute lung injury (ALI) with increase of inflammatory cells and mediators release following tissue damage. The hydroalcoholic extract of *Cissampelos sympodialis* (ECsy) has been used in the treatment of respiratory diseases due to its anti-inflammatory activity. **Aim:** To evaluate the pharmacological action of the ECsy on CS-induced ALI in mice. **Methods:** CEUA (16/2020). Male C57BL/6 mice (±23g) were divided into five groups: Control, CS, CS + 10, CS + 100 and CS + Dexa. The CS groups were exposed to 12 cigarettes/day for 5 days and treated with salina (CS), ECsy (10 or 100mg/mL by nebulization; 15min/day) or dexamethasone (0,4 mg/mL i.p) for 5 days. The control group was exposed to ambient air and treated with salina. After 5 days, lungs and BALF were collected to evaluate the inflammatory profile. Significant difference was considerate when $p<0.05$. **Results:** The CS group showed an increase of protein concentration in BALF when compared to control group (0.13 ± 0.01 vs. 0.04 ± 0.004; $p<0.0001$) and reduced in CS+10 (0.09 ± 0.01; $p<0.05$) and CS+100 (0.08 ± 0.01; $p<0.001$). The CS group showed an increased in the lung inflammation score when compared to the control group ($2,5 \pm 0,1$ vs.$1,7 \pm 0,1$; $p<0.01$) and reduced in CS+100 ($1,9 \pm 0,08$; $p<0.05$) and CS + Dexa ($1,77 \pm 0,09$; $p<0.001$). The CS group showed an increase of bronchoconstriction index when compared to the control group ($5,5 \pm 0,2$ vs. $2,4 \pm 0,2$; $p<0.0001$). and reduced in CS+10 ($3,6 \pm 0,2$; $p<0.0001$) and CS+100 ($3,3 \pm 0,2$; $p<0.0001$). The CS group showed an increase of ephitelial damage when compared to the control group ($3,0 \pm 0,2$ vs. $1,6 \pm 0,1$; $p<0.001$) and reduced in CS+10 ($2,3 \pm 0,2$; $p<0.05$) and CS+100 ($2,1 \pm 0,2$; $p<0.05$). **Conclusion:** These results demonstrate the anti-inflammatory potential of ECsy cigarette smoke-induced ALI. **Keywords:** inflamation;smoking;emphysema.

DO - 269 - **Elderly Lung Cancer Patients show Tumor-Infiltrating CD8+ T Cell Responses Enriched with PDCD1 and CXCL13 after Neoadjuvant Therapy with Anti-PD-1**

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Introduction. Aged individuals are significantly underrepresented in immunotherapy clinical trials for cancer. Little is known regarding the mechanisms that might regulate their responsiveness to immune checkpoint inhibitors (ICIs). Molecular signatures of T cells, such as immunosenescence and exhaustion are critical factors involved in the efficacy of antitumoral immunotherapy. **Objectives.** We asked whether age would be associated with poor outcomes in cancer patients undergoing anti-PD-1 immunotherapy. **Methods.** We screened public datasets from the *IMMUcan SingleCell RNAseq* and *TISCH2* databases. Only 1 dataset out of 334 met all inclusion criteria (patients over and under 65 years old, with similar tumor subtypes, treated with immunotherapy and with single-cell analyses of T cells from tumor tissues). We reprocessed the available data and molecular patterns of intratumoral T cells from 11 elderly patients (≥ 65 years) and 5 adults with NSCLC treated with neoadjuvant anti-PD-1 were investigated, contemplating 419,107 T cells. Statistical analysis was performed with *Fisher, Wilcoxon, Student* and *Mann-Whitney* tests, with p<0,05. **Results.** ICI responsiveness was achieved despite age. T cell immunosenescence was observed both in aged (≥ 65 years) and younger NSCLC individuals. Both elderly and adult individuals produced responses with a heterogeneous molecular program associated with tumor-reactive CD8+ TILs. Specifically, CD8+ T cells from elderly patients showed an enhanced expression of *PDCD1* and *CXCL13* (p<0.001) in comparison to younger subjects. **Conclusion.** Our findings demonstrate favorable molecular signatures in aged NSCLC individuals following anti-PD-1 treatment and suggest that the recruitment of older adults in immunotherapy clinical trials should not be dismissed solely on the grounds of age. **Funding:** CAPES. PRONON. CNPq. **Keywords:** Lung cancer;Aging;Neoadjuvant Immunotherapy.

DO - 270 - **Evaluation of the immunological mechanisms of the adaptive immune response induced by infection caused by Toxoplasma gondii**

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Toxoplasmosis, caused by the obligate intracellular parasite *Toxoplasma gondii*, is a disease with worldwide distribution. The immunological mechanisms involved in the development of ocular lesion during the infection are still unknown. *T. gondii* triggers IL-12 production by dendritic cells that in their turn and in combination with IFN-g produced by natural killer cells induce the development of a Th1 acquired immune responses aiming to control parasite burden. Therefore, we evaluated the $CD4^+$ T cell response in a longitudinal study of patients with toxoplasmosis stratified by the presence of ocular involvement, both at the early acute stage and six (early chronic) and twelve (advanced chronic) months later during chronic infection. Cells were acquired by flow cytometry and their frequencies defined manually (supervised) or by the unsupervised approach were calculated on $CD4^+$ T cells and subpopulations. For supervised analysis and unsupervised clustering we used packages FlowSOM. We found that patients with acute and early chronic *T. gondii* infection have higher frequencies of effector memory $CD4^+$ T cells when compared to unexposed controls (Ctl). On the contrary, patients in more advanced chronic phase have higher frequencies of central memory T cells when compared to acutely infected patients. Terminal effector T cells were less frequent in the circulation of patients with acute and advanced chronic toxoplasmosis without lesions when compared to Ctl. Unsupervised analyzes reveal that the frequencies of four cell clusters out of 50 obtained by FlowSOM are increased in patients with acute ocular when compared with patients without ocular lesion. These clusters are composed of effector and effector memory cells, most of which are committed to cytotoxic functions. Together, our analyzes show that acute toxoplasmosis is an inflammatory disease and that the combination of markers used here bring light on the understanding of the immunopathology of ocular involvement. **Keywords:** Toxoplasma gondii;immunology;ocular lesion.

DO - 271 - **RASSF9 regulates the Cell Death Machinery in Melanoma**

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The Ras-Association Domain Family (RASSF) is composed of Ras effector proteins that share a Ras-association (RA) domain. The family comprises ten members (RASSF1-10) that are divided into two groups depending on the location of the RA domain. RASSF9 is a member of the N-terminal group and is mostly expressed in the stratified epithelium, where it plays an important role in maintaining epidermal homeostasis. RASSF9 has also been identified as a gene whose expression is induced upon continuous exposure to UV radiation. Importantly, RASSF9 has been suggested to act as a tumor suppressor in some cancers by interfering with cellular proliferation. The role of RASSF9 in melanoma remains obscure. To address this point, we developed several RASSF9-deficient murine melanoma cell lines using the CRISPR/Cas9 system. Using these cell lines, we evaluated the role of RASSF9 on both proliferation and resistance to cisplatin, a chemotherapeutic drug used in the clinic, as well as to other cell death inducing drugs. RASSF9 elimination did not significantly interfere with proliferation but resulted in an increased resistance to cell death. Taken together, our results suggest that RASSF9 acts as a tumor suppressor in melanoma by contributing to the activation of the cell death machinery. **Keywords:** RASSF9;Melanoma ;Cell Death.

DO - 272 - **Lipoproteomics reveals profound alterations in the immune functions of lipoproteins during severe COVID-19**

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Patients who develop severe COVID-19 are affected by symptoms resulting from the hyperinflammatory process that promotes the emergence of decompensated mechanisms such as marked decrease in lipid profile. A strong correlation has been demonstrated between reduced levels of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) and an unfavorable prognosis. The dyslipidemia associated with severe clinical outcomes occurs through mechanisms that are not yet fully elucidated. However, there is evidence that lipoproteins undergo structural and functional modifications in response to viral infection. We investigated proteins associated with HDL and LDL in individuals with severe COVID-19 using a lipoproteomic approach. We employed a label-free proteomics approach to identify responses to SARS-CoV-2 infection in HDL and LDL isolated from human plasma. To assess differences in the lipoproteome, we distinguished the biological groups into critically ill patients with COVID-19 (survivors and non-survivors) and controls. Finally, computational analyses were applied to provide information on different (DEPs) and unique proteins (EPs) for each condition, as well as the biological processes related to the pathogenesis and severity of the disease. EPs and DEPs identified reflected the exacerbation of the immune response. HDL and LDL lipoproteome data in COVID-19 are mainly associated with complement activation and coagulation cascade. ApoA-I and PON1 are less abundant in COVID-19, and proteins related to inflammatory response and tissue injury (SAA, Protein S100) were associated with mortality from the disease since they were predominant in non-surviving patients. The data confirm that alterations in the lipoproteome of patients affected by severe COVID-19 reflect the pathophysiology of the disease, mediated mainly by immunological events of inflammation, and change in the composition of lipid particles which impact the progression and severity of the infection. **Keywords:** Proteomics;SARS-Cov-2;Lipoproteins.

DO - 273 - **Immunomodulatory Characteristics Acquired by Tumor Cells through In Vivo Selection**

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Metastasis is the leading cause of cancer-related mortality, and the formation of premetastatic niches plays a critical role in this process. Premetastatic niches are microenvironments established at distant sites before the arrival of tumor cells. These niches are orchestrated by tumor-derived factors like extracellular vesicles (EV) that can prepare the target organs and lead immune cells for subsequent metastatic colonization. During tumor development, tumor cells undergo dynamic changes driven by selective pressures from the immune system and the tumor microenvironment. Tumor cells that can evade immune responses and survive in the face of immunosurveillance gain a selective advantage and induce metastasis. Metastatic cells can be collected and used to induce another primary tumor to obtain more metastasis in a process called *In Vivo* Selection. *In vivo* selection has been used mainly to generate more metastatic cell lines in studies that investigate different genetic profiles and cell behaviors. Here, we want to investigate if the tumor cells acquire immunomodulatory characteristics that can contribute to the establishment of premetastatic niches facilitating subsequent metastatic outgrowth with focus on the EVs released by tumor cells. To this aim, we performed repeated injections of 4T1 metastatic cancer cells taken from lungs compromised by metastasis. We compared the capacity of EVs released by highly and less metastatic 4T1 cells to induce premetastatic niches in the lungs of mice. Elucidating the role of these characteristics in shaping the immune landscape of premetastatic sites will provide valuable insights into the mechanisms underlying metastasis and offer new opportunities for therapeutic interventions aimed at preventing or disrupting the formation of premetastatic niches. **Keywords:** Premetastatic Niche;Extracellular Vesicles;In vivo Selection.

DO - 274 - **Oral exposure to Escherichia coli toxin protects against lung allergen-induced inflammation**

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Recent studies suggest that the intestinal-derived components regulate lung immune homeostasis. On the other way, changes in the lung can modulate the gut immune system. Yet, mucosal-associated immune responses have been studied in a compartmentalized manner, as if there is no communication between different organs. Thus, the role of the gut-lung axis in mucosal immune responses is poorly understood. Using enterotoxigenic *Escherichia coli* (ETEC), we established a model of a gut restricted infection without translocation to any other tissue. In response to ETEC, type 2 innate lymphoid cells (ILC2s) and eosinophils accumulated in the intestinal lamina propria and persisted for the long-term after the bacterial clearance. In addition, oral ETEC transiently increased the numbers of ILC2 and eosinophils in the lung parenchyma. To test if oral ETEC could exacerbate lung immune responses to allergen, we exposed mice that recently cleared ETEC to intranasal papain/ovalbumin allergy induction model. Unexpectedly, ETEC-exposed mice were significantly resistant to allergen-induced lung inflammation, with reduced numbers of ILC2, Type 2 effector $CD4^+$ T cells (Th2) and eosinophils. Paradoxically, oral ETEC-induced protection against lung allergen exposure was dependent on IL-33: IL-33KO mice developed partial lung inflammation in this model, and no protection induced by ETEC exposure was observed. Importantly, protection from allergy was achieved with ETEC heat-labile toxin (LT) oral exposure; we also found LT increases the expression of IL-33 receptor in the ILC2 in the intestine, and conversely reduces the expression of this receptor in the ILC2 present in the lung. Paradoxically, in the absence of ILC2 (RORalpha flox/IL7Rcre) in mice, the protective profile is lost. Our results indicate that gastrointestinal bacterial infection mediators (toxins) negatively regulate the onset of allergic inflammation and may provide a novel immunological basis for the “hygiene hypothesis". **Keywords:** Allergy;Gut-Lung axis;ILC2.

DO - 275 - **PROTEOMIC ANALYSIS OF PERIPHERAL BLOOD POLYMORPHONUCLEAR CELLS FROM SEPTIC PATIENTS SHOWS MORE PRONOUNCED ALTERATIONS IN NON-SURVIVORS THAN IN SURVIVALS**

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Introduction: The immune response in sepsis is mediated by leukocytes, with neutrophils being the front line of defense. In cellular activation, energy metabolism supports function and in neutrophils, glycolysis is the main pathway. Here, we investigated the PMNs proteomic changes in septic patients, compared to healthy volunteers (HV). Methods: We selected 30 patients (21 - survivors and 9 – non-survivors) of patients admitted to the ICU and 11 healthy volunteers as controls. PMN samples were taken from the biorepository for processing. Proteins were extracted, reduced, alkylated, digested with trypsin, and labeled with TMT tagging. Raw data from mass spectrometry analysis were processed using Proteome Discoverer Suite and analyzed in R software. Differentiated abundant proteins (DAPs) were subjected to a pathway enrichment analysis in DAVID database. Results: Of the 1069 proteins identified, 684 are present in more than fifty percent of the samples. Patients with sepsis who survived present with 149 DAP sand non-survivors present 261 compared to HV. we found changes common to all of them, ranging from innate immune response to phagocytosis, but the main ones are in the metabolic field, such as the metabolism of fatty acids, amino acids metabolism and the cycle of tricarboxylic acids, while in glycolytic processes only the glycogen pathway was present. We investigated the relevance of these findings by comparing patients with sepsis who survived with those who died. All pathways found to be altered in the survival group are present in both conditions, but the group of patients who died has the highest number of altered pathways, suggesting a greater impairment of biological functions. Conclusion: Proteomic analysis reveals alterations in patients with sepsis, with a higher incidence in those who died. The altered pathways are mainly related to the immune response of neutrophils and energy metabolism, with emphasis on TCA, which appears with its altered components. **Keywords:** Neutrophil;Proteomic;Metabolism.

DO - 276 - **T lymphocytes antigen-specific response in patients with COVID-19 and Chagas disease coinfection**

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Approximately 30 to 40% of Chagas disease (CD) patients present the cardiac or cardiodigestive form of the disease, with majority of them being elderly or with comorbidities. A study at Evandro Chagas National Institute of Infectious Diseases/FIOCRUZ, showed that coinfection with SARS-CoV-2, compared to other causes of admission, was the higher risk of in-hospital mortality for CD patients. The underlying mechanism behind this remains unknown. Adaptive immunity plays a pivotal role in the resolution or not of both diseases depending on the profile of the response. Therefore, the aim of this study is to understand the impacts of COVID-19 infection in patients with CD monitored at Lapclin-Chagas/INI/Fiocruz and investigate the associations between different clinical forms of CD (indeterminate and cardiac) in the absence or presence of COVID-19. We analyzed different profiles of antigen-specific lymphocyte activation in CD4 T cells, regulatory T cells, and CD8 T cells after *in vitro* stimulation with SARS-CoV-2 'S' protein or total *T. cruzi* protein lysate. Production of profile-defining cytokines such as INFy, TNFa, IL-17, and IL-4, activation molecules such as CD69, CD137, and IL-2, as well as Granzyme, Perforin, and PD-1 were quantified by flow cytometry. Multiplex Cytokine (48plex) quantification of patient's serum were also done. Our preliminary analysis suggests that COVID-19 patients with the cardiac form of CD have more $CD69^{+}$/$IL\text{-}17^{+}$ and $CD69^{+}$/$IL\text{-}2^{+}$ CD4 T cells and $PD\text{-}1^{+}$ CD8 T "S" responding cells than non-coinfected patients. Ongoing analysis of the response to *T cruzi* proteins in COVID+ and COVID- patients are ongoing, as well as the multiparamtetric data processing for the cytokines. Our initial results suggest that COVID-19 can influence immune status of CD cardiac patients. **Keywords:** T lymphocytes;COVID19;Chagas disease.

DO - 277 - **Unraveling COVID-19 Resistance: Distinct Immune Profile in Uninfected Women highly Exposed to SARS-CoV-2**

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Global cases of individuals who have been directly exposed to SARS-CoV-2 but have remained uninfected. This intriguing phenomenon suggests the existence of biological mechanisms of resistance against COVID-19, similar to the natural resistance observed against certain pathogens in other human infectious diseases. To explore this intriguing possibility further, our study focused on investigating the innate and adaptive immune responses of six uninfected women who were exposed twice to their reinfected partners. We meticulously evaluated their type I/III IFN immune innate mediated responses, T lymphocytes, and Natural Killer (NK) cells' phenotypic characteristics. Additionally, we conducted an in-depth analysis of cytokine production and performed whole exome sequencing to identify any variants associated with susceptibility or resistance to COVID-19. Interestingly, our findings revealed a noteworthy upregulation in the expression of the IFIT3 gene following TLR3 stimulation in all six uninfected women, while no such effect was observed in either the men's group or the control group of infected women. Moreover, we observed a significant presence of NKG2C+ memory-like NK cells and an increased cytotoxic activity of T and NK cells in these uninfected women. Our compelling findings shed light on a distinctive immune profile in these naturally resistant women, despite repeated exposure to SARS-CoV-2. The study was approved by the Committee for Ethics in Research of the Institute of Biosciences at the University of São Paulo (CAAE 34786620.2.0000.5464) and supported by FAPESP (grant numbers 2013/08028-1, 2014/50931-3, and 2020/09702-1) and CNPq (grant numbers 465355/2014-5 and 404134/2020-3). **Keywords:** COVID-19;Natural resistance;SARS-CoV-2.

DO - 278 - **Study of the role of inflammatory monocytes in the control of experimental Leishmania major disease in mice**

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Leishmaniases are a group of diseases caused by intracellular digenetic parasites. Protozoa of the genus Leishmania are intracellular parasites in vertebrates, with the macrophage being the main host cell, while it is considered the cell responsible for controlling the disease. Our studies, however, have shown that these cells present difficulties in controlling experimental infection in vitro. Once infected, macrophages become incompetent and cannot eliminate the parasite, which makes it difficult to understand how natural infection control occurs. We recently demonstrated that inflammatory monocytes (GR1/Ly6C+ CCR2+) are capable of controlling experimental infection in mice, being the first cells to migrate to the site of infection, massively eliminating the parasites. This migration is dependent on CCR2 and C57BL/6 CCR2 Knockout mice show difficulty in controlling the infection, showing increased lesions in the paw. Thus, the objective of this work was to evaluate the role of inflammatory monocytes (GR1/Ly6C+ CCR2+) in the control of experimental disease in mice. For this, C57BL/6 mice were infected with *L. major* in the paw and compared with C57BL/6 CCR2 Knockout mice and with C57BL/6 CCR2 Knockout mice that received adoptive transfer of bone marrow cells from wild animals. Lesion control was observed in transplanted CCR2 Knockout mice when compared to non-transplanted CCR2 Knockout mice. In addition, experiments with parabiosis, combining wild-type mice (CCR2+) with CCR2 Knockout mice (CCR2-) led to the recomposition of CCR2+ monocytes in the circulation of Knockout animals and the control of footpad injury. Our results suggest that inflammatory monocytes participate in the control of experimental disease in mice infected with *L. major*. **Keywords:** Leishmania major;Inflammatory Monocyte;CCR2.

DO - 279 - **The Role of the Microglial Receptor CX3CR1 in Heme-Induced Brain Injury in Mice**

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The physiopathology of Intracerebral Hemorrhage is complex, and one relevant pathway is the damage induced by products of blood cell degradation, such as hemoglobin, heme, and iron, that have been shown to induce brain injury both by the production of reactive oxygen species and the activation of microglia. Our group has recently developed an ICH model in mice through the intracerebral injection of heme. Our objective was to investigate the importance of the CX3CL1 receptor CX3CR1 in heme-induced brain injury. We used 18-24 weeks old male C57BL/6 wild-type (WT) and CX3CR1 knockout (CX3CR1 KO) mice. Tests on female animals are under way. Animals were submitted to stereotaxic injection in the striatum of 5 ul of either a solution of heme (15 ug) or vehicle (sham). Motor function was evaluated using the Rotarod test 1 day pre-op and on days 3 and 6 post-op. Animals were euthanized by transcardiac perfusion with saline and 4% paraformaldehyde on day 7, and their brains were harvested and then sliced and submitted to thionine staining with analysis under an optical microscope to confirm the lesion in the Striatum. Statistical analysis was performed using GraphPad Prims v.9.0, and Two-way ANOVA and the Tukey's multiple comparisons test. Rotarod tests revealed a significant decline in the average time to fall on day 6 of WT heme-injected mice (mean 151.22 s – SD 87.65 s) in comparison to WT sham (mean 237.3 s – SD 108.74 s; $p = 0.022$). There was no significant difference on day 6 between the average time to fall of heme-injected CX3CR1 KO (mean 196.24 – SD 39.57) and sham mice (mean 201.12 s – SD 27.59; $p = 0.99$). The results show that heme injection can produce motor deficits, which are less intense in CX3CR1 KO animals. As this receptor controls microglia activation and mediates immune responses, this could indicate that microglia activation plays a key role in heme-induced brain injury. Further studies are required to better determine the role of microglia in ICH. **Keywords:** Microglia;Heme;Intracerebral Hemorrhage.

DO - 280 - **WILL WHITE BLOOD CELLS TELL? A POTENTIAL NOVEL TOOL TO ASSESS BROILER CHICKEN WELFARE**

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The search for new methods to assess animal welfare remains relevant. Our objective was to study leukocyte morphology for broiler chickens raised in dark houses to develop a morphological score for heterophils (HET) and lymphocytes (LYM). Blood (1 mL) was collected from 320 chickens from four barns via punction of the brachial vein. Samples were analyzed for total white blood cells (TWBC), HET, LYM, monocyte (MON), eosinophil (EOS), basophil (BAS), and the HET to LYM ratio (H/L) was calculated. The HET morphological score was 0 for typical cytoplasm with robust and fusiform granules; 1-2 for decreased granule density, rounded granules and decreased segmentation; 3-4 for basophilic and large granules or decreased granule density or agranulated, vacuolated cytoplasm and karyolysis. For LYM, score 0 for typical round nucleus with dense chromatin, moderately basophilic cytoplasm; and score 1 for increased basophilia and vacuolation. The TWBC ($6.42\pm0.08X10^3$) was normal; HET (50.20±0.94%) presented a discrete increase and 3-4 HET scores were observed in 291 chickens and 1-2 HET scores in 29 chickens. Some band forms, metamyelocytes and myelocytes were observed in 100 blood smears, reflecting decreased HET maturity. The high prevalence of HET scores 3-4 may indicate severe inflammation processes. The LYM value was 41.6±1.7%, which in 203 chickens presented normal morphological characteristics (40,6±1.1%, score 0), and in 117 chickens exhibited atypical form (0,9±0,1%, score 1), with one to at most six atypical cells per smear. The H/L (1.8±0.1) was higher than the reference range. MON (5.9±0.5%) and EOS (1.8±0.1%) percentages were normal. It is likely that the chickens faced moderate to severe inflammatory processes and general stress conditions. As low welfare levels were indicated by the high H/L, we conclude that scoring the morphological features of heterophils may be a promising indicator to assess the welfare of broiler chickens. **Keywords:** avian blood;inflammation;leukocyte.

DO - 281 - **Senescent CD4+ T-Lymphocytes as Potential Driver of Bone Loss during Periodontitis**

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Objectives: Senescence is a biological response to cellular stress, during which cells change their physiological functions, acquire new properties, and produce a senescence-associated secretory phenotype (SASP). An increase in senescent CD4+ T lymphocytes is frequently observed in distinct osteolytic diseases; however, their role has not been described in periodontitis. The aim of this study is to determine the presence and role of senescent CD4+ T-lymphocytes during periodontitis. Methods: Ligature-induced periodontitis model was used to evalute the co-expression of CD4, p16 and phospho-p38. In vitro, senescent CD4+ T-lymphocytes were induced by H2O2. The lysosomal and mitochondrial function was assessed using LysoTracker, senescence-associated β-galactosidase activity, MitoTracker, TMRE, MitoSOX and CellRox/H2DCFDA, and the production of SASP candidate (IL-17A, RANKL and TNF-a) by cytometry. To evaluate the influence of p38 MAPK, senescence induced CD4+ T cells were treated with the inhibitor BIRB-796. Results: CD4+ T lymphocytes with cellular senescence hallmarks were identified in periodontal lesions. Furthermore, we observed lysosomal and mitochondrial dysfunction in senescence-induced CD4+ T lymphocytes. We observed increase in the production of a Th17-type SASP in senescence-induced CD4+ T lymphocytes, which was inhibited by the p38 MAPK inhibitor - BIRB-796, as evidenced by decrease in mitochondrial mass, mitochondrial and cytoplasmic ROS production, as well as in the frequency of Th17-type lymphocytes during the induction of cellular senescence. Conclusion: Senescence-induced CD4+ T cells exhibit lysosomal and mitochondrial dysfunction along with the production of a potentially osteoclastogenic Th17-type SASP, orchestrated in part by the activation of p38 MAPK. This data suggest a possible participation of senescent CD4+ T lymphocytes during periodontitis lesions development. **Keywords:** periodontitis;senescence, CD4 T lymphocytes;p38, SASP.

IC - 009 - **Aging: NRF2 cellular signaling pathway has anti-inflammatory effect on resveratrol-mediated TNF and IL6 production**

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Introduction: One of the hallmarks of aging is both oxidative stress and inflammation. Resveratrol (RSV) may play a beneficial role against inflammation through the balance of cytokines regulated by the NRF2 pathway (BBA. 1863:585-597, 2017). **Objective:** To evaluate the effect of the NRF2 on the production of pro-inflammatory cytokines (TNF and IL6) and anti-inflammatory cytokines (IL10) in leukocytes from different age groups treated with RSV. **Materials and methods:** The project was approved by the UFMG Ethics Committee (CAAE: 33842420.4.0000.5149). The subjects were divided into three groups: 20-39, 40-59 and 60-80 years old. After separating the leukocytes, a 24-hour treatment was performed with and without inhibitor ML385 (Nature Com. 9:1-18, 2018) with the following treatments: Control, RSV, H_2O_2, H_2O_2+RSV. Cytokines were measured by the ELISA (*p<0.05). **Results:** Evaluating TNF and IL-6, the same profile was observed in both age groups. There was a decrease in the RSV *vs* Control, increase in H_2O_2 *vs* control and a decrease in H_2O_2 + RSV *vs* H_2O_2. Regarding IL-10, there was an increase in RSV *vs* control, decrease in H_2O_2 *vs* control and an increase in H_2O_2 + RSV *vs* H_2O_2. The same profile was observed comparing the same groups with inhibitor. When the NRF2 is inhibited, an increase in TNF and IL-6 values is observed in all age groups and treatments, especially in an oxidized environment compared to the same treatments without the addition of the inhibitor. IL-10 levels decrease in the presence of the inhibitor when RSV is added in the 20-39 and 40-59 age. It is important to highlight that with aging, the NRF2 begins to fail, as there was no difference in IL-10 levels in the H_2O_2+RSV without *vs* with inhibitor. **Conclusions:** The action of RSV is more effective at a preventive level, since it presents better results in younger age groups. Even so, this compound is a possible therapeutic in aging, since the NRF2 pathway is not silenced. **Keywords:** aging;NRF2;resveratrol.

IC - 010 - **Imunoensinando from UFRRJ –extension activities and social media as a tool for building knowledge in Immunology in schools**

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The ImunoEnsinando-UFRRJ is an extension project created in 2019, for which a social media channel was created since 2021. Our aim is to propagate scientific information for the public of Seropédica city, located in the Baixada Fluminense region of Rio de Janeiro state. Seropédica is recognized by 9 low socioeconomic indicators. According to 2019 IBGE data, the Basic Education Development Index for the first years of school was 4.9, for the last years of this same segment was 4.2 and for High School was 4.5. Science is an essential part of the citizen's life and must be popularized so that everyone can have the opportunity to access it, especially in topics focused on human, animal and environmental health known as one health concept. This project proposes to stimulate central immunological themes in our daily life by sharing scientific content through in person activities at public schools and posts on a social media. In person dynamics were carried out at schools using the concept of Problem-Based Learning (PBL), where situations present in everyday life such as the importance of vaccination, HIV infection in young people and the impact of fake news are discussed. The PBL activities were well received by high school students with active participation. In addition, the interaction with the contents published on our social media were followed up. A timeline of posts focused on Immunology were established. Data obtained from the social media channel, in August 2022 the number of followers was 558 and in June 2023 the number raised to 733 followers, an increase of 30%. Most of the followers are located at Rio de Janeiro (37%), Seropédica (25%) and Nova Iguaçú (8,5%). Most of them are young with ages between 18 to 24 years old (44,5%). Our data shows that initiatives like this can promote the popularization of science, highlighting the role of science as a transforming agent that contributes to the formation of critical thinking and opinion makers. **Keywords:** Education;Immunology;High School.

IC - 011 - **ROLE OF HISTONE DEACETYLASES IN NF-kB PATHWAY SIGNALING IN CISPLATIN-INDUCED LIVER AND KIDNEY INJURIES IN ZEBRAFISH**

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Introduction: Cisplatin, a drug used for solid cancer treatment, has as side effects the nephrotoxicity and hepatotoxicity, consequences of injuries induced by the drug, mainly from the release of reactive oxygen species caused by the attack on the CYP450 enzymatic complex. Considering this, we will use zebrafish (Danio rerio) as an organism model for injuries, because it has the advantage of having genetic compatibility and anatomical similarity with the human. Besides, the immortalized lineage Zebrafish Liver Cells (ZFLs), that will also be used for this project, is a good alternative to simulate human diseases. In this study, we aim to establish in vitro injuries models and investigate a potential protector effect of histone deacetylases inhibitors (HDACi) relying on autophagy and, from the degradation of IKKβ, a decrease in canonical NF-kB pathway activation and, consequently, leading to immunosuppression. **Methods and Results:** For an establishment of kidney primary cell culture, we will use a pool of 6 kidneys per group, which group using three different mediums with or without supplements, in a total of six groups. For this experiment, we will use AB wild type and the transgenic line LysC:dsRED to guarantee that the culture has only epithelial cells. After, tests with different concentrations of cisplatin on these primary cultures and in the ZFL cells to determinate in vitro injuries models will be carried out. To address the question regarding the protection effect of HDACis in these cells, we will evaluate some parameters using flow cytometry, qRT-PCR, and western blot techniques to analyze the interaction between the protein Keap1 and IKKβ and associate this to NF-kB internalization to the nucleus. **Conclusion:** With this project, we hope to understand and discover new protection mechanisms against treatments that lead to tissue damage, helping in possible therapeutic treatments aimed at improving the quality and life expectancy of patients. **Keywords:** Cisplatin;NF-kB;HDACi.

IC - 012 - **The role of CD4+ T cell on the efficacy of intranasal LaAg vaccine**

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The leishmaniasis are neglected tropical diseases caused by protozoan parasites of the *Leishmania* genus. In Brazil, *L. amazonensis* is the principal causative agent of cutaneous leishmaniasis. To mitigate the problem, developing a vaccine against leishmaniasis is significant and urgent. However, currently, there isn't an approved vaccine for humans use. Previous studies with *L. amazonensis* antigens (LaAg) by intranasal route induced partial protection. Therefore, we aim to characterize the mechanisms by which the LaAg vaccine acts in the immune system of mice, mainly the $TCD4^+$ lymphocytes. For the accomplishment of the experiment C57BL/6 WT, $CD4^{-/-}$, $MHCII^{-/-}$ and Interferon-gamma$^{-/-}$ ($IFN\text{-}y^{-/-}$) mice were immunized twice with LaAg (0.5 µg/µL) intranasally with an interval of one week between them, as the control group, we used two doses of PBS. After one week of the second dose, the challenge was carried out by infecting the footpad of the mice with $2x10^5$ *L. amazonensis*, following the progression of the lesion measuring of the infected paw. At the end of the experiment, the parasite burden of the footpad and lymph nodes was quantified by LDA. Our results showed that vaccinated WT mice had a reduction in the lesion when compared to PBS. However, the vaccinated $CD4^{-/-}$ group mice didn't protect against the lesion or decrease of the parasite load, showing no statistical difference between the vaccinated and PBS groups mice. Similar results were obtained using $MHCII^{-/-}$ that demonstrated the importance of $TCD4^+$ lymphocytes. We also vaccinated $IFN\text{-}y^{-/-}$, and no protection was observed in the lesion and parasite burden, suggesting the participation of IFN-y producing $CD4^+$ T cells. Experiments using $RAG^{-/-}$ reconstituted with $CD4^+$ $IFN\text{-}y^{+/+}$ or $CD4^+$ $IFN\text{-}y^{-/-}$ T cells are necessary to confirm the role of this cell. We concluded that the efficacy of the LaAg vaccine is associated with the presence of $TCD4^+$ lymphocytes which are essential for disease control in vaccinated mice. **Keywords:** Leishmaniasis;Vaccine;CD4 T cell.

IC - 013 - **Co-culture of human tumor cells with zebrafish blastoderm cells: Study of the tumor microenvironment**

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Breast cancer has a high mortality rate, and non-individualized treatments often have adverse effects. Precision oncology aims to improve treatment effectiveness by analyzing clinical, genetic, and molecular factors for personalized and preventive treatments. Zebrafish (Danio rerio) is a valuable tumor study model as it mimics the pathophysiology of various tumor types. Zebrafish and humans share conserved oncogenes and tumor suppressor genes. This study aims to establish a 3D co-culture of zebrafish blastoderm cells and MDA-MB-231 cells, which may offer advantages for xenotransplantation, evaluation of tumor progression, drug testing, assessment of the tumor microenvironment, and the study of tumor cell morphology and metabolism. The study will use 5,000 tumor cells and 2,500 zebrafish blastoderm cells to create spheroids. Nanoparticles will be used to magnetize the cells and facilitate spheroid production. Glucose and lactate levels will be measured, and confocal microscopy will be used to observe spheroid formation. The hybrid spheroids will be injected into adult zebrafish, some of which will be exposed to dexamethasone. In the results obtained so far, cell proliferation has been observed in the 2D single culture of tumor cells, the 2D single culture of blastoderm cells, and the 2D hybrid co-culture. There was a greater proliferation of zebrafish blastoderm cells when they were in hybrid co-culture with MDA-MB-231 cells compared to when blastoderm cells were cultured in 2D single culture. In the hybrid co-culture, both tumor cells and blastoderm cells showed a tendency to reduce mitochondrial mass. We observed a more pronounced decrease in mitochondrial mass in blastoderm cells in hybrid co-culture compared to tumor cells in co-culture. The modification in mitochondrial mass may suggest a metabolic change that needs further investigation. It was possible to infer an interaction between the studied cells, as they displayed intimate contact with each other. **Keywords:** Microenvironment;Zebrafish;3D culture.

IC - 014 - **IMMUNOLOGICAL RELATIONSHIPS BETWEEN AEDES AEGYPTI EMBIONARY CELLS AND WOLBACHIA PIPIENTIS BACTERIUM**

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Wolbachia sp. is an obligate intracellular bacterium that lives in more than 60% of insects. Its wMel strain was artificially inserted into the *Aedes aegypti* and has been used as a control transmission of the viruses that cause Dengue, Zika and Chikungunya. One of the effects of *Wolbachia pipientis* infection is the activation of mosquito immunity. In this way, exploring the immune potential becomes an important control tool. This study has the objective of analysis of the immunological interactions between embryonic cells of *Aedes aegypti and* the bacterium *Wolbachia pipientis*. For a better understanding of these mechanisms, the phagocytic capacity and the production of reactive oxygen species, important parameters in the activation of the immune system, will be evaluated in embryonic cells with and without *Wolbachia*. In addition, the expression of genes related to immune pathways (Toll and IMD) will be performed in order to confirm the activation of the immune system by *Wolbachia pipientis* in embryonic cells. To assess how much the presence of *Wolbachia* impacts the infection of cells by the ZIKA virus, cells, with and without *Wolbachia*, will be infected and the viral load will be evaluated. Preliminary results show that cells with *Wolbachia pipientis* have increased phagocytic capacity compared to cells without *Wolbachia*. Initially proving the activation of the mosquito's embryonic cellular immunity by the presence of *Wolbachia pipientis*. We believe that this study will contribute significantly to understanding the immune system of the *Aedes aegypti* mosquito. **Keywords:** WOLBACHIA PIPIENTIS;AEDES AEGYPTI ;EMBRIONIC CELLS;

IC - 015 - **Effect of treatment with roflumilast on Graft-versus-Host Disease (GVHD) induced in mice**

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Introduction: Graft versus host disease (GVHD) is a systemic inflammatory condition due to allogeneic bone marrow transplantation, causing morbidity and mortality among affected individuals. In this regard, harnessing the induction of endogenous pro-resolution molecules holds therapeutic potential for managing this disease. Selective inhibitors of the enzyme phosphodiesterase type 4 (PDE4), such as roflumilast, have been demonstrated to increase levels of cyclic AMP (cAMP), which is associated with the upregulation of annexin-A1 (ANXA1), a crucial regulator of inflammation resolution. **Objective:** This study aimed to assess the impact of roflumilast treatment on the resolution of the inflammatory response associated with GVHD. **Methods:** Balb/c recipient WT were lethally irradiated with 7Gys and injected i.v with $1x10^7$ bone marrow cells + $1x10^7$ splenocytes from C57BL/6. The control group received isogenic cells from Balb/c. After transplant the recipients were clinically evaluated with a standard scoring system. The experimental group (ROF), received daily roflumilast treatment at various doses, initiated from the onset of clinical signs. The target organs of the animals were collected, and the other analysis performed. **CEUA:** 22/2020. **Results:** Mice subjected to GVHD and treated with roflumilast at a dose of 1 and 3 mg/kg at the onset of clinical signs showed effective protection resulting in increased survival and improvement of clinical signs, however mice treated with roflumilast at a dose of 10 mg/kg showed an accelerated lethality. When roflumilast was administered concurrently with transplantation, mice were not protected and had impaired bone marrow engraftment. We also show that mice submitted to GVHD have an increased expression of cleaved ANXA1 in the target organs and mice treated with roflumilast reduced protein cleavage in tissues. **Conclusion:** Our data demonstrate an important contribution of roflumilast to regulating GVHD inflammation. **Keywords:** GVHD;RESOLUTION OF INFLAMMATION;PDE4 INHIBITORS.

IC - 016 - **POLYMORPHISMS OF THE ACE2, TMPRSS2, MX1, AND TLR7 GENES ARE ASSOCIATED WITH THE RISK OF COMORBIDITIES AMONG PATIENTS WITH COVID-19**

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Background: Comorbidities such as systemic arterial hypertension (SAH), obesity, diabetes mellitus (DM), and chronic obstructive pulmonary disease (COPD) are associated with increased risk for COVID-19 severity and death. Single nucleotide polymorphisms (SNPs) in the genes encoding molecules involved in SARS-CoV-2 cell entry and downstream cascade of innate immunity mediators can affect the antiviral response. We investigated the influence of 10 SNPs in ACE2, TMPRSS2, MX1, and TLR7 genes on the presence or not of relevant comorbidities for COVID-19. Methods: A total of 384 inpatients were followed at the COVID-19 Pandemic Hospital Center at INI/FIOCRUZ, RJ, Brazil, from 2020 to 2021. SNPs genotyping was determined by PCR. Associations were estimated by unconditional logistic regression models. Results: Among the genotyped SNPs, significant associations were observed for relevant COVID-19-related comorbidities. Specifically, C/C genotype (aOR=4.04;P=0.049) for women or carrier-C for men (aOR=2.07;P=0.0367) and both genders (aOR=2.36;P=0.0008) in the ACE2 rs4240157 were associated with an increased risk of SAH. Regarding risk for obesity, associations were observed with no carrier-A for men (aOR=2.95;P=0.022) or both genders (aOR=2.18;P=0.0099) in the TMPRSS2 rs734056, as well with no carrier-G in the TMPRSS2 rs2070788 for both genders (aOR=2.06;P=0.017). In addition, associations for both genders were observed for carrier-C (aOR=3.09;P=0.029) in the MX1 rs464397 with increased risk for DM, and A/A genotype (aOR=4.05;P=0.042) in the TMPRSS2 rs734056 with increased risk for COPD. Furthermore, other polymorphisms in the ACE2, TMPRSS2, MX1, and TLR7 genes were associated with protection for SAH, obesity, DM, and COPD, independently of gender. Conclusions: This study shows that genetic polymorphisms in key molecules involved in SARS-CoV-2 cell entry and downstream signaling molecules of innate immunity are associated with relevant comorbidities for COVID-19. **Keywords:** COVID-19;Comorbidities;Single nucleotide polymorphisms (SNPs).

IC - 017 - **Application of the Chagas-Flow ATE IgG1 serology for post-therapeutic monitoring and genotype-specific diagnosis of Chagas disease**

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Trypanosoma cruzi was subdivided in six genetic groups, Discrete Typing Units (DTUs), TcI-TcVI. These DTUs can impact parasite biological characteristics besides the Chagas disease (ChD) epidemiology, clinic and treatment. Thus, it is important to associate the *T. cruzi* genetic diversity with the methods used for the diagnosis and post-therapeutic monitoring of the ChD. In this context, was standardized the serological technique for detecting anti-amastigote (AMA-A), anti-trypomastigote (TRYPO-T) and anti-epimastigote (EPI-E) antibodies of *T. cruzi* by flow cytometry (Chagas-Flow ATE IgG1) for the genotype-specific diagnosis of the ChD. The present study evaluated the application of Chagas-Flow ATE IgG1 in post-therapeutic monitoring and genotype-specific diagnosis of the ChD. 100 serum samples were tested by this technique: 8 from *T. cruzi* non-infected individuals (NI); and 92 from Chagas disease patients (CH) in the chronic phase, being 32 samples from not treated patients (NT) and 60 samples from benznidazol treated patients (Bz-T) evaluated at two times: baseline study and after five years follow-up. The results demonstrated that 100% of serum samples of the CH and NI groups were segregated by Chagas-Flow ATE IgG1 at study baseline. Genotype-specific diagnosis of the ChD was realized by the same technique and classified the samples at study baseline in: 44 as TcII, one as TcI and one as TcVI. In the post-therapeutic monitoring of ChD, some antigens and serum dilutions showed outstanding performance to segregated the CH patients serum samples before and five years after treatment. The data still demonstrated that in Bz-T group there was alteration in the *T. cruzi* DTU in one of the samples: at study baseline as belonging to TcVI, while after five years follow-up was identified as TcII. Chagas-Flow ATE has shown to be a promising technique for *T. cruzi* genotyping, diagnosis and post-treatment monitoring of the Chagas disease. **Keywords:** Chagas disease;Genotype-specific diagnosis;post-therapeutic monitoring.

IC - 018 - **Circulating biomarkers associated with Visceral Leishmaniasis**

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Visceral leishmaniasis (VL) is one of the most lethal and neglected tropical diseases of the worldwide, with high prevalence in Brazil. The main etiological agent is Leishmania infantum, parasite with part of its cycle in mononuclear phagocytes of vertebrate hosts. The clinical outcome is result of the balance between parasite multiplication and the host's immune response. A mixed profile, with high production of regulatory cytokines (IL-10 and TGF-β) in the infection microenvironment, associated with exacerbated production of Th1 and Th2 axis cytokines, is characteristic of patients with classic VL, an immunological profile that can lead to inflammatory response syndrome system and lethality if the disease is not quickly treated. Thus, the analysis of circulating biomarkers profile present in VL is necessary for well understanding immunological mechanisms associated with disease outcome, contributing to clinical practice. Hence, the present study aims to evaluate the profile of circulating biomarkers in the context of human VL. For this, circulating biomarkers were quantified in plasma samples from uninfected (NI) and asymptomatic (AS) individuals, and from patients with classic VL, evaluated before (VL-PRE) and after treatment (LV-POS), through Luminex assay, using “Milliplex MAP Kit–Th17”, from Merck Millipore and the analyses were performed by Bio-Plex Manager™ software (6.1). A strong activity of Th17 axis was observed in the AS group; on the other hand, in VL-PRE, was observed an exacerbated production of pro-inflammatory and regulatory biomarkers. It is important to highlight the high production of IL-6, IL-10 and IL-27, cytokines associated with severe VL. Our data showed strong correlations between pro-inflammatory and regulatory biomarkers in VL-PRE patients, a greater number of negative correlations involving chemokines and regulatory cytokines and laboratorial data was noticed. Finally, the data indicate that Th17 axis is protective in the context of VL. **Keywords:** Visceral Leishmaniasis;Biomarkers;Leishmania infantum.

IC - 019 - **THE POTENTIAL IMPACT OF IMIDAZOPYRIDINE DERIVATIVES AS ANTI-INFLAMMATORY DRUGS IN SEPSIS TREATMENT**

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Introduction: Every year, sepsis affects more than 30 million individuals worldwide, with increasing rates of 13.7%, leading to over 8 million deaths. The balance between pro-inflammatory and anti-inflammatory events is crucial for regulating immunity and the development of diseases. Cytokines, such as TNF-α, play a major role in developing diseases like sepsis. Recent research has focused on investigating novel treatments for sepsis and inflammatory diseases by targeting inflammatory signaling pathways and specific cytokine inhibitors, such as anti-TNF-α therapies. Thus, in this study, we employed a novel treatment approach for sepsis, using anti-TNF-α drugs, specifically imidazopyridine derivatives. **Objectives:** This study aimed to investigate the inhibitory effects of two imidazopyridine derivatives (4B and 4J) in experimental sepsis model. **Methodology:** Two derivatives of hexahydroimidazo[1,2-α]pyridines were used to assess their inhibitory role in TNF-α production. U-937 cells and C57Bl6 mice were stimulated with LPS. The experiments were conducted for 24 h to evaluate cytokine production, cell viability, and mortality. **Results:** Both tested compounds exhibited inhibitory effects on TNF-α and IL-6 production in U-937 cells. Compound 4J demonstrated a 45.27% inhibition of TNF-α, which was similar to the inhibition of IL-6 secretion. Cell viability remained unaffected. *In vivo*, compound 4J also showed promise in reducing sepsis-induced mortality in pre-treated mice. **Conclusion:** The findings suggest that hexahydroimidazo[1,2-α]pyridine derivatives hold therapeutic potential as drugs to inhibit the production of inflammatory cytokines, such as TNF-α, in experimental sepsis. However, further studies are necessary to fully comprehend the capabilities and properties of these derivatives, emphasizing the need for additional research. **Keywords:** imidazopyridine derivatives;anti-TNF-α drugs;inflammatory response.

IC - 020 - **Imunogame - an inclusive game as a learning tool.**

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The National Curriculum Parameters (PCN) for Elementary and High School Education in Brazil ensure that all students acquire essential knowledge for the exercise of citizenship. Health is one of the cross-cutting themes established by the PCNs, with health education actions understood as a set of participatory and emancipatory pedagogical practices aimed at raising awareness, sensitization, and mobilization for health promotion. In this context, the contents related to the immune system are of utmost relevance for understanding the functioning of the body and the dynamics of health-disease and immunization mechanisms. The teaching and learning of Immunology in Brazilian Basic Education are usually described as difficult and complex processes, but the use of creative strategies has been associated with meaningful learning. In addition to the issues related to the teaching of immunology, pedagogical proposals need to be inclusive. Thus, this project seeks to develop a game that stimulates the desire to learn through the active participation of students in the construction of important meanings in the field of immunology. To this end, an inclusive board game has been developed: the cards have both conventional and Braille writing, each card and the board have QR codes for access to explanations in sign language, the pieces used for movement on the board have color and texture differences, and the dice also has accessible texture. The game features a path with stops where two different types of cards can be accessed: one type with direct questions and the other with descriptions of situations and curiosities. Constructing the game in this way allows for interactions and conversations among participants and the teacher, enriching the learning experience. A game evaluation form was filled out by the students after the activity. Preliminary analysis suggests that the expected objectives of the proposed activity were achieved. **Keywords:** board game;inclusive game;immunology game.

IC - 021 - **EVALUATION OF THE IMMUNOGENIC POTENTIAL OF A RECOMBINANT PROTEIN VACCINE AGAINST PNEUMONIA IN AN ANIMAL MODEL**

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Streptococcus pneumoniae is a Gram-positive encapsulated bacterium, responsible for causing diseases as pneumonia, meningitis and sepsis, being described at least 98 serotypes. Although current vaccines are effective, they only protect against the most common serotypes. In addition, an increase in infections with serotypes not included in the vaccine has been observed. The development of a vaccine based on conserved protein antigens among different serotypes of *S. pneumoniae* is a promising approach. The proposal is to use the protein Sortase A, which is present in the pathogen's membrane and plays an important role in the processing of surface proteins. Therefore, this work evaluated the immunogenic potential of a vaccine against pneumonia in an animal model, using a microemulsified system as an adjuvant containing the SrtA protein. Initially, an *in silico* step with computational modeling was performed. The recombinant protein was expressed in *E. coli* Rosetta-gami, purified by high affinity liquid chromatography, and confirmed by SDS-PAGE and Western blot. To evaluate the immunogenic potential of the vaccine, 92 mice received 3 doses of the formulation or placebo. The humoral immune response was evaluated by ELISA, showing high titers of total IgG (1:128,000), IgG 1 (1:256,000) and IgG 2b (1:128,000) in immunized animals, whose presence of specific antibodies was confirmed by Western blot. Histological analysis of the lungs showed tissue damage in control animals challenged with *S. pneumoniae*. Animal survival showed a significant difference between the immunized group compared to the placebo group ($p<0.05$). In conclusion, SrtA is highly immunogenic, generating a protective effect *in vivo*, which makes it a promising target for the development of a vaccine that can produce greater coverage against different serotypes of *S. pneumoniae.* **Keywords:** STREPTOCOCCUS PNEUMONIAE;SORTASE A;VACCINE.

IC - 022 - **TLR4 receptor response in particulate pollutant-induced intraperitoneal macrophage inflammation**

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Introduction: Macrophages are cells of the innate immune system with phagocytic capacity. They play a vital role in maintaining homeostasis and protecting against harmful agents such as particulate matter (PM). The TLR4 receptor participates in the activation of the immune system and the recognition of pathogens. Particles that are not recognized by the immune system, such as particulate matter, composed of several heavy metals, can cause constant inflammation. The components of PM depend on the pollution index of the localities since the more significant the pollution, the greater the amount of PM. Objective: To elucidate the TLR4 receptor response to inflammation caused by PM in two regions of Rio de Janeiro. Materials and methods: Peritoneal macrophages from C57 and TLR4-/- mice were incubated with PM from Copacabana (CP) and Santa Cruz (SC) for 16 hours and subsequently fixed for NO (DAF) and ROS (DHE) fluorescence analysis. Results: ROS production increased in TLR4-/- in different regions(p=0,0136; TLR4-/-CP=300,0; C57-CP=213,2; p=0,001; TLR4-/-SC=422,2; C57-SC=318,7). NO production increased in TLR4-/- in CP and decreased in SC compared to C57 (p=0,0214; TLR4-/-CP=4625179; C57-CP=6023169; p=0,0042; TLR4-/-SC=6106208; C57-SC=4299032). ROS production in TLR4-/- is increased in SC (p=0,0015; TLR4-/--CP=300,0; TLR4-/--SC=422,2) what also happens in C57 (p=0,0032; C57-CP=213,2; C57-SC=318,7). NO maintained the behavior in TLR4-/- and was increased in SC compared to CP (p=0,0240; TLR4-/-CP=4625179; TLR4-/-SC=6106208) and was opposite in C57 increased in CP compared to SC (p=0,0127; C57-CP=6023169; C57-SC=4299032). Conclusion: ROS production increased in both CP and SC regions in TLR4-/- but increased in SC in C57. NO production increased in SC in TLR4-/-, and in C57 it is higher in CP compared to SC. An increase of ROS is seen in SC in both species, and concerning NO, an increase in SC is seen in TLR4, while in C57, the production is higher in CP. **Keywords:** Macrophages;TLR4 receptor;particulate matter.

IC - 023 - **DEVELOPMENT OF INTRANASAL DNA VACCINE AGAINST STREPTOCOCCUS PNEUMONIAE USING LACTOCOCCUS LACTIS AS DELIVERY SYSTEM**

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Streptococcus pneumoniae is an encapsulated gram-positive bacterium part of the nasopharyngeal microbiota and can cause diseases including otitis media, pneumonia, meningitis and sepsis, which affect mainly children and elderly. The polysaccharide capsule is the main virulence factor of pneumococcus and 98 serotypes have been described. Current vaccines offer limited protection, which causes a redistribution of these serotypes. Furthermore, they induce a short immune response without inducing memory, making important the development of new vaccine strategies. Therefore, this work aims to develop an intranasal DNA vaccine, in which *Lactococcus lactis* will carry a preserved antigenic protein in different serotypes of *S. pneumoniae*. Initially, an *in silico* step was performed, in which the protein of interest was determined and the target gene was synthesized in the pET21a vector, and subsequently subcloned in the pExu vector, for expression in eukaryotic cells. Then, *E.coli* and *L. lactis* were transformed with the appropriated construction for clone expression and the vaccine formulation, respectively. Conventional PCR and NGS sequencing were performed for sequence confirmation. Concomitantly, expression of the recombinant protein was performed in *E. coli*, and purified by high affinity liquid chromatography, this step was confirmed by SDS-PAGE and Western Blot. Furthermore, the recombinant protein production were successful, with an average yield of 356µg/ml. Subcloning and *L. lactis* transformation were confirmed by conventional PCR and agarose gel electrophoresis which showed a band of the expected size. The sequencing of the construction pExu:target gene is in progress, afterwards experiments in an animal model will begin to evaluate the protection and immune response induced by our vaccine formulation. Our challenge is to develop a vaccine with national technology and high coverage against *S. pneumoniae.* **Keywords:** STREPTOCOCCUS PNEUMONIAE;LACTOCOCCUS LACTIS;VACCINE.

IC - 024 - **Scleritis as a manifestation of Acanthamoeba keratitis**

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Introduction: *Acanthamoeba* spp are protozoans with a life cycle containing trophozoites (infectious form) and cysts (resistance form). Whenever the environment is adverse the trophozoite become cyst. *Acanthamoeba* scleritis is a rare and painful complication of *Acanthamoeba* keratitis (AK), affecting 10% to 18% of patients. Scleritis is a devastating complication of *Acanthamoeba* keratitis with a poor prognosis and a high enucleation rate of 6%. The pathogenesis is still not fully understood but it seems to be an immune-mediated reaction against the parasite. Systemic immunosuppressive treatment, even with prophylactic antiamoebic agents, should always be used with the greatest caution. **Case discussion:** We report a 47-year-old scleral contact lens wearer patient with a proven bilateral Acanthamoeba keratitis who developed a diffuse scleritis in both eyes. Patient was initially treated with systemic prednisolone with limited response followed by systemic immunosuppression with azathioprine with strict maintenance of antiamoebic treatment. Fortunately, there was a good response to the treatment and although the patient lost significant scleral thickness there was no spread of the infection and visual acuity remained within normal limits, as did the rest of the ophthalmological examination. Left eye underwent white dyeing to reduce the dark aesthetic consequences of scleral thinning. **Keywords:** Acanthamoeba;Keratitis;Scleritis.

IC - 025 - **Evaluation of the immunomodulatory effects of omega-3 and its impacts on Salmonella typhimurium infection**

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The intestinal mucosa is exposed to antigens on a daily basis, originating from various sources such as diet, microbiota, and pathogens. These interactions can influence the modulation of the immune response and subsequently impact an individual's health. Numerous studies have indicated that diets rich in omega-3 fatty acids exhibit antioxidant and anti-inflammatory effects, leading to a reduction in the occurrence of non-infectious diseases. Thus, our research group aimed to investigate the immunomodulatory effects of omega-3 supplementation on the gut and assess whether this supplementation would affect the immunocompetence of the gut mucosal immune cells during *Salmonella typhimurium* infection. To conduct the study, we utilized 4-week-old female BALB/c mice, which were divided into two groups. The first, received the AIN93G diet, while the second group received the AIN93G diet supplemented with omega-3 for a period of 21 days. Consumption of an omega-3-enriched diet resulted in a decrease in the frequency of Th1, Th17, and Foxp3+ regulatory T cells in the mesenteric lymph nodes as well as reduced the frequency of Th2 and Th17 lymphocytes in the lamina propria of the small intestine. Notably, omega-3 supplementation improved the immunocompetence to infection, as evidenced by the survival curve of mice infected with 10^5 CFU of *S. typhimurium* via intragastric administration. While the control group succumbed to the infection within 7 days post-infection, the group that received the omega-3 diet survived for 20 days. Consequently, our findings suggest that the consumption of a diet enriched with omega-3 improves gut immune regulation without compromising immunocompetence against intestinal infections. Moreover, it enhances resistance to infection caused by *S. typhimurium*. Nonetheless, further studies are required to delve into this hypothesis and gain a comprehensive understanding of the immunomodulatory mechanisms of omega-3. Financial support: CNPq, CAPES, FAPEMIG. **Keywords:** Omega-3;Dietary supplementation;Immune modulation.

IC - 026 - **Analysis of CD8+ T cells immunomarkers in distinct clinical manifestations of COVID-19**

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COVID-19 is a highly transmissible respiratory disease with symptoms ranging from mild to severe. Considering reports describing a decrease in CD8+ T cells, with differences in the production of multiple markers, the present study aimed to investigate the immunomodulatory mechanisms mediated by CD8+ T cells in order to characterize the presence of immunomarkers. To this end, whole blood samples were obtained from recruited volunteers and distributed into control (CTL - n = 9), mild IgG - (n = 5), mild IgG+ (n = 6), and severe (n = 7) groups. Peripheral Blood Mononuclear Cells (PBMCs) were isolated and incubated under 4 different conditions: unstimulated (medium), stimulated with SARSCoV-2 peptides (Pool Spike CoV-2 and Pool CoV-2), and stimulated with Staphylococcal Enterotoxin B (SEB). CD8+ T cells were analyzed for activation/proliferation (CD38, CD69, Ki-67), cytokine production (IFN-γ), cytotoxic (granzyme B and perforin), and degranulation markers (CD107a). The analysis showed that individuals with mild COVID-19 (IgG+) and severe cases had a lower frequency of CD8+ T cells. The mild groups exhibited higher levels of CD38 compared to the control group in unstimulated conditions and stimulated with Pool CoV-2. In relation to the expression of CD69 both IgG+ and IgG- exhibited higher levels than the control and severe groups in the Pool Spike CoV-2 condition. Regarding the coexpression of granzyme B and perforin, the control group had higher levels than the IgG+ group in the Pool Spike CoV-2, while the mild IgG- group had the lowest level of CD107a expression in the same condition. Both mild groups presented higher levels of IFN-γ in comparison to the control group in the Pool Spike CoV-2 condition and, in the Pool CoV-2, both control and severe groups exhibited lower levels when compared to the IgG- group. These findings characterize potential CD8+ T cell markers as important mechanisms to control the severity of this disease in mild COVID-19 patients. **Keywords:** immune response;flow cytometry;SARS CoV-2.

IC - 027 - **Study of the modulation of heme-oxygenase-1 activity in the function of murine macrophages during interaction with the fungus Sporothrix brasiliensis**

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Sporotrichosis is a subcutaneous mycosis caused by traumatic inoculation into the skin or mucosa by fungi of the Sporothrix spp complex. In Brazil, the disease presents itself as a zoonosis that has expanded greatly in recent years and the predominant species is Sporothrix brasiliensis, considered the most virulent and pathogenic. The recognition of the fungus by macrophages induces the production of cytokines and the activation of microbicidal mechanisms. However, the activity of these cells is limited by strains that have evading mechanisms. Heme-oxygenase-1 (HO-1) is an enzyme involved in the degradation of free heme generating catabolic products that have anti-inflammatory effects. Depending on the infection, HO-1 activity can limit the host resistance mechanisms or contribute to tissue tolerance by limiting the effects of inflammation and infection. Given this scenario, little is known about the function of this molecule in the pathophysiology of mycoses, including sporotrichosis. Since the expression of HO-1 inhibits macrophage resistance functions, we aim to investigate the role of this enzyme in the activation of peritoneal macrophages from C57BL/6 mice during in vitro interactions with S. brasiliensis (ATCC MYA-4823). Initially, we observed the induction of HO-1 expression by western blot and RT-qPCR in macrophages after the interaction with conidia and yeasts of the fungus. Our hypothesis is that HO-1 expression reduces the microbicidal activity of macrophages. Therefore, our next objective is to evaluate the fungicidal capacity of macrophages in the presence or absence of cobalt protoporphyrin IX, an HO-1 inducer, or tin protoporphyrin IX, an inhibitor of the enzyme. In addition, we will evaluate the production of cytokines (TNF, IL-6, IL-1β and IL-10). Understanding the modulation of HO-1 activity in the infection of murine macrophages with the fungus S.brasiliensis may provide a better understanding of the pathophysiology of the disease. **Keywords:** HO-1;Sporothrix;Macrophages.

IC - 028 - **Clinical Case of Scleritis from Acanthamoeba Keratitis**

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Introduction: *Acanthamoeba* spp. are protozoans with two morphological classifications, with trophozoites as infectious form and cysts as a form of resistance in which the trophozoite becomes a cyst from the adversity of the environment. *Acanthamoeba* scleritis is a rare and painful complication of *Acanthamoeba* keratitis (AK), affecting 10% to 18% of patients. Scleritis is a possible complication of AK with poor prognosis and high enucleation rate of 6%. The pathogenesis is still not fully understood, but it is assumed to be an immune-mediated reaction against the parasite. **Case discussion:** We report a 32-year-old female patient, with *Acanthamoeba* keratitis who subsequently developed scleritis in the left eye, user of monthly disposable contact lenses for many years, misdiagnosed with herpetic keratitis previously and treated with acyclovir without improvement. With the collection of a positive corneal scraping for *Acanthamoeba* and inconclusive confocal, treatment was started with Moxifloxacin 0.5% eye drops every 6h, Biguanide 0.02% eye drops every 1h and Doxycycline 100mg orally. Without regression of the condition and with an increase in the number of cysts, Biguanide 0.04% every 1 hour was used, and still without improvement, Clorexidine 0.04% every 3 hours was used. With the evolution of the severity of the condition even after the use of strong medications, the patient underwent a penetrating transplant in the left eye due to a corneal ulcer refractory to treatment. The patient progressed with recurrence one month later, evolving with indication of a new corneal scleral transplant in the left eye due to ulcer recurrence associated with scleritis. **Keywords:** Acanthamoeba;Scleritis;Keratitis.

IC - 029 - **Effects of immunodominant peptides of SARS-CoV-2 on T CD8+ cells post vaccination**

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The emergence of the novel coronavirus, officially designated as SARS-CoV-2, has created a global public health urgency. While some pathophysiological mechanisms are understood, there are still gaps in understanding the development of long-lasting protective immunity against the COVID-19. This has led to the elaboration of strategies to understand the presence of immunodominant antigens and their correlation with the development of infection. In this context, the study aimed to analyze immune responses to SARS-CoV-2 peptide antigens in vaccinated individuals, focusing on the correlation of biomarkers in CD8+ T cells, which play an important role in the antiviral response and the establishment and maintenance of protective responses against the disease. Peripheral blood samples were collected from a group of mild recovered individuals who had been vaccinated (n=12). Peripheral blood mononuclear cells (PBMCs) were incubated under four different conditions: unstimulated (medium), stimulated with Spike glycoprotein-derived peptides (PS), stimulated with ORF1ab and nucleocapsid-derived peptides (PO), and stimulated with staphylococcal enterotoxin B (SEB). In this study, the expression of immunological markers in the CD8+ T cell subset was evaluated using flow cytometry. Compared to the control, the stimulation with PO and PS led to an increased expression of specific markers such as CD69, IL-10, IL-17, TNF-α, IFN-γ, and perforin. Conversely, the expression of CD137 and CD107a was not altered. These data highlight how cellular immune responses to some of these peptides can contribute to protection against SARS-CoV-2 through immunization against COVID-19. **Keywords:** SARS-CoV-2;IMMUNODOMINANT PEPTIDES ;BIOMARKERS .

IC - 030 - **Curcumin prevents microglial death mediated by Zika virus infection**

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The cell death and neuron dysregulation of the central nervous system (CNS) is a keypoint involved in the pathogenesis of microcephaly due to Zika virus (ZIKV) infection. Microglia has a fundamental role in CNS physiology, as well it responds against infections. Curcumin has different immunomodulatory effects and showed antiviral properties. Here, we evaluated the curcumin effect on microglia infected by ZIKV. To this, BV2 cells infected with ZIKV at a multiplicity of infection (MOI) of 0.1 and 1 were treated or not with curcumin at 0.31, 0.62 and 1.25 µM. The cell infection was evaluated by cytometry and electron microscopy. The MTT assay and LDH quantification were used to assess toxicity and cell disruption. Annexin and PI were used to evaluate the cell death mechanism. Caspase 3 expression was analysed by flow cytometry. The results showed that BV2 cells are permissive to ZIKV, although few infected cells were observed by electron microscopy. This fact was confirmed by cytometry analysis in which the result showed that only 0.1 to 1.0% cells were infected. After 24 hours post-infection, the percentage of infected cells was higher at MOI 0.1 than MOI 1, and according to MTT results, the viability of MOI 0.1 infected cells decreased drastically compared with MOI 1 infected cells. Probably, the ZIKV mediate cell death by a necroptic regulated mechanisms, once there was an increased quantity of late apoptotic/necrosed cells and increased LDH amount in the culture supernatant from infected cells, but no alteration of caspase 3 expression was observed. The curcumin did not influence BV2 infection, but it reversed the toxicity provoked by ZIKV by increasing the viability in practically all treatment concentrations and by decreasing the cell death quantity. This data shows that curcumin has an important potential of being used as a coadjutant treatment, as it can prevent cellular death caused by ZIKV infection, probably by regulating anti-apoptotic mechanisms. **Keywords:** Microcephaly;Cell death;Zika virus.

IC - 031 - **ROLE OF CD300A RECEPTOR DURING AN EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

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The protozoan *Trypanosoma cruzi* (Tc) is the etiologic agent of the neglected Chagas Disease (CD), whose main complications include cardiac, digestive, and neurological dysfunction. Those clinical forms are related to an unbalanced inflammatory response against the pathogen. The CD300 molecules are part of the immunoglobulin superfamily receptors, which among them CD300a is an inhibitory one, emerging to be important in modulating the leucocytes function. In this work, we investigated the influence of the CD300a receptor in the development of Tc-induced pathogenesis. BALB/c (WT) and CD300a knockout (KO) female mice, 8 to 9 weeks old, were infected ip with 10^3 trypomastigotes forms of Y strain. Parasitemia and body weight were evaluated at selected times and the survival daily. Furthermore, the animals were euthanized at 110 day-post-infection (dpi) and the heart was collected for histopathological analysis. In the acute phase, the result showed that the absence of CD300a increased the resistance to the infection since the survival rate was higher when compared to WT, although no significant differences were found in parasitemia and body weight between those groups. However, in the chronic phase of the disease, the KO mice presented severer tissue damage in the heart, with a higher amount of inflammatory infiltrate and amastigote nests. Collectively, these preliminary results suggest that the CD300a receptor is an important regulator in the development of CD chronic commitments. This helps portray new insights into the disease pathogenesis, still completely unknown, as well as can provide new potential therapeutic approaches and new knowledge concerning the onset of the chronic complications of the disease. **Keywords:** Trypanosoma cruzi;CD300a;Immunoregulation.

IC - 032 - **IMPACT OF EXTRACELLULAR VESICLES SECRETED BY (NIH3T3-L1) ADIPOCYTES ON MACROPHAGE ACTIVATION DURING Mycobacterium bovis BCG INFECTION in vitro**

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Introduction: Obesity is characterized by a chronic low-grade inflammatory state known as metainflammation. Factors secreted by adipocytes, including Extracellular Vesicles (EVs), have been proposed as modulators of macrophage function and profile, thereby intensifying the processes of metabolic inflammation. Among these EVs, transcription factors such as PPARγ play a significant role in regulating macrophage function and are involved in the biogenesis of lipid bodies (LBs) during BCG infection. LBs are dynamic organelles with functions in lipid storage, synthesis of inflammatory mediators, cellular signaling, and providing a survival niche for pathogens. However, the specific signaling pathways and metabolic processes regulated by adipocyte-derived-EVs, as well as their impact on the progression of infection by intracellular pathogens, remain poorly understood. **Objectives:** To analyze the interactions between EVs secreted by adipocytes in the macrophage activation during *Mycobacterium bovis* BCG infection, *in vitro*. **Methods:** LBs were quantified using fluorescence microscopy, cytokine levels were measured using ELISA. EVs were isolated using a series of centrifugation steps and characterized and quantified using flow cytometry, Zeta Size analysis, and Micro BCA. **Results:** Specific subpopulations of EVs, namely microvesicles and exosomes, positively influenced LBs formation and macrophage activation during BCG infection. They induced the synthesis of TNF-α and IL-10. microvesicles also induced KC synthesis, which was negatively modulated in the infected groups, whereas this effect was not observed for macrophages stimulated with exosomes. **Conclusion:** Our findings suggest that EVs secreted by adipocytes play a crucial role in BCG-infected macrophages by promoting LBs formation and the cytokines synthesis. However, further studies are required to fully understand the intricate interactions between adipocytes and macrophages during infections caused by intracellular. **Keywords:** Extracellular vesicles;microvesicles;lipid droplets.

IC - 033 - **The use of monoclonal antibodies for cancer treatment**

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Monoclonal antibodies may be defined as immunoglobulins resulting from the B lymphocyte clone, called a plasmocyte, to the epitope of an antigen. These antibodies are considered highly specific to the antigen and have few side effects, as they are produced to preserve healthy cells and cause fewer toxic effects compared to cytotoxic treatments used in certain cancers. The objective of the present study is to demonstrate the advantages of the use of monoclonal antibodies as a therapeutic ally in the fight against cancer, through a descriptive literature review using articles available in online databases published in the last ten years. The mechanism of action of monoclonal antibodies comprises, for example, the blocking of receptors or growth factors, binding on cellular targets, induction of programmed cell death, and instigating the production of cytotoxic particles. Some of these antibodies described in the literature are being used to suppress the immune system, such as Omalizumab®, and also to eliminate or inhibit tumor cells, such as Alemtuzumab®. The use of monoclonal antibody therapy is frequent in leukemias, breast cancer, lymphomas and colorectal cancer, in order to improve quality of life and achieve high survival rates of patients. This is due to their efficiency in selectively targeting and killing tumor cells. With this, we can conclude that monoclonal antibodies are considered important for the treatment in the fight against cancer, since in addition to efficacy, they have a low rate of adverse reactions, and immunological depression compared to conventional chemotherapy, resulting in improved quality of life of the patient. **Keywords:** Monoclonal antibodies;Tumor cells;Therapeutic.

IC - 034 - **Histological evaluation of the association of collagen matrix and gold nanoparticles with the indirect effects of oral tolerance on skin wound healing 60 days after the injury**

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Chronic wounds affect approximately 5 million Brazilians, generating high treatment costs for public health in Brazil (INSS). In this study, an excisional wound model (7 mm in diameter) and an incisional wound model (1 cm length) were used in male Swiss mice (8 weeks old) approved by the Ethics Committee on Animal Use under protocol No. 024/2020. Mice were divided into 5 topical treatment groups: ColH, ColH + OVA, ColH + AuNP, ColH + AuNP + OVA and saline, which received the dressing containing the respective treatment delimited to the group. Before surgery, animals in the experimental groups with topical treatment containing OVA, received orally a 1:5 egg white solution ad libitum for five consecutive days. The mice of the other experimental groups ingested water. After 7 days, the animals were anesthetized and after trichotomy the incisional and excisional wounds were performed. Then, topical treatments were applied to the wounds according to the experimental groups. After the surgical procedure, the lesions were covered with Micropore tape, which remained for 5 days and then was removed together with the suture of the incisional wound. The scar area was collected 60 days after injury and after histological processing was stained with Gomori trichrome for histopathological analyses. We found that topical application of ColH + AuNP + OVA reduced the scar area with better collagen deposition and resulted in the best aesthetic appearance. In conclusion, we demonstrate that the topical application of ColH + AuNP + OVA reduces the inflammatory infiltrate, improving the remodeling of the extracellular matrix. These data require further studies, but point to the possibility of use for post-surgical care and injuries. **Keywords:** Morphophysiology;Oral tolerance;Wound healing.

IC - 035 - **Study of heme-oxygenase-1 function during Cryptococcus neoformans infection.**

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Heme-oxygenase-1 (HO-1) is an enzyme involved in free heme catabolism that generates molecules with anti-inflammatory effects, providing protection to the host in case of infectious diseases. On the other hand, these effects can cause immunosuppression and reduce the action of microbicidal mechanisms. However, little information is known about the effects of HO-1 against fungal infections, including cryptococcosis. *Cryptococcus neoformans* is a fungus that mainly affects immunocompromised individuals. The lung is the first site of infection, and the most devastating consequence occurs when it reaches the brain, causing severe cases of meningoencephalitis. Several studies have demonstrated the ability of this fungus to adapt and survive within macrophages, addressing virulence factors and escape mechanisms that allow its proliferation in the intracellular environment. Our objective is to evaluate the function of HO-1 in peritoneal macrophages of C57BL/6 mice during interaction with the fungus *C. neoformans* (strain H99) in vitro and in the lungs of mice infected intranasally. Our western blot results demonstrated that the fungus, opsonized or not with antibodies, induced HO-1 expression in macrophages in vitro. In vivo, we observed an increased expression of the enzyme in the lung of mice after 7, 14 and 21 days of infection. Our next goals will be: (1) to evaluate the effect of cobalt protoporphyrin IX, an inducer of HO-1, and tin protoporphyrin IX, an inhibitor, on the fungicidal capacity and cytokine production (TNF, IL-6, IL-1B, IL-12 and IL-10) of macrophages in vitro; (2) to evaluate the amount of fungi in the lungs and brain of infected animals treated or not with these HO-1 modulators. Understanding the role of HO-1 in this model may provide a better understanding of the pathophysiology of cryptococcosis and contribute to the development of adjuvant therapeutic approaches. **Keywords:** HO-1;Cryptococcus neoformans;cryptococcosis.

IC - 036 - **The Transforming Role of Academic Monitoring: Strengthening Links and Potentializing Knowledge.**

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Introduction: Academic monitoring is based on carrying out teaching activities taught by the monitor, which provides the opportunity for mutual cooperation between the student body and the faculty, stimulating the exchange of knowledge, as well as allowing the evolution of fundamental aspects for the edification of the student monitor such as problem-solving skills, development of interpersonal relationships and methods for searching for quality information and content that can be shared with students**. Objective:** To highlight the importance of the subject of immunology in understanding and building solid knowledge for understanding the subject of pharmacology. **Methods and Results:** Descriptive observational study of the experience report carried out from the experience of the pharmacology monitor, from the undergraduate course at Estácio de Sá University - Angra dos Reis. Immunology, a subject of the basic cycle, brings with it important concepts to be used in pharmacology. It was evidenced with the resolution of exercises by the Kahoot platform that the students presented different degrees of difficulties, derived from the lack of an interdisciplinary reasoning, specifically regarding to immunology. It is necessary to understand concepts such as cytokines, receptors for inflammatory and anti-inflammatory mediators, antigens and tumor receptors, in order to understand where each drug acts. To this end, previous reviews of the immunological bases were carried out, highlighting the role of the monitor as a bridge in the relationship between knowledge and learning. **Conclusion:** At the end of the academic period, it was possible to verify that after the revisions encompassing the bases of immunology, noticeable advances were obtained in the student's academic performance, as they mentioned that this made them understand where each medicine acts, not being necessary to memorize the content, making teaching more effective, highlighting a point where the two sciences merges. **Keywords:** Immunology;Academic monitoring;Immunopharmacology.

IC - 037 - **N-(coumarin-3-yl)cinnamamide controls Toxoplasma gondii replication in macrophages acting in the host and parasite cells**

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Toxoplasmosis is a neglected disease caused by *Toxoplasma gondii* (Tg), a parasite with high prevalence in tropical regions. This parasite has the ability to disseminate all over the host's body residing in different tissue including brain scaping from the host immune response. The immune system cells including macrophages (MOs) are crucial in orchestrating the immune response during the infection. Coumarins, secondary metabolites synthesized by plants, have become the focus of investigation for development of analogues compounds with antihyperglycemic, anti-inflammatory and anti-neurodegenerative actions with potential for therapeutic use in various pathologies. Herein, the effect of *N*-(coumarin-3-yl)cinnamamide (amino coumarin; M220) in the invasion and replication of Tg in macrophages was investigated. MOs from Balb/c mice was cultured and infected with tachyzoites (Tg RH strain; 1:1 parasite:cell) and stimulated or not with M220 (400, 200, 100, 50, 25, and 12,5 mM). After 48h the nitric oxide production (Griess methods), lactate dehydrogenase (LDH), and the parasites counting were analyzed. Tg-infected MOs treated with M220 decreased the intracellular parasite replication at all tested concentrations without increasing NO levels and no presenting toxicity to host cells. Notably, the concentration of 100 mM induced the highest antitoxoplasma activity in MOs. A reduction of parasite replication was found during infection of MOs with tachyzoites pre-incubated with M220, and also in MOs pre-stimulated with compound before infection. Collectively, our data suggest that *N*-(coumarin-3-yl)cinnamamide M220 can be a new therapeutic target for studies of the treatment of Toxoplasmosis. **Keywords:** Toxoplasma gondii;N-(coumarin-3-yl)cinnamamide;macrophages.

IC - 038 - **Blockade of glutamine metabolism and its effects on the tumor microenvironment.**

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Tumor cells display metabolic reprogramming, being aerobic glycolysis the best characterized. However, many tumor cells concomitantly rely on glutaminolysis for energy and catabolite intermediates, a pathway mediated mainly by glutaminase. Tumor cells and immune cells share metabolic similarities and tumors are capable of modulating the immune response through the competition for nutrients and the generation of metabolites. There is evidence that glutaminase inhibition, in tumor associated macrophages (TMA), negatively impacts the M2 phenotype. In cervical cancer, macrophages have been shown to display M2 biased phenotype and to be important for tumor growth. Our hypothesis is that glutaminolysis inhibition will decrease tumor growth due to the direct negative effects on tumor cell metabolism, and by facilitating a change in macrophages towards a cytotoxic phenotype. We worked with the cervical cancer derived cell lines, SiHa and SW756, in co-culture with peripheral blood mononuclear cells (PBMCs). We used tumor spheroids, seeking to mimic the tumor microenvironment. Glutaminse was inhibited with BPTES or 968. Our results showed that both Inhibitors display toxic effects on tumor cells. That was the case in neat tumor cultures, both in monolayers and spheroids, and in co-cultures with PBMCs. Cell cycle analysis revealed that treatment with the inhibitors caused an accumulation of tumor cells in the sub-G1 phase (dead cells). We also observed that the treatment had immunomodulatory effects on macrophages. In addition, the preliminary results about the glutaminase expression in cocultures demonstrated that PBMCs, after treatment with glutaminase inhibitors, displayed an increase in the percentage of cells positive for glutaminase expression. In conclusion, our data indicates that glutaminase inhibition may be an useful tool in cervical cancer therapy. **Keywords:** cervical cancer;glutamine;macrophages.

IC - 039 - **IMMUNOLOGICAL OVERVIEW OF COVID-19 IN A COMMUNITY WITH SOCIAL VULNERABILITY IN FORTALEZA, CEARÁ**

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Introduction: COVID-19 (coronavirus 2019) is an infectious disease caused by the SARS-CoV-2 virus (severe acute respiratory syndrome coronavirus 2), which can range from mildly asymptomatic to severe illness, leading to the death of the patient. In view of this, the State of Ceará presented a very expressive number of cases, most of which in the capital, Fortaleza. In particular, the Barra do Ceará neighborhood in Fortaleza, Ceará, with a very low human development index (IDH), was directly affected by several epidemics that occurred in this State. In this study, we evaluated seropositivity to SARS-Cov-2 in a socially vulnerable community in Fortaleza, Ceará. Methods and Results: This is a prospective cohort study, carried out at the Casemiro Filho Health Unit, located in Barra do Ceará. Whole blood and nasal swab samples were collected for the diagnosis of SARS-CoV-2, in addition to the application of questionnaires for data collection. The collected samples were sent for analysis at the Oswaldo Cruz Foundation (Fiocruz-CE). A total of 214 samples were analyzed, which 99.5% (n=213) IgG seropositive by ELISA test and only 1.4% (n=3) were RT-PCR positive (Reverse transcription polymerase chain reaction) for virus detection. Regarding vaccination against COVID-19, out of the total, 94.8% (n=203) took the first dose, 90.2% (n=193) took the second dose and about the third dose, the value was reduced to 54.2% (n=116). Individuals who had symptoms amounted to 8.4% (n=18), and these included fever, cough, taste dysfunction, sore throat, among others. Conclusion: From the results, there was high adherence to vaccination against SARS-CoV-2 and almost all had an immune response to SARS-Cov-2, which could be related to the vaccine or direct exposure to the virus. Thus, this socially vulnerable population needs an active epidemiological surveillance. **Keywords:** SARS-CoV-2;SEROLOGY;RT-PCR.

IC - 040 - **Atazanavir prevent neuroblast death mediated by zika virus infection**

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Zika virus (ZIKV) can induce neuronal damage and death, involved in the pathophysiology of microcephaly. Atazanavir (ATZ) has activity against ZIKV, but its ability to prevent neuronal death is not well understood. The influence of ATZ on cell death was evaluated on Zika infected neuroblast. SK-N-BE(2) neuroblasts were infected or not with ZIKV at a multiplicity of infection (MOI) of 0.1 and treated or not with ATZ 1 µM. Cell viability and death was evaluated by MTT assay and by annexin V/propidium iodide method, respectively. The expression of key-proteins involved in programmed cell death mechanisms - MLKL (necroptosis), GSDMD (pyroptosis) and caspase 3 (apoptosis) - were assessed by flow cytometry. Cell infection was observed by electron microscopy and IL-1β production was quantified by a cytometric beads array method. Results showed that ZIKV infection decreased neuroblast viability. Probably, necroptosis is the main mechanism of neuroblast death, since MLKL expression increased in ZIKV infected cells. Additionally, necrosis and loss of cytoplasmatic continuity was observed by electron microscopy. GSDMD expression and IL-1β production were not altered by the infection, showing that pyroptosis is not the main mechanism of death. Activated caspase 3 and phosphatidylserine labelling by annexin V were not elevated, which also indicates that apoptosis probably is not a key feature in the infection. ATZ treatment improved cellular viability and decreased necrosis or late apoptosis of infected cells. Further, ATZ was able to decrease GSDMD, but it did not alter MLKL, caspase 3 nor IL-1β production by infected neuroblast. Together, data demonstrated the ZIKV cytopathic effect on neuroblasts, that can be reverted by treatment with ATZ, probably by regulating pyroptosis and other different cell death mechanisms, different from apoptosis and necroptosis. It shows that ATZ has a potential to be repositioned to prevent microcephaly since ATZ can be used by pregnant women. **Keywords:** Microcephaly;cell death;Zika.

IC - 041 - **Early immunological diagnosis for Parkinson's disease through alpha-synuclein**

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Alpha-synuclein is a key protein in the progression and etiology of Parkinson's disease (PD). This idiopathic disease is characterized by neuroinflammation, which plays an important role in its development. It is known that PD disrupts the expression and release of alpha-synuclein, which physiologically contributes to synaptic terminal plasticity, vesicular transport, and neurotransmitter release, but becomes pathological in this context. Inflammatory cells can accumulate aggregates of alpha-synuclein, called Lewy bodies, with astrocytes capable of transferring them from one cell to another and microglia acting as extracellular cleaners. The molecular patterns associated with damage (DAMPs) released by dying neurons and the chemokine CCL2, released by astrocytes, trigger the inflammatory response. PD patients are diagnosed based on clinical signs, and neurodegeneration associated with Parkinsonism can be diagnosed through studies using presynaptic dopaminergic radiotracers by Positron-Emission Tomography (PET) scan. However, multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) should also be considered. The objective of this work is to identify a target for early diagnosis of PD within the inflammatory process. To achieve this, a literature review was conducted using articles from the last 10 years to guide its practical development. There is evidence in the literature demonstrating the involvement of immune system components important for the inflammasome pathway in the pathogenesis of the disease, such as NLR, a receptor belonging to the NRLP3 family, responsible for sensing fibrillar alpha-synuclein found in microglial cytoplasm, which then activates the apoptosis-associated speck-like protein containing a CARD (ASC), which recruits pro-caspase 1. Therefore, analyzing the activity of these components could be a diagnostic breakthrough for Parkinson's disease. **Keywords:** Parkinson Disease;Diagnosis ;Alpha-synuclein.

IC - 042 - **Neonatally overfed infant rats exhibit reduced microglial activation and hippocampal damage in pneumococcal meningitis.**

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Pneumococcal meningitis (PM) is a severe disease caused by *Streptococcus pneumoniae*, that leads to inflammation in the meninges and ventricles, resulting in neuronal and progenitor cell death in the dentate gyrus (DG). Activated microglia play a crucial role in neuroinflammation and hippocampal damage associated with PM. Overfeeding in neonatal rats has been linked to cognitive decline and increased microglial activation in adulthood. This study aimed to investigate whether neonatal overfeeding affects the outcome of PM. Neonatal overfeeding was induced by adjusting the litter size to four or ten pups per dam after birth. On the 11th day, the rats received an injection of pneumococcal serotype 3 or saline. After 24 hours, the rats were euthanized, and their brains were analyzed. Histological analysis examined apoptosis in the DG, intensity of the inflammatory infiltrate, and microglial activation. RT-qPCR measured mRNA levels of *Aif1, Il6, Tnfα, Il1β*, and *Il10* in the hippocampus (HC). The overfed group exhibited a 34.8% greater mass gain compared to the control group. Surprisingly, overfed rats showed 50% lower BM-induced apoptosis in the DG compared to normally-fed rats. However, overfeeding had no impact on the intensity of the inflammatory infiltrate in the central nervous system. RT-qPCR revealed increased Aif1 mRNA levels in the HC of infected overfed rats. Morphological analysis of Iba1-immunostained cells indicated that microglia in the hippocampal region beneath the DG were less activated in response to PM in overfed animals. This finding was supported by a 5-fold increase in *Il10* mRNA levels in the HC of overfed infected animals. Overfeeding induced a minor increase in Il6 expression in response to the infection and had no impact on *Il1β* and *Tnfα* mRNA levels. In conclusion, overfeeding attenuates microglial activation in response to pneumococcal invasion of the central nervous system, thereby mitigating neuronal damage associated with PM. **Keywords:** Pneumococcal Meningitis;Overfeeding;Microglia.

IC - 043 - **Characterization and evaluation of the immunomodulatory potential of macrophages-derived extracellular vesicles stimulated with inactivated-SARS-CoV-2**

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INTRODUCTION: The COVID-19 pandemic, caused by SARS-CoV-2 infection, develops serious consequences for society. Extensive research has been carried out to understand the interactions between the virus and host cells, targeting to understand the infection process and pathogenesis. Extracellular vesicles (EVs) are a type of membrane-bound vesicles that carry crucial information and biomolecules from cells, playing a significant role in cell signaling. In this work, we seek to understand the immunomodulatory role of EVs secreted by macrophages during SARS-CoV-2 infection. **METHODS:** The concentration and size of the EVs was obtained by Nanoparticle Tracking Analysis (NTA) method, the zeta potential was measured using the Zeta Sizer. For untarget lipidomics, a liquid-liquid extraction was performed and the samples were injected into the LC-MS/MS. Cytokine dosages was taken using the ELISA method. **RESULTS:** Our analysis revealed that the predominant population of EVs ranged around 100 nm in size and had a zeta potential of -20. We evidenced the presence of oxidized arachidonic acid in EVs derived from classically activated (M1) and viral-stimulated macrophages (VM). Furthermore, EVs derived from VM showed elevated amounts of DHA-ômega 3 fatty acid. For immunomodulation effect, we showed that tumor necrosis factor (TNF) production abruptly decreases when macrophages are exposed to EVs from VM and then challenged with SARS-CoV-2 viral particles in contrast to non EVs-treated macrophages. Also, we demonstrate that viral particles did not induce a significant increase in interleukin (IL)-6 production in lung tissue cells culture. However, the treatment with EVs from VM induced high levels of IL-6 in H-460 epithelial cells. **CONCLUSION:** Our findings shed more light on the complex relationship between EV-mediated signaling and the immunological reaction to SARS-CoV-2 infection. Additionally, this study can benefit the development of news antiviral treatments. **Keywords:** SARS-CoV-2;Extracellular vesicles;Inflammation.

IC - 044 - **Evaluation of autophagy activation in macrophages stimulated by Leucurolysin-B (Leuc-B), a metalloproteinase from the venom of Bothrops leucurus: preliminary data**

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Leucurolysin-B (Leuc-B), a hemorrhagic metalloproteinase *Bothrops leucurus* snake venom, has been demonstrated to have antitumoral activity and the ability to bind to cell surface integrins to modulate immune responses. Our group has been studying the mechanisms by which Leuc-B modulates innate immunity and inflammation. Autophagy is a degradative mechanism that directs cytoplasmic substrates for lysosomal degradation. It has also been described as an innate immune mechanism essential to eliminate intracellular pathogens. In this project, we aim to evaluate whether Leuc-B is capable to stimulate antimicrobial autophagy in macrophages. For that, murine bone marrow-derived macrophages (BMDM) will be stimulated with crescent concentrations of purified Leuc-B, in addition to rapamycin or Tat-Beclin 1 peptide (known as autophagy stimulators; positive controls) in different time-points. The autophagy induction will be evaluated by counting the number of cytoplasmic autophagosomes by immunofluorescence and analyzing the LC3-I to LC3-II conversion by western blot. The autophagic flux will be determined by treating cells with bafilomycin A1, an inhibitor of autophagosome acidification. To determine whether autophagy induced by Leuc-B has the potential to control intracellular pathogen replication, cells will be infected with the intracellular bacteria *Salmonella typhimurium* and subsequently treated with Leuc-B, followed by quantification of bacteria replication by colony forming units (CFU) assay. Thus far, our preliminary data show that treatment of BMDM with 25 or 50 mg/mL of Leuc-B for 24 hr stimulated macrophage autophagy, as the number of autophagosomes detected in these cells significantly increased, compared to untreated cells. This preliminary data suggest that Leuc-B has the potential to be used as an autophagy-inducing agent, opening avenues for the development of new antimicrobial agents. Financial support: CNPq; CAPES; FAPEMIG. **Keywords:** autophagy;snakevenom;leucb.

IC - 045 - **Unexpected thermal modulation of microbicidal activity in macrophages**

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Recent data from our laboratory have revealed that body temperature regulates innate immunity in an intricate fashion by exerting opposite effects on the microbicidal activity of neutrophils and macrophages. Whereas neutrophils have their microbicidal activity increased at a fever-like temperature (38.5°C for rats), macrophages have their microbicidal activity increased at a hypothermia-like temperature (36.0°C for rats). The present study was conducted to investigate the mechanisms of the unexpected modulation of macrophages by temperature. We observed that the increased microbicidal activity of rat peritoneal macrophages in the hypothermia-like temperature was associated with heightened oxidative burst. Accordingly, inhibition of the NADPH oxidase complex by VAS2870 eliminated the inverse relationship between temperature and microbicidal activity. Next, we determined whether the inverse thermal modulation depended on the level at which the NADPH-activation cascade was stimulated: upstream stimulation was achieved using a TLR4 agonist (LPS); downstream stimulation was achieved using a protein kinase C activator (PMA). In both cases, oxidative burst was inversely proportional to temperature, indicating that the thermal modulation occurs downstream of protein kinase C. Because protein kinase C is known to activate NADPH oxidase by phosphorylating its regulatory subunit, p47phox, we sought to evaluate the involvement of this subunit in the thermal modulation of macrophages. Contrary to this expectation, though, overexpression of p47phox in RAW 264.7 macrophages did not amplify the inverse relationship between temperature and oxidative burst; rather, it turned it into a direct relationship. These findings indicate that the inverse thermal modulation of macrophages takes place at the NADPH oxidase complex, but not at its regulatory subunit activity p47phox. Putative targets of the modulation are the catalytic subunits: gp91phox and p22phox. **Keywords:** Macrophages;Phagocytosis;ROS.

IC - 046 - **Reinfection by COVID-19 in Brazil: current perspectives on clinical characteristics and new variants**

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Introduction: The SARS-CoV-2 pandemic permanently changed public health strategies in Brazil. It is estimated that a significant portion of the population has experienced at least one infection, increasing the likelihood of future COVID-19 reinfections (CR). This study aims to evaluate CR and the relationship between primary infections (PI), reinfection risk, and COVID-19 variants (VoCs) in Brazil. Methods: To identify studies assessing clinical outcomes in Brazilian CR patients, 2 authors independently searched the PubMed, Google Scholar, and SciELO databases until July 2023. The search strategy used terms SARS-CoV-2, COVID-19, reinfection, and second infection, combined with Brazil. The COVID-19 Epidemiological Bulletin (ISSN 9352-7864) was reviewed through a time-wise comparison. Results: The selected articles included patients from 9 out of 14 federative units in which CR has been reported (64.28%). In 2021, the VoC P1. (Gamma) accounted for 54.54% of CR cases, while VoC Omicron represented 71.25% of 80 new cases in 2022. Four articles reported mostly or all mild symptoms in PI, two studies associated reinfection with more symptoms, and one reported severe outcomes in CR patients. Discussion: While much attention has been given to the Immunity Fading (IF) phenomenon for public health, the decline in protection against reinfection is not the sole factor influencing CR risk. Most studies suggest a link between mild first COVID-19 infections and recurrence. Furthermore, a case-control study found a negative association between upper respiratory tract symptoms and CR (J Infect. 82(3):399-406, 2021). This evidence is also supported by the absence of a temporal humoral pattern in mild COVID-19 patients (World J Biol Chem. 14(2):40-51, 2023). Conclusion: This review provides circumstantial evidence that mild primary COVID-19 infections may offer only limited protection against CR. We propose considering individuals with mild COVID-19 as a group prone to reinfection risk. **Keywords:** Variants of Concern;COVID-19 Reinfection;Immunity Fading.

IC - 047 - **A low-cost process of lentiviral vectors production for cell therapy**

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Lentivirus production is a critical stage for the development of several cellular therapies, including chimeric antigen receptor (CAR) T cell generation. The quality of the lentiviral vectors produced can directly affect the effectiveness of the therapy, which may result in low CAR expression or high T cell cytotoxicity, with a consequent reduction in antitumor capacity. Moreover, the production of these vectors usually uses very expensive ultracentrifuges or high cost sedimentation reagents. In this context, this project aims to optimize a low-cost production of a lentiviral vector (G36/ZsGreen) for CAR T therapy, improving the efficiency of CAR transduction and expression, without compromising T cell viability. Lentiviral vectors will be produced by transient transfection of five plasmids into 293T cells using polyethyleneimine (PEI). Different variables were evaluated for optimizing the concentration of viral particles using a low-cost self-produced reagent that requires lower velocity for sedimentation in the centrifuge. Besides, we tested different values of a multiplicity of infection (MOI); cell densities for virus titration and filtration conditions. The evaluation of CAR expression in transduced cells was done by flow cytometry.Using different MOI values (1-20), we initially found that the T cell viability ranged from 10-40% 48 hours after transduction. Lower MOI values (1-0.0625) were evaluated using a pre-centrifugation condition, verifying that transduction levels varied between 15-25% of positive cells. Under these conditions, the viability levels reached 50-85% after 120 hours.With this project, we hope to optimize and cheapen the lentiviral vector production for CAR T therapy, improving its effectiveness and contributing to the development of lower-cost and high-efficiency treatments for cancer patients. The project was approved by the institutional review board and CIBio (Technical opinion number 6839/2020). **Keywords:** cell therapy vector;lentivirus;CAR T.

IC - 048 - **Methodologies, Challenges, and Joys of Teaching in the Monitoring of Medical Immunology.**

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Introduction: Academic monitoring is a teaching approach wherein a student who has previously completed a specific subject assists in the academic education of other students who are in earlier periods, under the guidance of the responsible professor. This practice of monitoring allows the student monitor to deepen their knowledge in the area of basic and medical immunology. Thus, the purpose of this study is to describe the methodologies and didactics employed by the monitor in their teaching practice, as well as the challenges and rewards associated with this experience. **Methods and Results**: This study is a descriptive observational analysis based on the monitoring experience in medical immunology within the undergraduate program at Estácio de Sá University - Angra dos Reis. In the process of developing didactics for teaching, certain difficulties were identified among the students, such as feelings of shame and fear when it came to expressing their doubts. However, having followed a similar path as them, I was able to demonstrate my understanding of their challenges, which helped establish a rapport and subsequently reduced their fear of expressing doubts. As part of the methodology, audio-visual materials were created through active monitoring. These materials required students to engage in clinical reasoning, reflection, and knowledge creation in tandem with our interactions in the classroom. This approach facilitated a better comprehension of the subject matter, fostering connections between cellular immunology and clinical practice. **Conclusion**: Throughout the process, students provided spontaneous feedback indicating that the didactics employed during monitoring had helped them gain a better understanding of their studies. This feedback demonstrates that the monitoring exercise not only provides an opportunity for the monitor to develop teaching skills but also brings satisfaction in guiding and facilitating the learning process of their fellow students. **Keywords:** Medical immunology;Monitoring;Learning.

IC - 049 - **The reappearance of eradicated diseases in Brazil: Measles - a literature review**

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Measles is a serious infectious disease caused by a virus that can lead to death and is transmitted when the patient coughs, talks, sneezes or breathes close to other people. To avoid measles, the most effective way is through vaccination. This study aims to analyze measles and its resurgence, seeking to answer the reasons why this disease, which had already been eradicated, returned to present prevalence in Brazil. A narrative analysis of the literature was carried out for the realization of this article, using the keywords: measles, vaccination, reappearance and Brazil. Through research, it was possible to observe possible causes for this event, among them, the anti-vaccine movements, incomplete vaccination coverage, fake news, misinformation of the population and poor management of public health. Therefore, the need for vaccination campaigns was concluded, not only for children, but also for adults, mainly in areas with lower previous coverage, in order to control this disease. **Keywords:** Measles;Vaccination;Reappearance and Brazil.

IC - 050 - **GENERATION OF ANTI-GD2 CAR-T CELLS BY SLEEPING BEAUTY TRANSPOSON SYSTEM**

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Immunotherapy involving T lymphocytes genetically modified with artificial receptors, called Chimeric Antigen Receptors (CARs), is one of the most promising antitumor therapies; once expressed on the T cell, the receptor is able to redirect it to a tumor antigen in a specific way. The CAR-encoding transgene can be inserted into the genome of T cells by means of the Sleeping Beauty (SB) system, which is formed by the bicomposite arrangement that is usually two plasmid vectors, one component is a vector containing the CAR gene and the other is the transposase expressing plasmid. It is known that some solid tumors commonly express the ganglioside GD2, which makes them a good target for CAR-T cell immunotherapy. The aim of this work was to synthesize and validate the anti-GD2 CAR (14G2A clone) plasmid and generate CAR-T cells from peripheral blood mononuclear cells (PBMCs). Initially the 14G2A plasmid was cloned into the PT4 transposon vector. The expected sequence was confirmed by EcoRV restriction enzyme digestion. We further electroporated the SB-transposase system carrying the GD2 CAR in the HEK 293FT cell line and could detect the CAR molecule in 7.04% of the cells after 24h. To validade the system in human primary T cells, 30 million PBMCs were electroporated and CAR frequency was assessed by flow cytometry 1, 8 and 12 days after in vitro expansion. It was observed an average (n=2) of 5.57%, 7.81% and 14.5% of anti GD2 positive CAR-T cells frequency after 1, 8 and 12 days of expansion respectively. The next steps of this project will be functional tests, such as lysis assay and in vivo validations. **Keywords:** Immunotherapy ;Sleeping Beauty ;anti-GD2 CAR-T cells.

IC - 051 - **Obtainment and characterization of TIM-3_ECD-FC in HEK293-T cells as promising tool to immunotherapy**

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T-cell immunoglobulin and mucin domain 3 (TIM-3) has emerged as crucial immune checkpoint receptor in the tumor microenvironment. The development of therapeutic strategies targeting TIM-3 holds great potential for enhancing antitumor immune responses. Despite the large amount of research developed about the TIM-3 role in the immune system, there is much contradictory evidence about specific ligand interactions and their relevance in the cancer perspective. Interaction inhibition of TIM-3 with its ligands by therapeutic antibodies showed promising results as an antitumor agent in preclinical and early-stage clinical studies. Previously we showed that recombinant TIM-3_ECD produced in bacteria provided expression gain of lymphocyte activation markers such as CD69, in activated human peripheral blood mononuclear cells (PBMC) showing a promising activation feature. The hypothesis raised here is that a TIM-3 ectodomain soluble form fused to a pharmacokinetically advantageous IgG1 Fc domain may be able to globally antagonize TIM-3 interactions. The synthetic pcDNA3.1-TIM-3_ECD-FC construction was transfected into HEK 293-T cells for transient expression, followed by the cell's supernatant protein G affinity chromatography to TIM-3_ECD-FC purification. The purification yield was 1.3 mg/L and analysis by SDS-PAGE of purified recombinant protein exhibited a single band at ~50kDa, suggesting high purity. The protein functionality was first analyzed by indirect ELISA, evaluating TIM-3-Fc recognition by conformational specific anti-TIM-3 and anti-human Fc antibodies, and confirming its proper folding state. Functional analysis to evaluate its ability to modulate immune responses is ongoing. Our partial results demonstrate the successful cloning, expression, and purification of functional TIM-3_ECD-FC in HEK-293T cells, if confirmed its ability to modulate immune responses, this molecule raises as a potential antitumor therapeutic tool. **Keywords:** TIM-3;recombinant protein;immunotherapy.

IC - 052 - **CONTROL OF CD8 T CELL EXHAUSTION BY THE POLYCOMB PROTEIN EZH2: IMPACT IN ANTI-TUMOR RESPONSE**

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In chronic infections and neoplastic processes CD8 T cells undergo progressive decrease of effector function and gain of inhibitory receptors. This loss of functionality, known as T cell exhaustion, also affects CAR (Chimeric Antigen Receptor) T cells immunotherapy for solid tumors, compromising disease control. Our prior data in exhaustion model, the murine LCMV clone 13 infection, revealed that early generated exhausted CD8 T cells expressed lower levels of the epigenetic modulator Ezh2 (Enhancer of zeste homolog 2) which is the catalytic subunit of the Polycomb Repressor Complex 2 that trimethylates histone 3 at the lysine residue 27, and has been reported as critical to CD8 T cell effector differentiation and anti-tumor response. Here, we aim to evaluate the role of Ezh2 in CD8 T cell exhaustion and the relevance of modulating its levels for advance in experimental immunotherapies. In vitro activated Ezh2 deficient CD8 T cells from Ezh2fl/fl Lck-Cre or CD4-Cre mice exhibited impaired proliferation and production of IFN-γ and TNF, along with increased expression of exhaustion associated molecules such as PD-1, TIM-3 and CD38. Similar phenotype was replicated by pharmacological inhibition of Ezh2 in wild type CD8 T cells. Analysis of tumor infiltrating CD8 T lymphocytes from B16-OVA melanoma bearing mice showed decreased cytotoxic and proliferative potential together with higher expression of inhibitory receptors and loss of Ezh2 in both endogenous and transferred OT-I cells, suggesting that Ezh2 downregulation is associated to the tumor microenvironment and is not dependent on antigen specificity. Lastly, in a preliminary experiment we observed that expression of Ezh2 in CD8 CAR T cells targeting mice B16 could improve T cell functionality by increasing proliferation and granzyme B production, along with the reduction in tumor size. Collectively, our results indicate Ezh2 as a potential target for rescuing CAR T cells immunotherapy benefits. **Keywords:** CD8 T cell exhaustion;Epigenetics;CAR T cell immunotherapy.

IC - 053 - **Role of polycomb group 1 protein Cbx4 in CD8 T cell effector function**

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We have previously demonstrated the novel role of epigenetic modulator Chromobox (Cbx) 4 in controlling cytotoxic CD8 T lymphocytes (CTLs) differentiation. CTLs are key players in the elimination of intracellular pathogens and tumors. During an acute infection, CTLs differentiate into a heterogeneous population composed of terminal effector and memory precursor cells. However, during chronic infections and tumors, CTLs can become compromised in different hypofunctional cellular fates, in a process known as exhaustion. We observed by knockdown and knockout approaches that Cbx4 deficiency promotes memory-associated phenotype in CTLs during acute LCMV (lymphocytic choriomeningitis virus) infection. Additionally, we observed reduced cytokine production and cytotoxicity in Cbx4-deficient CTLs activated in vitro. The phenotype of Cbx4-deficient CTLs during chronic settings as well as the impact of Cbx4 deficiency in virus and tumor control in vivo remains elusive. Here, we infected C57BL/6 Cbx4 fl/fl Cd4-Cre (Cbx4 TKO) mice, deficient in Cbx4 in T compartment, with the chronic LCMV strain clone 13 and observed increased frequency of virus specific exhausted cells with a progenitor phenotype, previously demonstrated to give rise to intermediate and terminal phenotypes (Immunity 52: 825-841, 2020), suggesting that Cbx4 regulates CTL stemness in both acute and chronic settings. Even though the effector function of Cbx4 KO CTLs is compromised in vitro, we did not observe changes in serum LCMV titers during acute infection of Cbx4 TKO mice. Moreover, our preliminary data showed that Cbx4 TKO mice display superior tumor protection in a murine model of melanoma (B16). Taken together, our results suggest that even though Cbx4 deletion can reduce effector functions, it does not compromise acute viral clearance. Moreover, the deletion of Cbx4 induces a T cell progenitor capacity with potential to better long term tumor control and application in immunotherapy strategies. **Keywords:** Epigenetics;CD8 T cell exhaustion;Immunotherapy strategies.

IC - 054 - **Capsular polysaccharides from Cryptococcus neoformans exert inhibitory effects on canine macrophages DH82 cell line**

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Cryptococcosis is a systemic fungal disease that affects different animal species. The infection is observed around the world and is caused by the genus *Cryptococcus*, which initially focuses on the respiratory system and can progress to the central nervous system. Infections in dogs are caused by the species *Cryptococcus neoformans* and *Cryptococcus gattii* (J Vet Emerg Crit Care. 23(5):489-497, 2013). One of the main virulence factors is the capsule, constituted by the polysaccharides glucuronoxylomannan (GXM) and galactoxylomannan (GalXM). Polysaccharides are immunomodulators and one of the target cells is macrophage (Front Med. 19(6):129, 2019). Polysaccharides from *Cryptococcus* exert immunomodulatory effects on murine macrophages favoring infection (Cell Microbiol. 10(6):1274-85, 2008). The scarcity of information on canine cryptococcosis led us to study the role of purified *C. neoformans* polysaccharides in canine macrophages. **Method**: DH82 canine macrophage cell line was cultivated in the presence of GalXM or GXM stimulated or not with LPS/INFγ. Cytotoxicity was assessed by measuring mitochondrial activity. Binding and phagocytosis were observed after incubation of macrophages with yeast for 40 minutes or 4 hours respectively by optical microscopy. The quantification of messenger RNA expression for cytokines was performed by quantitative real-time PCR and the expression of MHC II by flow cytometry. **Results**: Our results showed that the capsular polysaccharides GXM or GalXM did not present toxic effects on the DH82 macrophage. However, it was observed that phagocytic activity decreased after treatment with polysaccharides. In addition, yeasts recovered from macrophages treated with the polysaccharides, after phagocytosis, could be cultured, showing that their viability was not altered. The polysaccharides led to a reduction in IL-12 and IL-6 and MHC II expression. These data suggest a modulatory role of polysaccharides in the DH82 canine macrophage. **Keywords:** Canine macrophage;Cryptococcus neoformas;Capsular polysaccharides.

IC - 055 - **Artepillin C reduces lung inflammation by induction of monocytic myeloid derived suppressor cells (M-MDSC) expressing PD-L1 and apoptosis of eosinophils in experimental asthma.**

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Asthma is a chronic lung inflammatory disease characterized by eosinophil influx, increase in mucus production and bronchial hyperresponsiveness. Artepillin C (ArtC), a major compound of green propolis, is described as an immune regulatory and proapoptotic compound. Our group already showed that ArtC reduces eosinophil influx, lung inflammation and mucus production by induction of monocytic myeloid derived suppressor cells (M-MDSC) using an ovalbumin (OVA)-induced asthma model. However, the mechanisms by which ArtC-induced M-MDSC reduce pulmonary inflammation are unclear. We hypothesized that ArtC-induced M-MDSC reduces airway inflammation through PD-L1 expression and through induction of apoptosis in lung eosinophils after allergen exposure. Bone marrow cells from C57BL/6 mice were differentiated in vitro in MDSC with IL-6 and GM-CSF in the presence or absence of ArtC for 96 hours. ArtC induced M-MDSC differentiation and increased PD-L1 expression in those cells. Female C57BL/6 mice sensitized and challenged with OVA were treated with ArtC, 7 doses in 24 hour interval by intranasal route, and then re-challenged with OVA. ArtC-treated group exhibited an increase in M-MDSC PD-L1+ in the lungs (P>0.05) compared to non-treated group. To evaluate the proapoptotic effect of ArtC, lung cells from mice exposed to OVA were cultivated in vitro with ArtC for 24 hours. After stimulation with ArtC, eosinophils exhibited an increase in late apoptosis cell death (CD11b+SiglecF+AnnexinV+Live/Dead+ cells). Our results show that ArtC might negatively regulate pulmonary inflammation by two possible mechanisms and might represent a novel adjuvant therapy for patients with asthma. Financial support: FAPESP grants 2017/21629-5; 2022/16716-4. **Keywords:** MDSC;Artepillin C;Asthma.

IC - 056 - **Biological activity of DTL10: Stability study for a recombinant protein against human visceral leishmaniasis**

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Human visceral leishmaniasis (HVL) is a neglected tropical disease that remains one of the leading causes of morbidity and mortality worldwide, being endemic mainly among the poorest populations in Africa, Asia and Latin America. Currently, there are no therapeutic vaccines available for this zoonotic disease. Therefore, our research group developed in previous studies a chimeric protein denominated DTL10, containing Amastigota Protein 2 (A2) and a second Leishmania antigen (DTL8), which presented protection in an experimental animal model against *Leishmania infantum.* However, to qualify DTL10 for a human vaccine clinical trial, it is essential to develop a formulation that ensures long-term stability. Thus, this study aims to establish a protocol capable of quantifying the biological activity of the recombinant protein (DTL10) by indirect ELISA. The biological activity (in vitro) was determined by the EC50 parameter, which is defined by the necessary protein concentration to generate 50% of the maximum response obtained by a titration curve. For this, rabbits were immunized (CEUA, P-29/19-3, LW-39/19) with each subunit of the recombinant protein (A2 and DTL8) in a prime-boost regimen and serum was harvested from the carotid artery eight weeks later. ELISA plates were coated with DTL10 and tested against anti-DTL 8 and anti-A2 polyclonal serum, using an anti-rabbit-HRP as detection antibody. Biological activity tests were conducted over three days and the EC50 for each subunit was evaluated. The data collected so far determined an average of biological activity of 3.6X10^5 U/mg anti-DTL8 and 3.07x10^6 U/mg anti-A2. To further evaluate the long-term biological activity, repeatability tests will be conducted, comparing the data collected over time and providing insights into the protein´s stability. The study up to this point managed to establish a standardized protocol for stability studies of DTL 10 so that in the future it can fit in as a vaccine against HVL. **Keywords:** ELISA;Vaccines;Leishmania infantum.

IC - 057 - **Evaluation of the role of Legionella longbeachae capsule in the pathogenesis of infections in mice**

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Legionella are intracellular, gram-negative organisms that cause severe pneumonia known as Legionnaires' disease. The most studied specie of this genus is *L. pneumophila*, which has been widely used as a tool for activating several innate immune pathways. Among the most well-known species, *L. longbeachae*, has been highlighted as being responsible for part of the disease outbreaks. Interestingly, different from *L. pneumophila*, that not induce mice lethality, *L. longbeachae* induces a very strong disease in these animals, with a high mortality rate, however, it is not clear why this happens. *L. longbeachae* has virulence factors that differ from other bacteria of the genus, such as effector proteins secreted and the absence of flagellin, without NLRC4 inflammasome activation. This absence of flagellin is not the cause of the high mortality of *L. longbeachae*, since *L. pneumophila* deficient for flagellin also not induces mice lethality. Additionally, it was identified, by Carmen Buchrieser's group, two gene clusters encoding proteins that are predicted to be involved in the production of a capsule, including homologs of the ctrABCD capsule transport operon. Besides this characterization, there is no study exploring the role of *L. longbeachae* capsule in the pathogenesis of Legionnaires' disease. Therefore, in our study, we use *L. longbeachae* deficient for *ctrCD* and observed that capsule is essential for mice lethality since deleting this factor drastically reduces the animal's mortality. We also observed a high number of capsulated bacteria in the mice lung compared with the *ctrCD*$^{-}$, however, this didn't happen in BMDMs (bone marrow-derived macrophages). In addition, *in vitro*, capsule proved to be important for pro-inflammatory cytokines production, which may be related to the outcome of *in vivo* infections. Together, our data indicate that the *L. longbeachae* capsule has an important role in the pathogenesis of *L. longbeachae in vivo*. **Keywords:** Legionella longbeachae;Capsule;Pathogenesis.

IC - 058 - **ROLE OF THE FCYIIB RECEPTOR IN TRYPANOSOMA CRUZI EXPERIMENTAL INFECTION**

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Chagas disease is caused by the Trypanosoma cruzi parasite, which is endemic in Central and South America, but currently is a worldwide problem. Acute infection with T. cruzi leads to parasitemia and polyclonal activation that is linked to hypergammaglobulinemia and a delayed antibody response specifically targeting the parasite. Fc receptors are expressed on the surface of immune cells, including macrophages, and are responsible for binding to the Fc region of antibodies playing a crucial role in the host immune response. The FcyRIIB receptor is an inhibitory receptor that when bind to antibody delivery inhibitory signals to cells including macrophages, dendritic and B cells dampening their activation thus modulating immune response. The role of FcyRIIB receptor in the development of Chagas disease is unknow. Thus, our aim was to investigate the role of FcyRIIB receptor during experimental T. cruzi infection. For this, C57Bl/6 (WT) and FcyRIIB receptor knockout (KO) mice were infected with T. cruzi Y strain, and the following parameters; hematological profile, parasitemia, weight and survival were evaluated. Preliminary results showed that when compared with WT mice the FcyRIIB receptor KO mice anticipate the peak of parasitemia presenting decreased numbers of platelets and neutrophils, and increased number of leukocytes at the early phase of infection, but presented similar weight loss, clinic score, and survival rate. Notably, deficiency of FcyRIIB receptor resulted in increased hemoglobin levels at the basal levels. Collectively, our results suggested that FcyRIIB receptor is important during T. cruzi infection controlling the parasitemia and the profile of the blood circulating cells. Financial support: FAPEMIG, CNPq, CAPES. **Keywords:** Chagas Disease;Trypanosoma cruzi;Infectious Disease.

IC - 059 - **Standardization of Human Monocyte-Derived Dendritic Cell Differentiation**

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Introduction: Dendritic cells (DCs), characterized as antigen-presenting cells (APCs), have the function of presenting antigens to T lymphocytes. There are four main types of DCs classified according to phenotypic markers present on the membrane: myeloid DCs, plasmacytoid DCs, Langerhans cells, and monocyte-derived DCs. Monocyte-derived DCs are involved in inflammatory processes and can be obtained in vitro through differentiation with interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF) generating heterogeneous populations with different properties. These cells act in the early stages of infections, connecting the innate and adaptive immune response. The present study aims to evaluate the differentiation of human monocyte-derived dendritic cells. **Methodology:** Peripheral mononuclear cells (CEPEM-RO/CAAE: 6684.9123.7.0000.0011) obtained through cell separation using density gradient (Histopaque®) were cultured in Petri dishes containing RPMI/FBS. On the second and fifth days of culture, 50 ng/mL of IL-4 and 50 ng/mL of GM-CSF were added. After seven days of culture, differentiated DCs could be observed. On the eighth day, 1 mg/mL of LPS was added to induce DCs maturation. The viability of DCs was determined using 7AAD and immunophenotyped using CD14, CD83, and CD209 markers after seven days of differentiation. **Results:** DCs viability was not affected during the seven days of differentiation. DCs showed higher expression of CD14 and low expression of CD209, as well as a decrease in the expression of the maturation marker CD83, which became expressed after LPS stimulation. **Conclusion:** Together, the results obtained demonstrated the effectiveness of the method; the DCs displayed the dendritic cell marker (CD209) and had a little expression of the maturation marker, indicating that these cells are immature. LPS-induced DCs start expressing more of the mature cell marker CD83 at greater levels. **Keywords:** Dendritic cells;Monocytes;Immune response.

IC - 060 - **Characterization of the efficacy mechanism of the intranasal LaAg vaccine against leishmaniasis.**

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Leishmaniases are diseases caused by species of protozoa belonging to the genus *Leishmania* and are classified into tegumentary and visceral forms. Diffuse cutaneous leishmaniasis (DCL), although considered rare, is the most severe form of cutaneous leishmaniasis. In Brazil, *L. amazonensis* is the causative agent of DCL. Currently, there is no vaccine available to provide protection against *L. amazonensis* in humans. The vaccine candidate LaAg is a total lysate of *L. amazonensis,* which has shown partial protection when administered intranasally in murine models C57BL/6 and BALB/c. In this study, we investigated the protective mechanism of this vaccination. To accomplish this, we infected mice deficient in IL-10, IL-23, and B cells (µMT KO) with $2x10^5$ parasites subcutaneously, after immunizing them with LaAg or leaving them non-immunized (PBS control). We monitored the size of the lesions weekly and, at specific time points, sacrificed the animals to assess the parasitic load using LDA (limiting dilution assay). The results demonstrated that IL-10 KO animals exhibited similar lesions and parasite loads in both the control and immunized groups, with a lesion growth of 1.5mm and 10^4 parasites at the site of infection. Conversely, µMT KO animals displayed a significant difference between the control and immunized groups, with the immunized group reaching a maximum lesion size of 0.5 mm compared to 1.0 mm in the control group, although there was no difference in parasite load at the site of infection. Additionally, IL-23 KO animals showed a similar lesion profile and parasite load in both groups, with a lesion growth of 1.5mm and 10^5 parasites at the site of infection. These findings suggest that the vaccine's efficacy is dependent on IL-10 and IL-23 but does not rely on B lymphocytes. Ongoing experiments aim to determine which cells express IL-10 and IL-23 after LaAg immunization and identify the crucial cells for vaccine efficacy. **Keywords:** Leishmaniasis;Vaccine;LaAg.

IC - 061 - **Diagnostic performance of a protein A/G-based ELISA using rKLi8.3 kinesin from Leishmania infantum for detection of canine visceral leishmaniasis is improved by adding anti-canine IgG step.**

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Canine visceral leishmaniasis (CVL) represents a serious public health problem in Brazil and worldwide. Serological tests have been generally used to detect infected animals. Here, the serum IgG reactivity to a new diagnostic recombinant protein, rKLi8.3, containing 8.3 tandem repeat motifs of the 39 amino acids from a *Leishmania infantum* strain from Sudan, was evaluated through an immunochromatographic test (Lateral Flow (LF) Ingenasa, Spain) and an ELISA (NovaTec, Germany), both based on Protein A/G (Microbiol. Spectr. 11(3):e0433822). With the aim of improving the diagnostic performance of the Protein A/G-based ELISA-rKLi8.3, the effect of an additional step involving incubation with anti-canine IgG antibodies was investigated. Serum samples were grouped in the following way: (I) 38 parasitologically confirmed CVL cases divided in symptomatic (CVL-S, n=19) and asymptomatic (CVL-A, n=19), (II) non-infected healthy control (H-Ct, n=21) and (III) dogs diagnosed with other infections (I-Ct, n=17). The number of ELISA-rKLi8.3 positive sera was higher in the CVL-S group (16+/19) compared to the CVL-A group (9+/19). In the I-Ct group 3 sera were positive (3+/17), and in the H-Ct group all sera tested negative. Sera positive in the ELISA-rKLi8.3 also tested positive in the LF-test, with only three exceptions, one in the I-Ct and one in both the CVL-A and CVL-S groups. The sensitivity of the ELISA-rKLi8.3 to detect asymptomatic CVL increased with the addition of the anti-IgG step, with 7 out of 10 previously negative sera became positive with the anti-IgG. In contrast, all sera from the H-Ct group remained negative in the ELISA-rKLi8.3 plus anti-IgG. In the I-CT group only three sera converted to positive with the anti-IgG. These results showed for the first time the usefulness of rKLi8.3 antigen in the diagnosis of CVL in Brazil and that the addition of anti-IgG step can increase the sensitivity of the protein A/G-based ELISA-rKLi8.3 without affecting its specificity. **Keywords:** Visceral leishmaniasis;Serodiagnosis;Improved protein A/G-based ELISA.

IC - 062 - **The Mer receptor regulates splenic redox processes but has little effect on melanoma-associated immune cell subsets in hyperglycemic mice**

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The role of the Mer receptor in tumor cell proliferation and survival has been explored; however, just a few studies have focused on its role in efferocytosis (EF) in the tumor microenvironment. In parallel, hyperglycemia also favors tumor development through some pathways that are likewise stimulated by Mer receptor. Wild-type C57BL/6 (WT) and Mer-deficient (M) mice were treated with streptozotocin (STZ) to induce hyperglycemia (H and HM) or PBS (control), and after 15 days, they were inoculated with melanoma of the B16-F10 lineage (M and HM). Cells from the primary tumor (PT) and from the cervical lymph node (CLN) were collected for analysis by flow cytometry. Splenic tissue was collected for analysis of oxidative stress biomarkers (OSB). STZ treatment was able to induce hyperglycemia in mice from groups H and HM. We showed that PT grows less in M-M and HM when compared to WT. PT cytometry did not reveal significant changes in the number of macrophages, monocytes, and neutrophils between the groups, but we found a significant decrease in the number of $CD3^+$ T cells in M-HM when compared to WT-HM. Furthermore, when compared to the WT-M, the M-M had a large increase in the number of $CD8^+$ T cells, whereas the M-HM had a considerable decrease. We also did not find alterations in the number of $CD4^+$ and $CD8^+$ T cells in the CLN. Finally, in M, the number of Tregs increased considerably in the M and HM. Even though there were no changes in the number of T cells in the spleen, the OSB analysis revealed that M-M and HM showed an increase in lipid and protein oxidation, as well as a decrease in the tissue's antioxidant capacity, despite increased activity of the antioxidant enzymes superoxide dismutase and catalase. Collectively, our data suggest that the Mer receptor is critical for controlling oxidative stress in the spleen but has little role in T cell recruitment in PT or SCL. **Keywords:** Efferocytosis;Hyperglycemia;Melanoma.

IC - 063 - **Hyperglycemia impairs infected neutrophil efferocytosis and dendritic cell activation during MRSA infection**

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Neutrophil recruitment and abscess formation during skin methicillin-resistant *Staphylococcus aureus* (MRSA) infection is crucial to host defense. Previous studies have shown that there is extensive neutrophil apoptosis in the abscess, and dendritic cells (DCs) play an important role during the clearance of dead neutrophils, called efferocytosis. However, diabetic patients are unable to form the abscess and clear the MRSA infection properly. In diabetic mice, Langerhans cells are unable to migrate to the lymph nodes and activate T cell response during MRSA infection. Therefore, this study aimed to characterize neutrophil cell death and DC function during efferocytosis in diabetic and non-diabetic mice during MRSA infection. We treated male C57BL/6 mice with STZ (i.p.) for 5 days. Mice were considered diabetic with glycemia >250mg/dL. We injected $5x10^6$ CFU of MRSA (s.c.), and after 18h, the skin biopsies were collected, digested and DCs were isolated by magnetic separation with CD11c microbeads. Cells were intracellularly stained with anti-Sirp1α and Ly6G and extracellularly stained with anti-CD86, anti-MHC-II and anti-CCR7 and assessed by flow cytometry. The negative fraction was labeled with Ly6G, Fixable Viability Stain and Cleaved-Caspase 3 to determine cell death. Neutrophil recruitment to skin was similar (~85%) between normoglycemic and hyperglycemic mice. The STZ group had a higher rate of necrotic neutrophils than normoglycemic mice (35% and 20%, FVS+ Caspase3-). We also detected decreased efferocytosis in the diabetic group (~35% Sirp1α+ Ly6G+) compared to normoglycemic mice (~45%). Also, there was a modest accumulation of cDC2 (Sirp1α+) in the skin of STZ mice as well as a lower expression of CD86, MHC-II, and CCR7 compared to normoglycemic mice, suggesting the reduced capacity for migration and antigen presentation. These preliminary data indicate that hyperglycemia inhibits the cDC2 activation during efferocytosis of iAC thus impairing host defense. **Keywords:** MRSA;Dendritic cells ;Diabetes .

IC - 064 - **Evaluation of the metalloprotease leptolysin from pathogenic leptospiras as a vaccine antigen in hamsters**

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Leptospirosis is a neglected zoonosis caused by bacteria of the genus *Leptospira*, with an estimated 1 million cases and 58.000 deaths per year. Once infection occurs the leptospires reach the bloodstream, and the non-pathogenic are rapidly eliminated by Complement System (CS), an important component for the innate and adaptive immune response. Pathogenic species have developed evasion mechanisms, as secretion of metalloproteases which blocks CS activation, favoring their dissemination in the host. Exoproteomic analysis of *L. interrogans* indicated the existence of leptolysin - a novel metallopeptidase conserved in the *Leptospira* genome. Leptolysin is expressed abundantly by pathogenic *Leptospira*, cleave C3, and interacts with extracellular matrix components. This project aims to evaluate the use of leptolysin as a possible vaccine candidate, analyzing whether previous immunization would protect hamsters when challenged with pathogenic *L. interrogans* species. Three doses of 60µg of recombinant leptolysin induced a significant production of specific IgG. The End-$Point_{50}$ (EP_{50}) doses of pathogenic *L. interrogans* serovar Copenhageni L1-130 and Canicola LO-4 (more virulent) were determined. Leptolysin-immunized hamsters were challenged with $5xEP_{50}$ of Copenhageni L1-130 and the survival rate was similar to bacterin-vaccinated hamsters. However, unexpectedly, 60% of non-immunized animals survived. No protection was observed when leptolysin-immunized hamsters were challenged with $10xEP_{50}$ of Canicola LO-4. Future experiments will be performed to determine the best conditions to evaluate the role of anti-leptolysin antibodies to protect hamsters against pathogenic *Leptospira.* **Keywords:** Metalloprotease;Vaccine;Leptospirosis.

IC - 065 - **Reprogramming dendritic cell metabolism through nanostructured lipid carriers**

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Recent findings have shown that treatment regimens can drive immunogenic (ICD) or non-immunogenic cell death (NICD). NICD inducers might promote the accumulation of apoptotic cells (ACs) in the tumor microenvironment (TME), and dendritic cells (DCs) play an important role during the clearance of these cells through a process termed efferocytosis. Efferocytosis of tumor-ACs leads to anti-inflammatory mediators production that suppresses the TME. Metabolic activity is essential for cytokine production by immune cells. Recent findings from our group demonstrated that glycolysis is triggered during the efferocytosis of Renca-ACs, leading DCs to a tolerogenic profile. Therefore, we hypothesize that Nanostructured Lipid Carriers (NLC) incorporated with metabolic inhibitors could switch DCs profile to an immunogenic feature during the efferocytosis of tumor-ACs. BALB/c mice were injected with Renca cells (s.c) and after around 30 days animals were treated with doxorubicin (doxo) (10mg/kg i.p). After 3 days, DCs from the TME were isolated by magnetic microbeads, and MHC-II, CD86 and PDL-1 expression was evaluated by flow cytometry. DCs from mice doxo-treated group expressed lower levels of MHC-II and CD86 (~50% and ~20%, respectively) compared to DCs from untreated mice (~85% and ~80%, respectively). Moreover, IL-6 production was 40% higher in the tumor from doxo-treated mice compared to the untreated group. Considering our previous findings that demonstrated glycolysis involvement in PDL-1, IL-10 and IL-6 expression during the efferocytosis of Renca-ACs, an approach of targeted delivery of glycolytic inhibitors through NLC was attempted to reprogram DCs profile. DCs were incubated with NLC loaded with DiOC18(3) (DIO), and we observed that 95% of viable DCs were able to incorporate NLC-DIO. Next, additional experiments are ongoing to incorporate sodium oxamate, a glycolytic inhibitor, and evaluate the reprogramming of DCs tolerogenic profile in vitro and within TME. **Keywords:** Dendritic cells (DC);Immunometabolism;Nanostructured Lipid Carriers (NLC).

IC - 066 - **The Axl receptor protects against systemic damage in mice with hyperglycemic melanomas.**

MENDES, C.M.; FERREIRA, J.R.M.; PINTO, K.G.; RAMOS, L.R.; CIPRIANO, C.; LIMA, J.N.H.; DA COSTA, A.L.A.; PINTO, V.S.; TODESCHINI, A.R.; FILARDY, A.D.. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

The Axl receptor contributes to tumor cell proliferation and survival, but its role initiating the antitumor response is still unknown. In parallel, hyperglycemia also favors tumor development through some pathways that are likewise stimulated by TAM receptors. To investigate the role of the Axl in systemic changes caused by primary melanoma in hyperglycemic mice, wild-type C57BL/6 (WT) and Axl-deficient (A) mice were treated with streptozotocin (STZ) to induce hyperglycemia (H and HM), and after 15 days, they were inoculated with melanoma of the B16-F10 lineage (M and HM). We found that the primary tumor is significantly larger in A (M and HM) than in WT mice. Blood lactate and nitrite levels did not differ between groups, but there was a significant increase in LDH (A-M and HM) and γGT (A-M) as compared to the respective WT groups. Furthermore, we did not find differences in the levels of lactate and LDH in the peritoneal cavity across the groups, but we did find increased glucose and decreased nitrites levels in the A-HM compared to the WT. Although the numbers of monocytes, B cells and peritoneal macrophages (PM) were the same, we found a decrease in the number of neutrophils in the A-H and HM and in the number of PM $CD206^+$ in the A-M and HM, as well as an increase in the number of PM $MHCII^+$ in the A-M and HM, when compared to their respective WT groups. When compared to the corresponding WT groups, oxidative stress biomarkers analyses revealed that there was more oxidation of lipids and proteins in the hepatic tissue (HT) of A-M and HM and, to a lesser extent, in visceral adipose tissue (VAT). Furthermore, we found no differences in the activity of antioxidant enzymes in HT, but there was a substantial decrease in the activity of superoxide dismutase in A-HM VAT as compared to WT-HM. Collectively, our data suggest that the Axl receptor expression in non-tumor cells is important to restrain the tumor growth and some systemic damage during hyperglycemic melanoma. **Keywords:** Hyperglycemia;Melanoma;Axl receptor .

IC - 067 - **Breast cancer immunophenotypes: correlation with bone metastases development**

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Breast cancer is one of the human tumors that frequently metastasize to bone. Despite improvement in systemic therapies, survival remains poor for women harboring bone metastases (BoMet). The prognosis of breast cancer is influenced by the nature of tumor infiltrating lymphocytes (TILs), especially those from triple negative breast cancer (TNBC) subtype known for its poor prognosis and its resistance to conventional treatments. Once converted into tumor promoters, by immune surveillance and immune editing principles, these immunophenotypes control the development and progression of breast cancer. In the last few years, accumulating evidence suggest that bone colonization is preceded by changes in bone marrow (BM). In this context, our group reported that spontaneous BoMet development, originated from 4T1 TNBC model, depends on RANKL production by tumor primed CD3+ T cells, which establishes a pre-metastatic niche, inducing an early osteolytic disease, before the establishment of BoMet. In contrast, animals that receive 67NR in situ carcinoma, a 4T1 sibling cell lineage, have an increase in bone mass, dependent on CD19+ B cells producing OPG and CD8+ T cells expressing IFN-□ and IL-10, which regulate CD3+ T cells RANKL+, inhibiting the formation of the pre metastatic niche, and preventing the establishment of BoMet. In order to investigate whether these phenotypes are present in human disease, samples from patients, harboring the TNBC subtype, who have or have not developed BoMet were analyzed, retrospectively. Our results show that the frequency of CD3+ T cells expressing RANKL is significantly higher in primary tumors and sentinel lymph nodes from patients that developed BoMet, as compared to those who have not developed. Moreover, CD3+ T cells RANKL+ were also found in BM samples from those who developed BoMet. The confirmation of these immunophenotypes in human disease would be a valuable tool for predicting and/or inhibiting the development of BoMet. **Keywords:** Breast cancer;Bone metastasis;Immunophenotypes.

IC - 068 - **NEW INSIGHTS INTO THE ANTIMICROBIAL ACTION FROM HTLV-1-INFECTED INDIVIDUALS TOWARD ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS**

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Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1) infection is endemic in Brazil and is linked with pro-inflammatory conditions including HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). This chronic neuroinflammatory incapacitating disease culminates in loss of motor functions and an increase in susceptibility to opportunistic infections, such as *E. coli* and *S. aureus* (Fron Med. 9:812016, 2022). The aim of this work was to investigate the antimicrobial immunoglobulins and antibody-mediated responses from HTLV-1-infected patients. **Methods and results:** Sera were obtained from HAM/TSP patients, asymptomatic carriers (AC), and uninfected individuals (NI), and levels of IgG total, anti-*S. aureus* (ST30) and anti-LPS were evaluated by ELISA. HTLV-1-infected patients had a higher total IgG level compared to uninfected individuals. Preliminary results indicated that HAM/TSP patients presented higher titer of IgG anti-*S. aureus* than AC individuals. Moreover, HTLV-1-infected individuals presented higher levels of IgG Against LPS (38%) compared to NI donors. Following, we investigate the opsonophagocytosis capacity of monocytes and neutrophils using *E. coli* (ATCC-25922) previously stained with the SYTO9 probe. Labeled bacteria were incubated with heat-inactivated serum from HAM/TSP, AC and NI donors for 15 minutes at 37ºC. Opsonized and non-opsonized *E. coli* were incubated with monocytes or neutrophils for 30 minutes at 37ºC, then phagocytosis was evaluated by flow cytometry. The results showed that the bacteria opsonization induced an improvement in phagocytosis. However, the opsonization promoted by serum from HTLV-1-infected patients induced lower phagocytosis than serum from NI individuals. **Conclusion:** Although, the results suggested that HTLV-1-infected individuals have high levels of IgG against bacterial antigens, the opsonophagocytosis capacity was lower than NI, corroborating the bacterial susceptible infection state of these patients. **Keywords:** HTLV-1;E; coli;S; aureus.

IC - 069 - **Biochemical and Functional Characterization of a Novel Phospholipase A2 Isolated from Bothrops mattogrossensis Venom**

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Introduction: Phospholipases A_2 (PLA_2s) are proteins widely found *Bothrops* snake venoms and play a crucial role in the inflammatory response and activation of immune cells in victims of snakebites. So far, there are no reports in the literature regarding the action of PLA_2s from *Bothrops mattogrossensis* venom on the inflammatory response. Thus, the aim of this study was to isolate and characterize an acidic phospholipase A_2 from *B. mattogrossensis* venom and evaluate its action on neutrophils activation, as well as its involvement in local myonecrosis and inflammatory response through the release of creatine kinase (CK) and lactate dehydrogenase (LDH), activation of the NLRP3 inflammasome and IL-1β release, respectively. Methodology: PLA_2 was isolated from 100 mg of *B. mattogrossensis* venom through three chromatographic steps: cation exchange on a CM-Sepharose FF column (10 x 600 cm), followed by hydrophobic interaction on a n-butyl-Sepharose-HP column (1 x 15 cm), and finally a reverse phase on a C-18 column (25 mm x 4.60 mm, Kinetex). The resulting fractions were monitored at 280 nm and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Preliminary results: The butyl fraction has an acidic characteristic, and the interested protein exhibited a single band at approximately 15 kDa, corresponding to a PLA_2. The synthetic substrate 4N3OBA was used to determine the phospholipase activity, which demonstrated that the isolated toxin is enzymatically active. Conclusion: The isolated PLA_2 from *Bothrops mattogrossensis* is novel, exhibits acidic characteristics and enzymatic activity typical of an Asp-49 PLA_2, and was named $BmPLA_2$-A. It is worth noting that biochemical characterization studies and cell function assays are currently underway. **Keywords:** Snake venom;Bothrops mattogrossensis;BmPLA2-A;.

IC - 070 - **The potential of microbiota in regulating T cells associated with breast cancer bone metastases**

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Using the 4T1 model of metastatic breast cancer, our group demonstrated that tumor-specific $CD3^+$ T cells expressing $RANKL^+$ favor the formation of a pre metastatic niche in the bone marrow (BM), inducing an early osteolytic disease, before the establishment of metastases at this site. Currently, we seek to understand the correlation between the pattern of breast and/or intestinal microbiota and the generation of T and B pro and anti-metastatic phenotypes, since there are clear evidences that germ free mice have osteopetrotic alterations. Moreover, the estrogen deficiency-related bone loss is dependent on the intestinal microbiota, and on the expansion of pro-osteoclastogenic Th17 T lymphocytes. Herein, we are showing that the treatment of mice orthotopically inoculated with 4T1 tumor cells, with a broad spectrum of antibiotics, reduces the size of primary tumor and the number of metastatic clones into draining lymph nodes and BM. Moreover, these experimental group change the osteolytic phenotype to a regulatory one in both lymph nodes and BM. A more detailed understanding of this scenario would support new therapeutic and/or prophylactic strategies for breast cancer patients. **Keywords:** microbiota;bone metastases;T cells.

IC - 071 - **Recombinant human antibodies fragment (Fab) selection against heat-labile (LT) toxin produced by enterotoxigenic Escherichia coli.**

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Enterotoxigenic Escherichia coli (ETEC) causes diarrhea and diarrheal death among young children and travelers in developing countries. The major virulence factors of diarrhea-causing ETEC strains are enterotoxins, specifically a heat-labile toxin (LT) and a heat-stable toxin, making these toxins excellent targets to detection for diagnosis. The LT is an AB_5 toxin, it binds to ADP and ribosylates the guanyl-nucleotide alpha regulatory binding protein of the adenylate cyclase system, thereby increasing the levels of cyclic AMP. Antibodies are an excellent molecule for the design of high-affinity, protein-based binding reagents to be use as detection tools for immunodiagnosis. Using recombinant DNA technology and methods such as Phage Display, it is also possible to obtain fragments of recombinant antibodies (rAbs), such as Fab, with in vitro immunological repertoires, without the need for direct immunization of live hosts. Here, we used a human synthetic Fab fragment library, to select antibody fragments against LT by phage display, to be use as tools for ETEC detection. Antibodies selection was performed by phage display with a naive human Fab fragment library panned against immobilized native LT. The selected phages were analyzed for affinity by single-point competitive phage ELISA assay and sequencing. The sequences showed the presence of 34 distinct clones, of which only 12 had CDRs with functional sequences. Furthermore, among these 12, two had responded with saturation at the lowest antigen concentration in the competition ELISA, the most promising being 10C and 12G. The 10C and 12G Fab sequence was amplified, cloned, and expressed in a bacterial system, followed by purification by Immobilized-metal affinity chromatography. The purification yield was 3 mg/L and 6,5 mg/L to 10E and 12G respectively. The ETEC diagnostic assays are ongoing. These results showed promising tools for specific diagnosis of ETEC. **Keywords:** Recombinant Antibody;Immunodiagnostic;Phage Display.

IC - 072 - **Possible involvement of HMGB1 in dendritic cell reprogramming in severe sepsis**

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Dendritic cells (DCs) play an important role in the origin of the immune response and maintenance of the inflammatory process, and their dysfunction can contribute to the establishment of immunosuppression in sepsis. Benjamim et al (2005) showed that after 15 days of a severe sepsis episode, bone marrow-derived DCs (BMDCs) from septic mice remained dysfunctional. However, all the agents involved in the DC reprogramming have not been fully elucidated. HMGB1 is an alarmin that has already demonstrated participation in prolonged systemic inflammation in septic conditions. Recent studies in animal models have shown that inhibiting its action is beneficial in a sepsis scenario. Therefore, the project aims to characterize the involvement of HMGB1 in DC reprogramming through the action of glycyrrhizin (GL), an HMGB1 antagonist, on an acute sepsis frame. In this project, C57BL/6 mice were submitted to sepsis by the CLP model, treated or not with GL and euthanized after 7 days. BMDCs were differentiated for 7 days with medium containing IL-4 and GMCS-F, stimulated and subsequently analyzed. The results we have are that all septic animals treated with GL survived compared to septic animals without GL (57%). Additionally, we observed a distinguish cytokine profile with a decrease of IL-10 and IL-6 levels in the liver of GL-treated animals, and an increase of IL-10,IL-6 and TNF-a in the lung of septic animals. Furthermore, we observed an increase of splenic DCs in GL-treated animals ($4x10^6$) compared to sham ($1x10^6$), and a reduction of activated DCs in the mesenteric lymph node of septic animals (MFI 800) compared to sham (MFI 400). Finally, we observed that the BMDCs of septic animals, with and without GL treatment, are reduced (MFI 600) compared to sham (MFI 1300). In conclusion, our data suggest that, 7 days after sepsis induction the DCs are already reprogrammed with reduced differentiation and activation, even though GL has shown protective effects in septic animals. **Keywords:** Sepsis;Dendritic cells ;HMGB1.

IC - 073 - **Vaccines against leishmaniasis in times pre and post COVID-19. A PubMed narrative review**

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Introduction: *Leishmania* genus protozoa are used as models to develop new vaccine strategies, since the disease transmission occurs under a complex biological system composed of a human host, reservoir, parasite, and vector, and manifests as tegumentary (TL) and visceral leishmaniasis (VL). **Methods:** A bibliographic search was performed in PubMed for the last 10 years for VL and 25 for TL. A database was built with title, abstract, authors, journal, year, antigen, delivery systems, species, immunogenicity, and DOI. **Results:** Among the fifty publications included, most were developed in Brazil (42%) followed by Iran (14%), United States and India (12%), Spain (8%), and to a lesser extent in the United Kingdom, China, Germany, Canada, Tunisia and Portugal (2%). Most immunogenicity assays were performed in mice (52%), human Purified Blood Mononuclear Cells (14%), dogs, hamsters, bioinformatic modulations, transfected mice with human Major Histocompatibility Complex (MHC) class I (6% each), *Rhesus macaques* (4%), and in low proportions in humans and *in Silico* (2% each). Within the twelve publications for TL, it is highlighted the race from a first-generation vaccine of *Leishmania* (*Viannia*) *amazonensis* with the recombinant IL12 (rIL12) and Alum adjuvants *in Rhesus macaque*s to the most recent *L.* (*V.*) *amazonensis* formulation of Mapping Epitopes proteins associated to human MHC Class I in mice. Besides, it should be noted that in the vaccination race against VL, inside the thirty-three articles, one outstands the recent vaccination strategy using recombinant chimeric proteins associated with Monophosphoryl lipid A and Saponin adjuvants in vaccinated mice and challenged with *L. infantum*. **Conclusions:** The potential expansion of this disease and the absence of effective control, make it necessary to develop a human vaccine with high efficiency, taking advantage of the technological advances and emergent authorizations already shown during the vaccine race against COVID-19. **Keywords:** Visceral Leishmaniasis;Tegumentary Leishmaniasis;Vaccines.

IC - 074 - **CD300a MEDIATES CYTOKINE KINETICS AND PARASITE ELIMINATION IN EXPERIMENTAL STRONGYLOIDIASIS**

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Strongyloidiasis, a neglected tropical disease, affects about 600 million people worldwide, caused by Strongyloides stercoralis in humans. Experimental data and evidence from infected individuals indicate that type 2 immune response mechanisms are crucial to the control of migrating larvae and the intestinal expulsion of worms, averting fatal cases of hyperinfection and parasite dissemination. Receptors with inhibitory function such as CTLA-4, PD1, and CD200 play a key role in modulating T-cell-subset response. CD300a, another inhibitory receptor, is expressed on myeloid and lymphoid lineages and suppresses cellular migration, activation, and pro-inflammatory cytokine production. Its participation in helminthic infections, however, remains mostly unknown. To explore the possible role of CD300a activation during experimental Strongyloidiasis, we compared parasite burden and tissue cytokine profiles in Strongyloides venezuelensis-infected wild-type (WT) and CD300a knockout (CD300a−/−) BALB/c mice. CD300a−/− mice exhibited a markedly higher parasite burden throughout the infection, with more larvae in the lungs at 2 days post infection (dpi) and higher amounts of worms in the duodenum at 7 dpi. Adult worms infecting knockout mice also presented higher fertility, resulting in a 3-fold increase in the number of eggs shed at 7 dpi. Accompanying this difference in the parasitological profile, our preliminary data indicate a reduction in the production of Th2-related cytokines, such as IL-4, and an increase in IFN-γ and IL-17 in the lungs of infected CD300a−/− mice compared with WT mice. Knockout mice also presented a significant increase in intestinal IL-10 levels at 7 dpi. Thus, the absence of CD300a implicated in an altered immune response and increased parasite load during S. venezuelensis infection. Subsequent studies are being conducted to further investigate the role of CD300a in innate and adaptive immunity during Strongyloidiasis. **Keywords:** CD300a;Strongyloides;Type 2 Immune Response.

IC - 075 - **Distribution of the prevalence of malignant immunoproliferative diseases in Brazil from 2013 to 2022.**

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Introduction: Malignant immunoproliferative diseases are disorders characterized by uncontrolled production of lymphocytes, abnormal proliferation of primary cells of the immune system or excessive production of immunoglobulins. They have the ability to originate malignant cells in the bone marrow and migrate to the peripheral blood, manifesting as leukemias or as solid tumors in the lymph nodes or other secondary lymphoid tissues. These are serious diseases whose diagnosis and treatment measures are a major public health challenge. Objective: To verify the distribution of the prevalence of malignant immunoproliferative diseases in Brazil. Methodology: The number of cases of malignant immunoproliferative diseases was obtained from the database provided by DATASUS, technology and information at the service of SUS, according to the following search criteria: (1) panel - oncology - Brazil, (2) region of residence, (3) measures cases (3) available period, 2013 to 2022 and (4) detailed diagnosis - C88 malignant immunoproliferative diseases. The data were grouped by Brazilian region considering the period evaluated. Results: The prevalence of malignant immunoproliferative diseases increased by 5,919% in 2022 compared to 2013 in Brazil. Through the distribution by Brazilian region, an exorbitant increase of the disease in the Northeast was observed, a large peak in 2019 (83.96%) made it the region with the highest concentration of the disease, reaching 98.6% in 2022. The other regions (North, South, Southeast and Midwest) did not have such significant increases in the disease, reaching a total of 1.4% of cases in Brazil in 2022. Conclusion: Based on the results obtained in this study, it is evident the need for more specific studies to understand the epidemiological behavior of this pathology in the Brazilian regions, since there was an occurrence of these diseases in all regions, with great emphasis on the Northeast region, which concentrates the highest number of cases. **Keywords:** immunoproliferative diseases;prevalence;Brazil.

IC - 076 - **SNAKE VENOM METALLOPROTEINASES MODULATE MACROPHAGE ACTIVATION INDUCED BY TOLL-LIKE RECEPTOR 2 AGONIST**

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Toll-like receptor 2 (TLR2), expressed in macrophages, is crucial to the innate immune response through the recognition of molecular patterns associated with pathogens (PAMPs) or damage (DAMPs). TLR2 is involved in the pathogenesis of infectious diseases, thrombosis, as well as in the inflammatory response induced by *Bothrops atrox* snake venom. The snake venom metalloproteinases (SVMPs) are the major component of Viperidae snake and play a relevant role in the envenomation by activating platelets, coagulation cascade, fibrinolysis and inflammation. In the present work, we have analyzed the modulating role of SVMPs in the activation of macrophages induced by Pam3CSK4, a TLR2 agonist. For this, murine bone marrow-derived macrophages (BMDMs) were stimulated with Pam3CSK4 and treated with atroxlysin-I (Atr-I) or atroxlysin-III (Atr-III), which are respectively classes PI and PIII SVMPs, isolated from *B. atrox* venom. The production of the main pro-inflammatory cytokines IL-6, IL-1β, and TNF-α was quantified by ELISA and the activation of transcription factors involved in the immune response was evaluated by Western Blot (WB). Our results showed that Atr-I reduced IL-6 production and Atr-III decreased IL-1β production by BMDMs stimulated with Pam3CSK4. These proteinases also inhibited TNF-α expression induced by the TLR2 agonist. Furthermore, Atr-I and Atr-III inhibited the activation of NF-κB, which modulates the transcription of inflammatory genes, and potentiated the activation of AKT and ERK1/2, transcription factors involved in chemotaxis and cell proliferation. On the other hand, these proteinases did not interfere with p38 MAPK activation. These results suggest that SVMP modulate macrophage activation and may be further evaluated in models of inflammation or thrombo-inflammatory diseases. **Keywords:** Toll-like receptor 2;snake venom metalloproteinases;inflammation.

IC - 077 - **EVALUATION OF THE INFLAMMATORY MEDIATORS PRODUCTION BY MACROPHAGES TREATED WITH TOXINS ISOLATED FROM BOTHROPS LEUCURUS VENOM**

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Bothrops snake envenomation is characterized by an acute inflammatory response, including edema, leukocyte migration, endothelial damage, and necrosis at the bite site. Bothrops snake venoms are mainly composed of metalloproteinases (SVMPs), serine proteinases (SVSPs), phospholipases A2 (PLA2), disintegrins, and C-type lectins. The roles of these toxins in the inflammatory response still not fully understood. Therefore, this work aimed to evaluate the production of inflammatory mediators by macrophages treated with proteins isolated from Bothrops leucurus venom. For this, RAW 264.7 cells were stimulated with Leuc-a (SVMP-PI), Leuc-b (SVMP-PIII), and PLA2. After 24 hours of cell cultured with the treatment, the supernatants were collected for IL-6, IL-1β, and TNF-α quantification by ELISA. To verify whether these toxins could modulate macrophage activation, Raw 264.7 cells were pre-stimulated with Pam3CSK4 or LPS, respectively toll-like receptor (TLRs) 2 and 4 agonists, and then treated with Leuc-a, Leuc-b and PLA2. The results showed that all toxins evaluated impacted on the reduction of TNF-α production induced by LPS and Pam3CSK4. Furthermore, Leuc-a decreased IL-6 production induced by TLR 2 and 4 agonists. Leuc-b decreased IL-6 production induced by LPS whereas increased IL-6 production induced by Pam3CSK4. The toxins had induced a slight increase in IL-1β production when macrophages were pretreated with Pam3CSK4 or LPS. In conclusion, our data suggest that SVMPs and PLA2 could be involved in the inflammatory response induced by B. leucurus snake venom. The modulation of macrophage activation by SVMPs, mainly by Leuc-a, a non-hemorrhagic SVMP-PI, demonstrates a strong biotechnological potential of this toxin applied for the treatment of inflammatory diseases. **Keywords:** macrophages;snake venom metalloproteinases;inflammation.

IC - 078 - **CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES IN MURINE BREAST CANCER CELL LINES**

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Metastasis development is responsible for the majority of deaths rather than the primary tumor, especially for breast cancer, the most frequent type of cancer in women. The mechanism in which metastasis happens with different site specificity is still unknown. Cell lines subcloned from a murine spontaneous carcinoma have the capacity to metastasize to different sites. The cell line 67NR is the only non metastatic, 168FARN is described with low potential to develop metastasis but can metastasize to lymph nodes. Whilst 66cl4 metastasized majority to lungs and 4T1 to lungs, liver and bone. It's known that metastasis development in the 4T1 is mediated by T cells that produce RANKL. Thus we aim to find out genesets in the primary tumor cells that when upregulated influences lymphocytes to produce cytokines that facilitate metastasis development. A joint analysis with public deposited samples from Gene Expression Omnibus and a bulk RNA sequencing we performed for each cell line were analyzed using a pre-established pipeline of differential gene expression. The comparison was performed between each metastatic cell line and the non-metastatic one. We found specific gene signatures for each cell line, furthermore we will perform GeneSet Enrichment Analysis. Our results suggest that site-specific metastasis could be related to genesets that are upregulated in each cell line individually and their immune mediating process in which T cells produce facilitating cytokines. Identifying the molecular mechanisms of which site specific metastasis happens is essential to the design of new therapeutic strategies. Therefore we seek to design RNAi and investigate if by silencing these candidate genes we can impact metastasis development *in vivo*. **Keywords:** breast cancer;metastasis;T cell.

IC - 079 - **The Mer receptor helps maintain peritoneal cavity homeostasis in mice with hyperglycemic melanoma.**

LIMA, J.N.H.; FERREIRA, J.R.M.; PINTO, K.G.; RAMOS, L.R.; CIPRIANO, C.; DA COSTA, A.L.A.; MENDES, C.M.; PINTO, V.S.; TODESCHINI, A.R.; FILARDY, A.D.. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

The Mer receptor (MR), expressed by tumor cells, contributes to cell proliferation and survival, but its role in initiating the antitumor response by immune cells has not been shown. In parallel, hyperglycemia also favors tumor development through some pathways that are likewise stimulated by MR. To investigate the role of the MR in systemic changes caused by primary melanoma in hyperglycemic mice, wild-type C57BL/6 (WT) and Mer-deficient (M) mice were treated with streptozotocin (STZ) to induce hyperglycemia (H and HM) were inoculated with melanoma of the B16-F10 lineage (M and HM) 15 dpi. Systemic damage biomarkers were quantified in blood and peritoneal cavity (PC), the immune cells profile by flow cytometry (FC) and oxidative stress biomarkers (OSB) from liver and visceral adipose tissue (VAT). First, we found that primary tumors grow slower in M (M and HM) than in WT. There were no variations in blood lactate, LDH, or nitrite levels across groups, however there was a significant increase in γGT in M-M compared to WT-M. Furthermore, lactate and LDH levels in the PC M-M were significantly lower than in the WT-M, whereas nitrite levels in the M-HM were lower than in the WT-HM. By FC, we found an increase in the number of monocytes and a decrease in the number of neutrophils and peritoneal macrophages (PM), mainly with anti-inflammatory phenotype in the M-HM when compared to the WT-HM. OSB analysis revealed that there was greater oxidation of lipids and proteins in the liver tissue of M-M and to a lesser extent in VAT when compared to the WT-M. Finally, when compared to the WT-HM, there was a considerable increase in total antioxidant capacity and catalase activity in both tissues, but a decrease in superoxide dismutase enzyme activity in the M-HM. Collectively, our data suggest that the MR expression in non-tumor cells is important to restrain the tumor growth and to maintain homeostasis in the PC of mice with hyperglycemic melanoma. **Keywords:** TAM receptors;Hyperglycemic;melanoma.

IC - 080 - **Hemopressin, a cannabinoid peptide, prevents acute and late cognitive impairments of sepsis in mouse.**

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Sepsis can be described as an organ dysfunction mediated by a dysregulated host response to an infection condition. Even with substantial mortality rates, survivors show a series of long-term impairments such as immunosuppression, cognitive impairments, and other complications. Despite the rich literature and knowledge about sepsis, there is no effective treatment that can control the acute insults and, consequently, protect sepsis survivors against late impairments. In our previous studies, we found that hemopressin (HP), a cannabinoid peptide antagonist/inverse agonist of CB1, attenuates the early inflammatory response and improves cognitive and metabolic late impairments in mice. Therefore, the aim of this study is to evaluate protective effects of HP in sepsis-associated encephalopathy (SAE) and its consequences. The *in vivo* sepsis model used was cecal ligation and puncture (CLP) with 9 punctures in C57BL/6 mice. The treatment was performed in the time points of 5, 24, 48, and 72 hours after surgery and consisted of intraperitoneal injection of ertapenem (75 mg/kg) plus HP (250 nmol/kg) or vehicle 20 minutes after antibiotic treatment. In the *in vitro* model we used Bv2 cells, a microglial lineage, which were stimulated with LPS (100 ng/mL) and treated with HP 1, 3, and 10 µM for 24 hours. Firstly, in the *in vivo* approach, HP showed a tendency to increase of synaptophysin, a synapse marker, expression in the mouse cerebral cortex. In addition, HP also up-modulated CB2, but not CB1, expressions observed in mice brains during SAE ($p < 0.05$). Furthermore, in our *in vitro* approach HP restored and further increase CB1 and CB2 microglial expression after stimulus, but did not show any effect on the release of TNF and NO. Therefore, our early data showed that HP seems to modulate the cannabinoid system in microglia and by regulating the inflammatory response in SAE could support the protection against cognitive impairments. **Keywords:** Sepsis;Hemopressin;Cognitive Impairments.

IC - 081 - **Impact of efferocytosis of Zika virus-infected cells on dendritic cell activation and differentiation of self-reactive T lymphocyte clones**

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Severe viral infections can disrupt immune tolerance and induce autoimmune diseases. Previous studies have demonstrated that efferocytosis of Citrobacter-infected apoptotic cells by dendritic cells (DCs) can be crucial in developing autoreactive CD4+ T cells. Therefore, we hypothesized that efferocytosis of ZIKV-infected apoptotic cells (ZIKV-iAC) could active DCs and leads to autoreactive CD4 T cell activation. To address the hypothesis, the neuroblastoma cell line (SH-SY5Y) was infected with ZIKV, and the infection rate was determined after different time points using an anti-4G2 antibody. At MOI of 1, ~ 40% of cells were positive for ZIKV, and 48h p.i. is more effective in achieving a high infection rate than longer periods. After the ZIKV infection, the cells were irradiated with 5mJ (UVC) to improve the number of apoptotic cells, and 35-40 % of cells were positive for cleaved caspase-3. To improve the enrichment of ZIKV-infected cells, cells positive for 4G2 were sorted by BD FACSAria. After sorting, we obtained 86% ZIKV+ cells (named high-rate ZIKV-iAC). The impact of efferocytosis of ZIKV-iAC on DC activation was evaluated by co-cultured DC with high-rate ZIKV-iAC for 18h, and molecules expression and cytokines were determined by flow cytometry and ELISA. We observed a significant increase of MHC-II+/CD86+ (51,56%) expression on DCs compared with DCs only (22,28%) or DC+ACs (28,62%). Moreover, the efferocytosis of high-rateZIKV-iAC promoted high-level production of IL-10, IL-6, and IL-1β, when compared with DCs or DC+ ACs. Interestingly, the efferocytosis of low-rate ZIKV-iAC (lower than 30%), there was no impact on MHC-II/CD86 expression or cytokine production by DCs. Taken together, these data suggest that the activation of immunogenic DC occurs in the presence of a high-rateZIKV-iAC; otherwise, the efferocytosis of a low-rateZIKV-iAC does not impact DC activation and possibly does not break down self-tolerance mechanisms mediated by DCs. **Keywords:** Zika virus;Dendritc Cells;Self-reactivity.

IC - 082 - **Treatment with mimetic peptides from Tityus serrulatus venon regulates immune response and improves outcome in experimental severe malaria.**

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Plasmodium berghei ANKA (PbA) infection in mice mimics many aspects of severe malaria in humans, including cerebral malaria. The components of the *Tityus serrulatus* - TsV (yellow scorpion) venom and their derivatives present immunomodulatory, antimicrobial, antitumor and antiparasitic capacity. Herein, our objective was to test the therapeutic and neuroprotective potential of TsV-derived peptides (characterized and synthesized by our group) in the treatment of experimental severe malaria. C57Bl/6 mice were inoculated with 10^5 PbA-parasitized red cells, and were treated or not intraperitoneally with the peptides (pep 1a, 2a and 2b -1mg/kg), and orally with chloroquine (CQ-30mg/kg) initiating at 8 hours or 3 days after infection, once a day, until the seventh day. Our results showed that the treatment peptides was able to prolong survival, decrease body mass loss and alleviate the pathological effects caused by malaria. In the novel object recognition test, animals treated with the peptides had a better result compared to chloroquine. There was no difference in parasitemia, indicating that the effects of the peptides come from some interaction with the immune system, since the pre-incubated PbA-parasitized red cells with the peptides showed better results when compared to only infected group. Notably, flow cytometry analyses demonstrated a significant increase in CD4+IFNγ+ lymphocytes in animals treated with 2a. Treatment with 2a associated or not with chloroquine decreased the levels of transaminases in the blood (which can be associated with hepatic and systemic damage) and reduced the pulmonary dysfunction caused by the disease, when compared to untreated infected group. Collectively, our results suggest that the peptide 2a have potential immunomodulatory activity, controlling disease development, being a promising compound for treatment and co-treatment during severe experimental malaria. **Keywords:** peptides;malaria;immunoregulation.

IC - 083 - **EVALUATION OF ANTITUMORAL EFFECTS OF SOLUTIONS TREATED WITH PLASMA AT ATMOSPHERIC PRESSURE ON THE MIGRATION AND PROLIFERATION OF MURINE MELANOMA CELLS (B16F10) IN VITRO.**

DAS NEVES, D.V.[1]; DE OLIVEIRA, E.C.[2]; DA CRUZ, N.C.[3]; RANGEL, E.C.[3]; HERGESEL, K.G.[4]; DO NASCIMENTO, G.G.[5]; DE LIMA, D.M.[1]; FRAGOSO, M.V.F.[5]; DE MOURA, K.A.S.[6]; DOS SANTOS, L.M.B.[7]; LIMA, A.D.D.R.[7]; FERRARI, B.B.[7]; PRADELLA, F.[7]; DOS SANTOS, J.P.[8]. 1. CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA, CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA VOTORANTIM - SP - BRASIL; 2. CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA, CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA CAMPINAS - SP - BRASIL; 3. UNIVERSIDADE ESTADUAL PAULISTA- CÂMPUS DE SOROCABA – UNESP, UNIVERSIDADE ESTADUAL PAULISTA- CÂMPUS DE SOROCABA – UNESP SOROCABA - SP - BRASIL; 4. CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA, CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA MAIRINQUE - SP - BRASIL; 5. CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA, CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA SOROCABA - SP - BRASIL; 6. CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA, CENTRO PAULA SOUSA (CEETEPS)- FACULDADE DE TECNOLOGIA DE SOROCABA- FATEC SOROCABA ITU - SP - BRASIL; 7. DEPARTAMENTO DE GENÉTICA, EVOLUÇÃO, MICROBIOLOGIA E IMUNOLOGIA- UNICAMP, DEPARTAMENTO DE GENÉTICA, EVOLUÇÃO, MICROBIOLOGIA E IMUNOLOGIA- UNICAMP CAMPINAS - SP - BRASIL; 8. UNIVERSIDADE PAULISTA- SOROCABA- UNIP, UNIVERSIDADE PAULISTA- SOROCABA- UNIP SOROCABA - SP - BRASIL.

Plasma at atmospheric pressure (APP) is a new modality of cancer therapy and research. APP is a partially ionized gas operated at room temperature, the reactive species of oxygen and nitrogen (ROS/RNS) produced by APP has been studied in recent literature for treat eczema, bedsores, skin cancer (Melanoma) and helping in wounding healing. Melanoma is one of the most common types of cancer in the world, the main cause being excessive exposure to UV light with cumulative effect, causing the malignant transformation of the melanocytes. This study started analyzing two types of solution, sodium chloride 0,9% and ringer lactate exposed to APP at different times (30, 45, and 60 minutes). Melanoma cell line and the non-tumor control cell (L929) were exposed to the treated solutions for 120, 60, and 30 minutes and cell viability, the morphology of cells, and gene expression for cytokines were analyzed after 24 and 48 hours. Both treated solutions have a significant reduction in the viability of B16F10 cells when compared with non-treated solutions. Solutions treated with APP have been stored at -20 ºC for use in another experiment and tested in different times. More than 75 days later, frozen solutions maintained their effect on tumor cells, mainly ringer lactate. In flow cytometry assay, both solutions in different times of treatment and frozen induced apoptosis and necrosis of the tumor cells, but it´s depending on the time of exposure of the solutions to the APP and the time of contact with cells. Solutions without treatment did not modify the cell morphology in vitro, but those solutions APP treated modified the cell structure and decreases the B16F10 cell migration, but not the L929 cells. These results showed that the APP can be used for the treatment of physiologic solutions for adjuvant cancer therapy. **Keywords:** Atmospheric plasma;melanoma;tumor imunology.

IC - 084 - **EVALUATION OF DIMETHYL AND MONOMETHYL FUMARATE EFFECT ON GLIOBLASTOMA CELL IN VITRO**

DE LIMA, D.M.[1]; DAS NEVES, D.V.[1]; FRAGOSO, M.V.F.[2]; DE MOURA, K.A.S.[1]; DOS SANTOS, J.P.[3]; HERSEGEL, K.G.[1]; DO NASCIMENTO, G.G.[1]; LIMA, A.D.D.R.[4]; FERRARI, B.B.[4]; DOS SANTOS, L.M.B.[4]; DE OLIVEIRA, E.C.[1]. 1. FACULTY TECHNOLOGY OF SOROCABA - FATEC, FACULTY TECHNOLOGY OF SOROCABA - FATEC SOROCABA - SP - BRASIL; 2. FACULTY TECHNOLOGY OF SOROCABA - FATEC, FACULTY TECHNOLOGY OF SOROCABA - FATEC SORACABA - SP - BRASIL; 3. PAULISTA UNIVERSITY - UNIP, PAULISTA UNIVERSITY - UNIP SOROCABA - SP - BRASIL; 4. UNIVERSITY OF CAMPINAS – UNICAMP, UNIVERSITY OF CAMPINAS – UNICAMP CAMPINAS - SP - BRASIL.

Glioblastoma multiforme (GBM) is the most frequent and aggressive malignant tumor among all brain tumors. This disease has shown resistance to conventional treatments, with a recurrence in 90% of cases of primary glioblastoma. So, it is essecial to explore treatment options to respond to the rapid evolution and carcinogenic development. Many therapeutic agents are tested in clinical trials involving recurrent glioblastomas. Dimethyl fumarate (DMF) is a drug with several biological actions as modulating immune functions for autoimmune diseases such as relapsing-remitting multiple sclerosis and psoriasis. When ingested, DMF is rapidly metabolized to its biologically active form, monomethyl fumarate (MMF). Studies reveal that many glioblastoma patients treated with DMF have had to stop treatment due to disease progression. This work aims to evaluate the effects of DMF and MMF on glioblastoma cell lines in vitro. Human glioblastoma cell line NG-97 was exposed to different concentrations of DMF and MMF (5, 10, 20 µg/mL) and incubated for 24 and 48h. The non-tumor cells L929 (mouse fibroblast) were used as control. We evaluated the cytotoxic effect using MTT method and analysing morphologic alterations after treatments. The preliminary data show that DMF significantly decreases the cell viability of NG-97 in vitro, while MMF increases de growth of cells in the same conditions. The morphological evaluation showed that MMF altered the growth pattern of NG-97 and modified its cellular structure when compared with untreated cells. The main challenge to the success of GBM therapies is to reduce the ability of drugs to alter the molecular characteristics of this type of tumor, increasing its heterogeneity. Other experiments, such as the expression of the immune checkpoints PD-L1, PD-L2, and gene expression for TGFβ and IL-10 will help to understand the role of monomethyl fumarate in the progression of this type of tumor. **Keywords:** Glioblastoma;Dimethyl Fumarate;Monomethyl fumarate.

IC - 085 - **Analysis of the interaction between feline macrophages of the Fcwf-4 lineage and the fungi Sporothrix brasiliensis and Sporothrix schenckii.**

TAVARES, M.L.A.[1]; DOS SANTOS, R.Q.[2]; BONILLA, J.J.A.[3]; LIMA, I.D.[3]; DA SILVA JUNIOR, E.B.[3]; DE OLIVEIRA, J.C.G.[3]; DA SILVA, L.H.P.[1]; FREIRE-DE-LIMA, C.G.[3]; DECOTE-RICARDO, D.[1]; NASCIMENTO, D.D.O.[1]. 1. UFRRJ, UFRRJ SEROPÉDICA - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO SEROPÉDICA - RJ - BRASIL; 3. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

Sporotrichosis is a fungal and zoonotic disease caused by species belonging to the pathogenic clade of the genus *Sporothrix,* classified as a neglected disease by the World Health Organization (WHO). This pathogenesis has a worldwide distribution but is predominantly prevalent in tropical and subtropical regions. It is considered the most frequent subcutaneous mycosis in the world and is currently widespread in Brazil, where *S.brasiliensis* represents the predominant species. Its transmission has been primarily linked to contact with sick cats. The current characteristic of zoonotic transmission in Brazil, associated with the fungus *S.brasiliensis*, has shown a correlation with more severe symptoms in human patients, leading to an increased reporting of severe cases in immunocompetent individuals and also in felines, which develop multiple lesions and disseminated disease upon infection. Despite its importance, there is little available data on the role of the immune system in cat infection, such as the involvement of macrophages during the early stages of infection. Therefore, we aim to initially evaluate in vitro infection by the fungi *S.brasiliensis* and *S.schenckii* in the Fcwf-4 lineage of cat macrophages. For this purpose, cultures with 10^5 cells of the Fcwf-4 lineage were infected with yeast cells. We observed that the fungus *S.brasiliensis*, when compared to *S. schenckii,* induced more cell death in the cat cell lineage, while not altering nitric oxide production and resulting in a greater number of viable intracellular yeasts. Based on these findings, we evaluated the profile of these stimulated macrophages. Our data suggest that *S.brasiliensis* yeasts have higher infectivity compared to *S. schenckii*. **Keywords:** Sporotrichosis;Sporothrix brasiliensis;Sporothrix schenckii.

IC - 086 - **FUNCTIONALIZED MULTI-WALLED CARBON NANOTUBES POTENCIALIZE EFFECT OF PHOTODYNAMIC THERAPY OF MURINE MELANOMA IN 2D AND 3D CULTURE**

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Photodynamic therapy is a minimally invasive health technology used to treat cancer and other non-malignant diseases. Multi-walled carbon nanotubes (MWCNTs) have wide biomedical application prospects but exhibit notable biotoxicity associated with your hydrophobic character. In this study, we functionalized the MWCNT by plasma ablation process for use in photodynamic therapy with LED. Plasma treatment reduced the hydrophobic character of MWCNT and improved the dispersion in water and PBS. Carbon nanotubes were functionalized by 30 and 60 seconds (f-MWCNT-30´ and 60´) using 3 sccm of O_2 with 800 V, and the nanotubes not functionalized (MWCNT-0) were internalized by mouse melanoma (B16F10) and non-tumor control cells (mouse fibroblast cells - L929) in 2D and 3D culture. After 24h, cells were exposed to the LED 660 nm for 5 minutes for two consecutive days. Cell viability, qPCR, and flow cytometer were used to evaluate the cells after treatments. In 3D culture, we evaluated de internalization of f-MWCNT and MWCNT-0, spheroids morphology, size, and invasion and migration capability in vitro. The results showed that the plasma ablation process increased the dispersion of f-MWCNT 30´ and 60´ in an aqueous medium without affecting its crystalline structure. Plasma treatment reduced the hydrophobic character of f-MWCNT-30´ and f-MWCNT-60´, therefore, they were more internalized by cell lines. LED treatment decreases cell viability. Treatment of f-MWCNT 60´+ LED decreases the expression of PD-1 and PDL-1 in B16F10 cells. Gene expressions revealed that there was a decrease in TGF-β cytokines and VEGF after treatment in the tumor cells. In 3D melanoma tumor models, the treatment with f-MWCNT 30´+LED and f-MWCNT 60´+ LED resulted in morphological alterations and decreases migration capability of the spheroids in vitro. The results are interesting to understand how functionalized MWCNT and LED can be used how cancer therapy in the future. **Keywords:** Mouse melanoma;Carbon nanotubes;LED treatment.

IC - 087 - **Immunoinformatic approaches to building multi-epitope proteins from SARS-COV-2 structural proteins**

DE ARAÚJO, L.P.; SANTOS, N.C.D.M.; AUGUSTO, H.V.; DA SILVEIRA, N.J.F.; CORSETTI, P.P.; DE ALMEIDA, L.A.. UNIVERSIDADE FEDERAL DE ALFENAS, UNIVERSIDADE FEDERAL DE ALFENAS ALFENAS - MG - BRASIL.

COVID-19 is a severe acute respiratory syndrome caused by the SARS-CoV-2 coronavirus, which has already resulted in the death of approximately 6.5 million people worldwide. Currently, there is no specific therapy, making early diagnosis and treatment one of the best alternatives in its combat. The use of vaccines for prevention is the most effective method for COVID-19, studies aimed at improving the immunological durability of these vaccines are constantly developing. From this perspective, the main objective of this study was to develop a multi-epitope vaccine candidate derived from the structural proteins of SARS-CoV-2. For this purpose, five epitopes obtained from the structural proteins of SARS-CoV-2 were used, as identified by our research group, along with two adjuvants: β-defensin and PADRE (pan DR epitope). These components were concatenated in three versions, using rigid, flexible, and cleavable linkers, resulting in 3 chimeric proteins. Subsequently, the proteins were evaluated for their antigenicity, allergenicity, and stability through online servers, as well as subjected to analyses of molecular modeling, molecular dynamics, and molecular docking with TLR2, TLR4, and TLR8 receptors. Finally, the prediction of the immunogenic response was performed for three vaccine doses, and the cloning prediction was carried out in an *E. coli* expression system. The results indicated that the 3 proteins have potential for *in vitro* and *in vivo* analyses, as they demonstrate good stability, antigenicity, and absence of allergenic risks. Additionally, a good interaction with the receptors was observed, given that the chimeric proteins are capable of interacting with the adjuvant and epitope portions. Therefore, vaccine safety and confirmation of *in silico* analyses should be conducted in vitro and in vivo. **Keywords:** Bioinformatics;Reverse Vaccinology;COVID-19.

IC - 088 - **EVALUATION OF PVA'S HYDROGEL AS A RELEASE SYSTEM OF BEE VENOM (APITOXIN) ON TUMORS CELLS IN VITRO**

FRAGOSO, M.V.F.[1]; DE OLIVEIRA, E.C.[1]; BOTARO, V.R.[2]; SILVA, J.O.[2]; DE LIMA, D.M.[1]; DAS NEVES, D.V.[1]; DE MOURA, K.A.S.[1]; DOS SANTOS, J.P.[1]; LIMA, A.D.D.R.[3]; FERRARI, B.B.[3]; DOS SANTOS, L.M.B.[3]; PRADELLA, F.[3]; HERSEGEL, K.G.[1]; DO NASCIMENTO, G.G.[1]. 1. FATEC SOROCABA, FATEC SOROCABA SOROCABA - SP - BRASIL; 2. UFSCAR, UFSCAR SOROCABA - SP - BRASIL; 3. UNICAMP, UNICAMP CAMPINAS - SP - BRASIL.

Apitoxin (APX) is a name given to the venom produced by Apis mellifera bees, used for individual or colony defense. APX and its components, such as melittin, induce cytotoxic, antitumor, immunomodulatory, and apoptotic effects in different cells *in vitro* or *in vivo*. However, different doses of these venoms present high toxicity, a crucial problem to solve. Hydrogels are materials with different characteristics in their composition and synthesis. Their ability to absorb water and polar fluids makes them attractive for use in different areas of science, such as the medical field. Polyvinyl alcohol hydrogel (HPVA) can be an exciting alternative for drug delivery due to its biocompatibility and ability to absorb and release substances. This work aims to evaluate the effects of APX conjugated to HPVA on the murine melanoma cell line (B16F10) using culture in two dimensions (2D) and three dimensions (3D). FTIR, thermogravimetric analysis, and SEM/EDS characterized the synthesis of HPVA. UV-VIS evaluated the ability to absorb and release APX by HPVA at different times. The B16F10 strain was exposed to different concentrations of APX conjugated to HPVA in 2D and 3D cultures. In the 2D culture, we evaluated the cytotoxicity by MTT, the type of cell death (apoptosis or necrosis), morphology, cell migration, and expression of PD-1, PDL-1, CD31, and CD34. In the 3D culture, we evaluated the size of the spheroids exposed to APX and HPVA in different concentrations. The murine fibroblast cell line (L929) was used as a control. Preliminaries results showed the APX cytotoxicity on the tumor cells, the concentrations 10 and 5 µg/mL the most effective, considering the same concentrations for the control. In addition, the HPVA demonstrated good absorption of the bee venom, mainly the components melitin and phospholipase A2. **Keywords:** Apitoxin;PVA"S Hydrogel;Tumor cells.

IC - 089 - **Establishment of an infection model with Leishmania amazonensis employing microneedles**

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Leishmaniasis encompasses a group of diseases caused by protozoa of the Leishmania genus, transmitted by phlebotomine sand flies. Research groups unable to utilize phlebotomine sand flies for natural infection must establish infections using intradermal and subcutaneous needles. However, these artificial infection methods do not accurately replicate the conditions induced by phlebotomine sand flies during blood feeding. Microneedles are widely employed in the field of aesthetics, but their potential use as an infection apparatus has not been investigated thus far. Therefore, the objective of this study is to evaluate a transdermal infection model using microneedles as an alternative to intradermal needles, while also determining the optimal quantity of *L. amazonensis* parasites required to establish transdermal infection in BALB/c mice ears. To achieve this, BALB/c mice ears were infected with 2x10^6 *L. amazonensis* promastigotes using microneedles of different depths (1.0 mm and 1.5 mm) for transdermal infections, as well as intradermal infections. Additionally, a trial was conducted where different quantities of parasites were inoculated in each group (2x10^6, 2x10^5, 2x10^4, and 2x10^3) employing microneedles with a depth of 1.0 mm. Preliminary results indicate that transdermal infections, in contrast to intradermal ones, induce similar lesion sizes. Moreover, when compared to the parasite quantity necessary for establishing the infection, 2x10^6 promastigotes appeared to be the optimal dose for inducing larger lesions and parasite loads. Furthermore, infections with lower parasite doses led to proportionate parasite loads in the spleen, ear, and draining lymph nodes, despite the smaller lesion sizes induced. Thus, one potential application of this study is to facilitate experimental evaluations of the efficacy of new drugs and vaccines, as it allows for experimental infections that closely resemble the quantity of parasites inoculated during natural infections. **Keywords:** microneedle;leishmania amazonensis;leishmaniasis.

IC - 090 - **Evaluation of B lymphocytes in Leishmania amazonensis infection**

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On this work, we tried to understand the responses of B lymphocytes between susceptible (BALB/c) and partially resistant (C57BL/6) models to infection by *Leishmania amazonensis.* For this, we infected both models in the ear. After 38 days of infection, a period in which the lesions of both C57BL/6 and BALB/c models were starting to show statistical difference in terms of size, an equal parasite load was observed in the ear and in the draining lymph node, but no load was observed parasite in the spleen. After 59 days, a period in which the BALB/c animals had larger lesions than the C57BL/6 animals, the parasite load was also higher than the C57BL/6 model in the lymph node and ear, and once again the spleen did not show a parasite load. We evaluated some cell populations of B lymphocytes in the draining lymph node and in the ear. In he lymph node, after 38 days, animals BALB/c showed higher levels of B lymphocytes in the germinal center and B lymphocytes that produce IL-10 and express PD-L1 than animals C57BL/6. After 59 days, BALB/c animals showed lower levels of total B lymphocytes and B lymphocytes that express CD1d than C57BL/6 animals in the lymph node, but higher levels of B lymphocytes in the germinal center. In the ear, no differences were observed in the levels of these populations. We performed an ELISA and we observing BALB/c animals showed higher production of IgG1 and IgM than C57BL/6 animals, but showed a lower production of IgG2c. After the infection on C57BL/6 WT, IL-10 -/- and µMT animals, which are deficient in B lymphocytes, we observed that the B lymphocyte in C57BL/6 animals does not seem toparticipate in the development and not directly associated with the resolution of the lesionand parasite load, on the other hand, IL-10 seems to be important for the lesion control andparasite load Our data demonstrate that IgG1 production may be associated with pathogenesisin BALB/c, and IgG2c production may be associated with a better prognosis of the disease. **Keywords:** Leishmania amazonensis;B lymphocite ;Antibody.

IC - 091 - **The IL-17 axis and Neutrophil Elastase in the immunopathology of C57BL/6 mice infected with Leishmania amazonensis**

DE SOUSA, L.N.; DOS SANTOS, J.S.; GUEDES, H.L.D.M.. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a group of neglected diseases caused by protozoa of the genus *Leishmania*, such as *Leishmania amazonensis*, which is the etiological agent of diffuse cutaneous leishmaniasis in Brazil. Neutrophils play a significant role during *Leishmania* infection. This project aims to investigate the role of neutrophil elastase in C57BL/6 wild-type (WT), IL-17R and elastase knockout (KO) mice. The mice were subcutaneously injected in the right hind footpad with 2x10^6 *L. amazonensis* parasites of the MHOM/BR/75/Josefa strain. The progression of the lesion was measured weekly throughout the entire experiment using pachymetry. The parasite loads were determined using the Limiting Dilution Assay (LDA). The immune profile was observed using flow cytometry; however, the data is still being analyzed. The absence of elastase and IL-17R may be associated with an improvement in the lesion profile during the peak of infection, resulting in a smaller lesion size. However, during the chronic phase, both C57BL/6 wild type, IL-17R and elastase KO mice exhibited similar resolution of the infection. The parasite burden found in the footpad and draining lymph nodes of C57BL/6 WT, IL-17R and elastase KO mice was similar, this was observed during the peak of infection and chronic phase. Further experiments are necessary to understand how elastase and IL17 contribute to the promotion of the disease. **Keywords:** cutaneous leishmaniasis; elastase;IL-17R .

IC - 092 - **EVALUATION OF THE PROTECTIVE EFFECTS OF THE ADMINISTRATION INTRANASAL OF LACTOBACILLUS DELBRUECKII UFV H2B20 IN THE CONTROL OF ASPERGILLUS FUMIGATUS INFECTION**

DE ANDRADE, A.C.M.M.; SILVA, A.E.N.E.; FAGUNDES, D.D.S.; VIEIRA, L.Q.; SENA, I.R.R.. UFMG, UFMG BELO HORIZONTE - MG - BRASIL.

Aspergillus fumigatus (AFU) is a fungus present especially in environments containing decomposing organic matter. Conidia are normally dispersed in atmospheric air and are constantly inhaled by humans. However, immunosuppressed are susceptible to infection. These patients allow the adherence and growth of in the bronchi. Adhesion can lead to formation of aspergillomas, and to a chronic inflammatory and fibrotic process. Recent studies by our group show that the probiotic species ***Lactobacillus delbrueckii*** UFV–H2b20 (LAC), when administered orally, improved the immune response to pathologies such as asthma and AFU. In this work we hypothesized that intranasal administration of LAC protects against AFU infection. To test this hypothesis, BALB/c mice received 5x10^8 colony forming units (CFU) of LAC intranasally every 48 hours for 20 days. Control group received sterile saline. On day 21, animals were infected with 1x10^8 CFU of AFU. After infection, mice were followed for 7 days for weight loss and survival. We found that the group treated with LAC had better survival rate (52%) and reduced weight loss, indicative of less infection severity; and better weight recovery, indicative of better conditions to fight infection and return to homeostasis. In addition, 24 hours after infection we found a decrease in fungal loads in lungs in the LAC-treated group, showing higher efficiency to eliminate the pathogen. Despite fighting infection better, we found that treated animals had more controlled production of reactive oxygen species (ROS) we found fewer cells producing ROS and that treated mice had a more controlled production of ROS when compared to untreated animals. These results suggest that intranasal treatment with LAC has protective effects against AFU infection, helping to eliminate the fungus, while containing the collateral tissue damage by microbicidal activity. **Keywords:** Microbiota;Aspergiloma;Lactobacillus delbrueckii.

IC - 093 - **The use of adjuvants for oral LaAg vaccine against leishmaniasis.**

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Leishmaniasis is a complex of neglected diseases in the world. Due to its high annual incidence, the search for vaccine compounds is urgent, as there are currently no available anti-leishmaniasis vaccines for humans, and the therapeutic treatment is highly toxic. In 1970 came a non-prophylactic vaccine that came closest to a possibility for humans made from promastigotes total antigen lysates of L. amazonensis (PH8) – LaAg- providing partially protective immunity by increasing IFN-γ levels in mice and monkeys. Clinical trials in humans did not succeed, and therefore, production was discontinued. Vaccine studies conducted by our group not only aim to improve previous studies but also seek to incorporate new immunization routes that provide a stronger immune response and greater potential for mass vaccinations and patient adherence, with reduced economic impact. Thus, this study aims to evaluate the use of antacids as enhancers of an oral vaccine, while assessing adjuvants such as aluminum hydroxide. To achieve this goal, the animals received two doses of the vaccine orally, with an interval of seven days between them, subdivided into groups that received an association of LaAg with antacids such as sodium bicarbonate $NaHCO_3$, bismuth salicylate, $Al(OH)_3$ and magnesium hydroxide Mg(OH)2, or control. Seven days after the second dose, the challenge with L. amazonensis (Josefa) was performed intradermally in the right paw. Weekly, lesion sizes were mensurated and euthanasia (133dpi) was performed to assess the parasite load from the paw, draining lymph node, and the spleen. Preliminary observations allowed us to infer, the vaccinated group that received only orally administered LaAg showed partial protection compared to the control group, however, without protection in the chronic phase. Yet, the results of parasite load are ongoing. Furthermore, additional animals have been requested to perform a new repetition of the experiment. **Keywords:** vaccines;leishmania;leishmaniasis.

IC - 094 - **Pentoxifylline modulates the inflammatory profile of γδ T-cells from patients with Chagas cardiomyopathy**

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The response of gamma-delta (γδ) T-cells in the chronic phase of Chagas disease is unclear, despite their high ability in producing cytokines and exerting cytotoxic functions. By leveraging publicly available transcriptome data from heart tissue of patients with Chagas disease cardiomyopathy (CCC), the most severe consequence of *Trypanosoma cruzi* infection, we identified upregulated γδ gene expression. We aimed to investigate the functional characteristics of γδ T-cells in CCC, focusing on inflammatory and cytotoxic responses, and assess the potential of FDA-approved drug pentoxifylline to modulate their inflammatory profile. We demonstrate the presence of an activated CD8+ γδ T-cell subpopulation in the peripheral blood of CCC patients. Moreover, γδ T-cells from CCC patients exhibit a pro-inflammatory profile characterized by elevated TNF-alpha and TNFR expression, in contrast to cells from chronic indeterminate Chagas patients (IND). Additionally, we observed that *T. cruzi*-derived glycolipid-rich fractions strongly stimulate CD8+ γδ T-cells from CCC, leading to exacerbated IFN-gamma expression, compared to IND. Notably, CD8+ γδ T-cells from CCC express cytotoxic molecules that correlate with worse disease prognosis and display inflammatory cardiotropic chemotactic receptors, suggesting their potential recruitment to the heart. PCA and heatmap analysis using immune molecules expressed by γδ T-cells successfully segregated CCC and IND clinical forms. Myocardium gene expression analysis showed that TCRgd gene in CCC positively correlates with inflammatory cytokines and CD8 gene expression, confirming their migratory and mainly inflammatory profile. Finally, our findings demonstrate the ability of pentoxifylline to attenuate this inflammatory profile by mainly reducing TNF-alpha, TNFR and cytotoxic molecule expression in different γδ T-cell subsets, indicating the potential repurposing of this compound for controlling CCC and other inflammatory cardiomyopathies. **Keywords:** Gamma-delta;Chagas disease cardiomyopathy;Pentoxifylline.

IC - 095 - **Evaluation of endocrine and immune mediators in ex vivo human placental cells during Zika virus infection**

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Zika virus infection (ZIKV) in pregnancy is associated with a variety of birth defects. Functional changes in the maternal-fetal interface that contribute to the fetal pathogenesis are unclear. We investigated the hormone and cytokines production by primary cultures from amniochorionic membrane (chorion and amnion) and villous explant, in addition to the trophoblasts lineage (JEG-3). The endocrine and immune factors were measured in the supernatants of ZIKV-infected cultures using ELISA and CBA kits. Results showed that ZIKV infection led to a significant reduction in beta-hCG levels in villous (26405 pg/mL) and trophoblasts (19720 pg/mL) compared to the respective uninfected cultures (27565 pg/mL and 23492 pg/mL). Preliminary data from the evaluation of cytokines production revealed that only TNF-alpha and Interleukin 6 (IL-6) were produced and modulated in our ex vivo model. The levels of TNF-alpha were significantly increased only in ZIKV-infected villous (1.8 pg/mL) compared to uninfected cultures (0.7 pg/mL). Regarding the IL-6 results, although villous cultures produced high levels of cytokine (2378 pg/mL), ZIKV infection did not alter its production (2410 pg/mL), but led to significantly increased levels during the temporal kinetics of the infection in the trophoblast and chorionic cells. The IL-6 levels observed in the trophoblast cultures were 2.5 pg/mL at 24 h, 3.2 pg/mL at 48 h and 4.5 pg/mL at 72 h after infection, compared to 1.6 pg/mL in uninfected cultures. In the chorionic cultures, the values were 24 pg/mL for uninfected and 16 pg/mL, 29 pg/mL and 70 pg/mL for infected cultures at 24, 48 and 72h, respectively. Together, the results suggest the modulation of endocrine and immune mediators by ZIKV infection in human placental cells. References: Cell Host & Microbe, 20(2):155-66, 2016; J. Interferon Cytokine Res., 37(7): 287–294, 2017; J. Reprod. Immunol., 119: 62-66, 2017; Sci. Rep., 19(6):35296, 2016. **Keywords:** placenta;ZIKV;mediators.

IC - 096 - **ZIKV infection triggers OxPHOS and cytoskeleton remodeling in the brain of immunocompetent mice and boosts mitochondrial function and metabolism in human microglial cells.**

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Introduction: ZIKA virus (ZIKV) is a neurotropic arbovirus associated with the establishment of neuroinflammation in the Central Nervous System. Although ZIKV-induced cell death in neuronal cells is well known, the description of the metabolic alterations in response to ZIKV infection is under investigation. Thus, this project aimed to investigate the effects of ZIKV infection on brain proteins from immunocompetent mice along with cell activation parameters, mitochondrial function in microglial cells. **Methods:** C57/BL6 mice and human microglial cells were infected or not with ZIKV, separately. A proteomics analysis was performed in the brain of mice. In microglial cells, metabolic profile was assessed by GC-MS; cell viability was assessed by MTT; AXL expression, ROS generation, cell proliferation, and cell activation were analyzed by flow cytometry; Mitochondrial abundance and function were analyzed by confocal microscopy and high-resolution respirometry, respectively; Cell localization of NFkB and lipid droplets (LD) were analyzed by confocal microscopy. **Results:** Our data showed that ZIKV enhanced the abundance of proteins associated with oxidative phosphorylation and cytoskeleton remodeling in the brain of infected mice. Moreover, in microglia infection in vitro, ZIKV enhanced cell activation and proliferation, induced NFkB translocation towards the nucleus, increased LD biogenesis and mitochondria perinuclear localization. Interestingly, ZIKV increased mitochondrial capacity, even though it led to an augment of oxidative stress at later stages of infection. Finally, ZIKV infection alters the metabolic profile, leading to an accumulation of citric acid cycle metabolites such as succinate, malate, fumarate, citrate. Conversely, it resulted in a decrease in amino metabolites, especially glutamine. **Conclusion:** Our data highlights the complex interaction between ZIKV and cellular metabolism, providing insights behind metabolic shift in response to viral infections. **Keywords:** ZIKV;IMMUNOMETABOLISM;MICROGLIA.

IC - 097 - **Behavior of serum adipokines in healthy aging.**

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Healthy aging, accompanied by successful remodeling of the organism and immune system, represents a paradigm for modern societies. Understanding the functions that deteriorate and those that can compensate for them, as well as identifying the biological markers (immune, genetic, and epigenetic) that are determinants of healthy aging, can enable interventions to promote it. Therefore, in this study, we aimed to analyze immune factors related to adipose tissue (adiponectin, resistin, and leptin) that may be associated with "healthy aging."

This was a cross-sectional study involving 54 healthy individuals of both sexes, divided into three groups: 19-60 years (n= 18), 60-75 years (n= 19), and >75 years (n= 17). The concentrations of adiponectin, leptin, and resistin in serum were determined using the ELISA. The findings indicated a significant decline in serum leptin levels in individuals over 75 years of age, irrespective of nutritional status, which was not observed for resistin and adiponectin. Further research is required to elucidate the influence of adipose tissue on the homeostatic functions of the immune system involved in aging. **Keywords:** healthy aging;elderly;adipokines.

IC - 098 - **MICROBIOTA-DEPENDENT MACROPHAGE RESPONSE AGAINST TRYPANOSOMA CRUZI IN VITRO**

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The microbiota affects physiological functions of its host organism by establishing mutualistic relationships with mammals. It varies according to environmental conditions, antibiotic use, host diet and immunometabolic dysfunctions. The beneficial potential of the microbiota demands a homeostatic balance within the microbial communities, in addition to the balance between these microorganisms and the intestinal interface of the host. In a healthy host, the microbiota can help to prevent the installation of pathogens, and changes in its composition generally have the potential to influence parasitic infections, such as Chagas' disease. In-depth studies on the interaction of *T. cruzi* with the microbiome of hosts could favor the development of prevention and effective treatment against this infection. Macrophages are one of the host cells of *T. cruzi* but, when activated by inflammatory cytokines, can kill the parasite. We studied the role of the microbiota in the function and activation of macrophages to evaluate if the modulating effect induced by the microbiota occurs permanently or transiently in myeloid cells, and how these cells behave during an in vitro infection by *T. cruzi*. Conventional and mice with antibiotic-depleted microbiota, mimicking the "germ-free" condition, were studied. Macrophages were derived from the bone marrows. Microbiota-depleted-derived macrophages showed lower phagocytic capacity and increased production of IL-10, and reduced production of TNF, indicating that the depletion of the microbiota promotes a reduction in inflammatory signaling. Analysis by flow revealed that macrophages derived from antibiotic-treated mice have a greater expression of CD206, confirming that the depletion of the microbiota induces an M2 polarization. We conclude that the microbiota induces, in an unknown way, an inflammatory profile in macrophage precursors and can influence the control of *T. cruzi* infection. **Keywords:** Microbiota;Macrophages;Trypanosoma cruzi.

IC - 099 - **ANTITUMORAL POTENTIAL OF TWO RESVERATROL ANALOGUES, AR26 AND AR33, ON MELANOMA CELLS**

ALMEIDA, A.P.G.; MACHADO, L.F.; PENIDO, B.E.R.; BOLOTARI, M.; GUEDES, M.C.M.R.; DOS REIS, L.B.; DE PAULA, W.T.; DA SILVA, A.D.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, UNIVERSIDADE FEDERAL DE JUIZ DE FORA JUIZ DE FORA - MG - BRASIL.

Introduction: Melanoma is an aggressive malignant neoplasm with high metastatic potential and mortality rates. Although existing, therapeutic strategies for melanoma are limited, which implies a need for new treatment approaches. In this context, resveratrol (RVT), a phytoalexin abundant in grapes and red wine, has shown promise. Regarding melanoma, RVT is capable to reduce tumor size and modulate pathways related to survival, proliferation, and resistance. However, resveratrol's use *in vivo* is limited due to its low bioavailability. Thus, this study aims to evaluate the antitumoral potential of two synthetic resveratrol analogs, AR26 and AR33, in melanoma treatment. **Methods and Results:** Firstly, the analogues cytotoxicity was evaluated *in vitro* on J774A.1 cell line (murine macrophages), using the MTT method. Subsequently, the lethal concentration 50 (LC50) of analogues was determined. Finally, the antitumor potential of the analogues was evaluated on the metastatic murine melanoma cell line (B16F10) by the MTT assay. The results showed that compound AR26 exhibited acceptable cytotoxicity (viability higher than 70% - ISO 10993-5:2009) up to a concentration of 250µM. Differently, RVT and AR33 were more toxic, presenting acceptable cytotoxicity only up to 50µM. Consistently, the LC50 presented by AR26 analogue (279.6µM) was significantly higher than that showed by AR33 (84.13µM) and RVT (157.7µM). Regarding antitumor activity, at the highest non-toxic concentrations, compound AR26 was capable of reducing B16F10 cell viability by 76.8%±3.34 (250µM), while AR33 reduced viability by 60±3.8% (50µM), and RVT by 56.4±2.9% (50µM). **Conclusion:** The results suggest that, at non-toxic concentrations, compound AR26 exhibits significant antitumor activity, demonstrating a potential to compose new therapeutic strategies against melanoma. Further studies are being conducted to confirm this antitumor activity. Financial support: FAPEMIG, CAPES and CNPq. **Keywords:** MELANOMA ;RESVERATROL;ANTITUMORAL POTENTIAL .

IC - 100 - **Gene expression signature related to low-density neutrophils in viral and bacterial, acute and chronic infections.**

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The LDNs (Low Density Neutrophils) are a neutrophil subpopulation with fewer nuclear lobules, larger intracellular vacuoles and smaller enzyme granules. They may have increased functions such as the production of NETs, phagocytic and ROS generation. On the other hand, they are associated with the suppression of T cell function and proliferation. Here, we looked for molecular signature related to LDNs in PBMC microarray datasets from acute and chronic diseases. Microarray datasets were collected from the Gene Expression Omnibus database, with the criteria of human PBMC, infection and presence of healthy controls. Thirteen datasets were imported into R (version 4.0.2) and analyzed with the limma, affy, tydiverse, dplyr and MetaVolcanoR packages. Through the vote-counting function (ndeg ≥5) of MetaVolcanoR, we obtained a list of 2798 genes that were imported into the Cytoscape program and performed a "multiscale community analysis" with the "STRING" and "CyCommunityDetection" extensions. A "specific granule lumen" module with 120 genes was obtained, which were imported into R and merged with the 13 sets, using their p-values and LogFC. Heatmaps were generated for the sets with acute (N = 8) and chronic (N = 4) infections using the pheatmap package in R. After a new filter, only genes with a maximum of 30% of LogFC values absent in the sets remained. 35 genes were obtained for the acute sets and 39 for the chronic sets. In the acute sets, genes with increased expression predominate, such as RNASE2 and RNASE3 (antibacterial activity), ELANE and MMP8 (inflammatory response), LTF (regulation of cell growth), MPO (granulopoiesis) and S100A9/12 (alarmins). Among the chronic ones, those with reduced expression predominate, such as CXCL8 (neutrophil attraction and activation), CCL2 (immune cell recruitment) and PTX3 (immune response modulation). In conclusion, LDN signature was identified in several infectious diseases, with different profiles in acute and chronic infections. **Keywords:** LDN;Neutrophils;Infections.

IC - 101 - **Protein undernutrition alters location and subpopulations of T cells in the thymus of BALB/c mice infected with Leishmania infantum**

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Our group previously showed that undernourished mice infected with Leishmania infantum suffer drastic thymic atrophy, but the thymocytes migratory capacity against chemotactic stimuli ex vivo is preserved. Besides, while well-nourished infected mice (CPi) show an increase in the thymic cortical zone, malnourished-infected mice (LPi) exhibited a significant reduction in the cortex:medulla ratio, followed by increased levels of extracellular matrix proteins in the cortical region. Such results suggested that (i) T cells could be accumulating in the medullar region of LPi mice, and/or (ii) immature T cells are migrating precociously from cortex to medulla in these animals. To evaluate this, we analyzed thymic T cells subpopulations in undernourished mice infected with L. infantum, verified the presence of double-positive (DP) T cells in the medullary thymic region and peripheral blood (PB), and analyzed the levels of molecules involved in mature T cells attraction from cortex to medulla, or to periphery in those animals. We observed a general reduction of DP cells in undernourished mice LP and LPi, while these cells were increased in the thymic medulla and in the PB. Furthermore, there was significant reduction of CCL21 levels in LP and LPi mice. Increased S1P1 levels were observed in the cortical region of LP and LPi mice while its ligand S1P was reduced in peripheral blood of infected animals CPi and LPi. qPCR assays revealed a significant increase in the thymic parasite load in LPi mice compared to CPi. Moreover, effector T cells (CD44+CD62l-) are increased in the thymus of CPi mice but not in LPi mice. Together, these results suggest that undernutrition induces a dysfunctional thymic microenvironment where the differentation and migration of thymocytes is compromised. Such defects may negatively impact the exit of mature T cells to the periphery and the control of parasite load in the spleen, aggravating/accelerating the immunopathological events in infected mice. **Keywords:** Protein undernutrition;Thymus;Visceral Leishmaniasis.

IC - 102 - **Listeria monocytogenes induces early biogenesis of LDs in murine macrophages dependent on lipid remodeling.**

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Listeria monocytogenes is a gram-positive pathogenic bacterium and the etiological agent of listeriosis. Although listeriosis usually results in mild gastroenteritis, severe L. monocytogenes infection can result in complications such as septicemia, meningitis, endocarditis, or spontaneous abortion, especially in at-risk populations (immunocompromised, elderly, and pregnant women). During invasion, remodeling of lipids and cellular structures are key factors in the success of intracellular L. monocytogenes infection. Lipid droplets are complex and dynamic organelles involved in energy and lipid homeostasis of eukaryotic and prokaryotic cells. Numerous studies over the last few decades discuss which pathogens target host LDs to increase both immune evasion and microbial proliferation. Our objective in this work was to analyze the dynamics of accumulation of LDs and their involvement in the pathogenicity of L. monocytogenes in macrophages. For this, bone marrow-derived macrophages were infected in vitro with L. monocytogenes (MOI 10) for 1 h. The biogenesis of LDs was evaluated 1h, 6h, 24h and 48h after infection. Our results show that after 1h of infection by L. monocytogenes induced LD biogenesis in both types of macrophages, this elevation remained at all times analyzed. In addition, by microscopy, we observed that there was also an increase in the bacterial load in this period. Furthermore, using inhibitors of important metabolic pathways linked to lipid remodeling that could lead to the induction of LDs in macrophages by Listeria sp. We observed that L. monocytogenes-induced LD biogenesis is an event dependent on the activity of DGAT-1, DGAT-2 and cytosolic phospholipase A2. To better elucidate the nature of this relationship and the contribution of LDs to the intracellular proliferation of L. monocytogenes, further experiments will be carried out. **Keywords:** Lipid droplets ;Listeria monocytogenes ;Lipid remodeling.

IC - 103 - **Ras guanine exchange factor RasGEF1b mediates the steady-state and signal-dependent transcriptional activation of the SerpinB2 gene in macrophages**

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SerpinB2, also known as plasminogen activator inhibitor type 2 (PAI-2), regulates innate immunity, macrophage survival, and plasminogen activator activity. Its expression is induced in macrophages upon exposure to inflammatory triggers like lipopolysaccharide (LPS) and mitogenic triggers like Phorbol-12-myristate 13-acetate (PMA). However, the transcriptional regulation mechanism of SerpinB2 remains unclear. In our recent global RNA sequencing (RNA-seq) study, we observed that SerpinB2 expression is significantly reduced in LPS-stimulated bone marrow-derived macrophages (BMDMs) when the guanine nucleotide exchange factor RasGEF1b is absent. The cellular function of RasGEF1b is also unknown. This prompted us to investigate the transcriptional activation of SerpinB2 and identify the promoter regions regulated by RasGEF1b under resting, LPS, or PMA-induced conditions. To achieve this, we transfected cells with a RasGEF1b expression plasmid and luciferase-based reporter constructs containing the 5'-flanking region of the murine SerpinB2 gene, including mutants. Additionally, we evaluated SerpinB2 transcriptional activity in RasGEF1b-silenced RAW264.7 macrophages before and after LPS or PMA stimulation. Our findings reveal that RasGEF1b alone induces SerpinB2 transcriptional activity and enhances it in response to PMA. RasGEF1b knockdown impairs both basal and PMA or LPS-induced transcriptional activation of SerpinB2. Mutation analyses indicate that a CCAAT enhancer binding (C/EBP) element and an activator protein 1 (AP-1) response element in the SerpinB2 proximal promoter are essential for optimal RasGEF1b-mediated basal transcriptional activation. These results provide new insights into the regulation of SerpinB2 expression and highlight the important role of RasGEF1b in maintaining constitutive and signal-induced gene expression in macrophages. **Keywords:** macrophages;SerpinB2;lipopolysaccharide.

IC - 104 - **THE ROLE OF OMEGA-3 DHA IN WHITE AND BROWN ADIPOSE TISSUE MODULATION AND ITS FUNCTION ON THE INFLUENCE OF THE CARCINOGENIC PARAMETERS OF MELANOMA CELLS**

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Introduction: Long-chain polyunsaturated fatty acids of the n-3 series (n-3 PUFAs), such as docosahexaenoic acid (DHA), have protective mechanisms against the development of inflammatory diseases, among them obesity and cancer. DHA was described as an inductor of pyroptosis cell death in certain types of tumor cells. However, the role of n-3 PUFAs in the communication between melanoma and adipose tissues (AT) is unclear. This study aimed to investigate the role of n-3 PUFAs in modulating AT and its function on the carcinogenic parameters of melanoma, particularly in the induction of pyroptosis. Methods: C57/BL6 mice were supplemented or not with omega-3 at a concentration of 1g/kg. Serum, peritoneal lavage, AT, liver, and spleen were analyzed. Additionally, the AT-conditioned medium (CM) obtained from those animals was used to stimulate the B16F10 melanoma cell line in vitro. After stimulation, cell viability and death, and cytokine quantification were assessed. Additionally, the human melanoma cell line MeWo was stimulated with DHA for 48 hours in vitro. Secretion of lactate dehydrogenase (LDH), membrane pore formation, and caspase-1 activation were analyzed. Results: Our data demonstrated that supplementation with omega-3 reduced AT weight and led to an increase in lipid droplet biogenesis of peritoneal cells, in addition to a reduction of reactive oxygen species (ROS). Furthermore, stimulation of B16F10 cells with brown adipose tissue (BAT)-CM from omega-3 DHA-supplemented mice resulted in decreased cell viability and increased cell death. Moreover, the treatment with DHA induced LDH release, increased membrane pore formation, and triggered caspase-1 activation. Conclusion: This study demonstrated the potential of omega-3 supplementation in modulating AT, as well as suggesting the ability of DHA to induce pyroptosis in melanoma cells in vitro. Thus, it provides new perspectives for the use of omega-3 DHA as an adjuvant in the treatment of melanoma. **Keywords:** Cancer;omega-3;adipose tissues.

IC - 105 - **In vitro effect of Monomethyl and Dimethyl fumarate on dendritic cells**

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Introduction: Dimethyl fumarate (DMF; Tecfidera, Biogen) is an oral medication utilized to treat Relapsing-Remitting Multiple Sclerosis (MS), being associated with reduced disease activity in MS patients. DMF is converted into its active form, the monomethyl fumarate (MMF), by dendritic cells (DCs) of the gut. However, the mechanisms of action of DMF are not fully understood. Objective: To analyze the effect of DMF and MMF on the in vitro generation of dendritic cells. Methods: DCs were generated from bone marrow precursors extracted from femurs and tibias of C57BL/6J mice. Red blood cells were lysed and cells were cultured for 12 days in RPMI medium with 10% fetal bovine serum, 10ng/ml GM-CSF, 1% antibiotic and 1% L-glutamine. From day 2 of culture, part of the cells received DMF at a concentration of 70uM, another group received MMF at a concentration of 50uM, and another group did not receive any treatment. The medium was changed every 2 days or sooner if necessary. After 12 days, the immature DCs were be induced to maturation with LPS (5ng/ml, for 24h). Cells were then destinated to flow cytometry and qPCR analysis. Results. Both forms do not alter the proportion between CD11b+ and CD11c+ DC's. Additionally, they do not modify the expression of auxiliary factors CD80, CD86 and MHC II in CD11c+ and CD11b+CD11c+ DCs. However, it was observed that DMF and MMF increase the expression of CD80, CD86 and MHC II in CD11b+ DCs. Cytokine expression is modulated by the treatment with MMF. Conclusions. Although preliminary, our results suggest that MMF acts, at least in part, inducing a tolerogenic profile characterized by the expression of CD11b in DCs, to the detriment of the immunogenic profile, characterized by the expression of CD11c. Thus, more Treg cells would be generated, which would attenuate MS in mices. **Keywords:** CELLULAR IMMUNOLOGY;BASIC IMMUNOLOGY;MULTIPLE SCLEROSIS.

IC - 106 - **Sialylated and asialylated forms of Fetuin-A interfere with in vitro SARS-CoV-2 infection**

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COVID-19 is a highly impactful global infectious disease caused by SARS-CoV-2. The molecular mechanisms involved in this infection are not fully elucidated. Glycans containing sialic acid (N-acetylneuraminic acid, Neu5Ac) may participate in the virus–host cell interaction process (*Biomolecules.* 11(6):831, 2021). Interestingly, the N-terminal domain of the spike protein of SARS-CoV-2 can bind to Neu5Ac, and a sialylated protein (Fetuin-A) is considered a severity biomarker in COVID-19 (F1000Res. 9:1078, 2020; Clin Transl Med. 12(1):704, 2022). In this study, we explored the potential impact of spike protein's recognition of sialic acid on experimental SARS-CoV-2 infection. Through bioinformatics analysis and molecular simulation, we demonstrated the existence of evolutionarily conserved sites for interaction with Neu5Ac in the Receptor Binding Domain (RDB) of the spike protein from various types of beta coronaviruses. The identified sites were found suitable for binding glycans containing sialic acid based on molecular docking. Experimental validation of the in silico findings was performed by infecting H460 cells (human lung carcinoma cells) with the Wuhan strain of SARS-CoV-2 (multiplicity of infection equal to 1), in the presence or absence of sialylated or asialylated fetuin-A (1 and 10 µg/mL). The cellular viral load was determined by real-time PCR. Sialylated fetuin-A (1 µg/mL) and asialylated fetuin-A (10 µg/mL) promoted an approximate tenfold increase in viral load compared to the control. These results suggest that the glycoprotein fetuin-A potentiates in vitro infection by SARS-CoV-2, and sialic acid plays a relevant role in this biological event. This study provides perspectives for a better understanding of the molecular mechanisms involved in the infection process of host cells by SARS-CoV-2. **Keywords:** COVID-19;Fetuin-A;Sialic acid.

IC - 107 - **High mobility group box-1 assists on renal recovery stem cell-based therapy after acute kidney injury**

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Factors that initiate cellular damage and trigger the inflammatory response cascade and renal injury are not completely understood after renal ischemia-reperfusion injury (IRI). High obility group box-1 protein (HMGB-1) is a damage-associated molecular pattern molecule that binds to chromatin, but upon signaling undergoes nuclear cytoplasmic translocation and release from cells. We investigated the role of HMGB-1 by daily injection of glycyrrhizinic acid (GL) in a rat model of bilateral renal ischemia-reperfusion (IR) injury during bone marrow-derived mesenchymal stem cell (BM-MSC) therapy. GL administration was able to modulate transcription of inflammatory mediators: TLR-2, TRL-4, RAGE and IL-18 after 3 and 7 days after IR. The administration of BM-MSCs was able to promote a significant improvement in functional and structural parameters of renal tissue as well as decreased expression of IR injury-related inflammatory mediators such as IL-18, MCP-1 and, IL-17. Increased mediators related to cell regeneration and anti-inflammatory action, such as IL-12, INF-γ and IL-10 were also observed. In addition, an increase in cell proliferation was promoted. Thus, our results suggest an important role of HMGB-1 on immunomodulation and on repair mechanisms promoted by BM-MSCs in the model of acute renal injury induced by IR. **Keywords:** Cell therapy;HMGB-1;Acute kidney injury.

IC - 108 - **ANTITUMORAL ACTIVITY OF THE RESVERATROL ANALOGS, TIN46 AND TIN50, IN MURINE AND HUMAN MELANOMA CELLS**

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Introduction: Melanoma is a malignant neoplasm originating from melanocytes, with high metastasis and mortality rates. Therapeutic interventions involve surgical removal, chemotherapies, radiation therapies, and immunotherapies. However, in the advanced stages of the disease, these interventions have reduced efficiency, encouraging the search for new melanoma treatment strategies. In this context, resveratrol (RVT), a phytoalexin found in grapes and red wine, has shown promise, presenting excellent antitumor activities. Despite these, RVT use is limited due to its low bioavailability. However, the resveratrol molecule is a prototype for the synthesis of analog compounds capable of overcoming this issue. Thus, the present study aimed to evaluate the antitumor activity of the analogs TIN46 and TIN50 on murine and human melanoma skin cancer**. Methods and Results**: First, the cytotoxicity of the compounds was determined on RAW264.7 (murine macrophage). After that, the antitumor potential of the analogs was evaluated in metastatic cell lines of murine melanoma (B16F10) and human melanoma (WM1366) by MTT. The results showed that both compounds present acceptable cytotoxicity at concentrations up to 50µM (viability greater than 70% - as recommended by ISO10993-5:2009). Regarding antitumor activity, when used in non-toxic concentrations, both TIN46 and TIN50 were able to reduce the viability of murine melanoma cells (B16F10) by 47,4%± 4,85 (50uM) and 13,12%± 3,89 (50uM) respectively. However, in the human melanoma cell line (WM1366), only TIN46 was effective, reducing the cell viability by 82,4%± 1,5 (50uM). **Conclusion:** The results showed that the TIN46 analog has an important cytotoxic activity against the evaluated melanoma cell lines, demonstrating a potential to compose new therapeutic strategies against melanoma. More studies are being conducted to confirm and elucidate its antitumor activity. **Keywords:** resveratrol analogs;antitumoral activity;melanoma.

IC - 109 - **CHARACTERIZATION OF THROMBOINFLAMMATION DURING CHIKUNGUNYA INFECTION**

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Platelets play a crucial role in hemostasis. However, they can also act as a link between the inflammatory and thrombotic responses, playing a key role in a commonly observed phenomenon in many viral infections. Thromboinflammation is characterized by dysregulation of the coagulation cascade, intense platelet activation, and increased recruitment of leukocytes in microvasculature regions. The specific role of platelets during Chikungunya virus (CHIKV) infection is not well understood. Typically, acute CHIKV symptoms resolve within approximately 15 days without medical intervention. However, some individuals develop a chronic condition. These persistent symptoms, primarily musculoskeletal and often resembling autoimmune inflammatory arthritis. Given the evidence of platelet activation associated with CHIKV infection, our objective was to evaluate the role of platelets and thromboinflammation in this particular context. Plasma was extracted from both healthy donors and infected patients. ELISA assays were conducted to analyze inflammatory mediators and clotting factors. Our analysis revealed distinct release patterns of cytokines associated with the inflammatory response between the two groups. Additionally, factors associated with platelet activation processes, were found to be significantly elevated. We also observed that components related to endothelial activation were expressed at high levels. However, when comparing these same mediators between chronic and non-chronic patients, no notable differences were observed. The findings indicate elevated levels of inflammatory cytokines, platelet activation mediators, and endothelial activation mediators. However, it is important to note that these results alone cannot be considered as definitive predictors of chronicity. The existing body of evidence suggests that viral infections have the ability to influence essential elements of hemostasis, potentially triggering thromboinflammation in affected individuals. **Keywords:** platelets;chikugunya fever;thromboinflammation.

IC - 110 - **Characterization of the murine Schlafen-4 gene promoter and its transcriptional activation by Interferons (IFNs)**

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The gene encoding the myeloid differentiation factor Slfn4, also known as Schlafen-4, has its expression induced in macrophages by inflammatory agonists of Toll-like receptors, interferons (IFNs), and in collagen-induced arthritis. Besides, Slfn4 mRNA is down-regulated during macrophage differentiation, thus suggesting a role in myelopoiesis. Therefore, the regulation of its expression at the transcriptional level might represent a further key element for pathogenesis implicating myeloproliferative diseases. However, the transcriptional mechanisms that govern the expression of Slfn4 are still unknown. In this work, we aimed to identify and functionally characterize the murine Slfn4 putative promoter activity. Using Chip-seq publicly available database for epigenetic markers such as H3K4me1, H3K4me2, and H3K27a, we identified an active chromatin region of 1,764 nucleotides in BMDMs. By using rVISTA program, the sequence was aligned with the rat promoter sequence and identified binding sites for activator protein 1 (AP-1), and interferon-responsive transcription factors including STATs and IRFs. Besides, microsatellites were also found, located from -937 to -546 from the transcription start site (TSS). The DNA sequence comprising -1670 thru +94 nucleotides of the gene (NG_023386.1 Reference Sequence Gene) was used for the construction of luciferase reporter pGL3-basic plasmid. HEK293 cells were transfected with pGL3-basic or pGL-Slfn4P construct and co-transfected with pRL-TK plasmid for normalization of the results. Transfected cells were also treated with IFN-A1 (1,000U/mL) for 18 hours, and the cell lysates were harvested for measurement of the luciferase activity. The reporter assay showed that the DNA sequence of the Slfn4 gene presented promoter activity below the activity of pGL3-basic plasmid, but increased in response to IFN-A1 treatment, indicating that the putative DNA sequence of Slfn4 is transcriptionally activated under inflammatory conditions. **Keywords:** macrophages;schlafen-4;transcriptional activity.

IC - 111 - **Antioxidant effect of N-acetylcysteine promotes lung tissue repair in CS-induced emphysema in mice.**

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Introduction: Cigarette smoke (CS) is the major cause of Chronic Obstructive Pulmonary Disease (COPD). It is able to cause lung damage including an important change in lung parenchyma. NAC (N-Acetylcysteine) is an amino acid that is able to reduce mucus secretions, oxidative damage and inflammation. However the effect of NAC in lung tissue reaper is not clarified. Methods: CEUA (16/2020). Male mice (C57BL/6, ±23 g) were divided into three groups (n= 7/group): CONTROL, cigarette smoke (CS) and CS + N-Acetylcysteine (CS+NAC). The CS and CS+NAC groups were exposed to the smoke of 12 cigarettes/day for 60 days. Sham group was exposed to ambient air for 60 days. The Sham and CS group received treatment with saline by inhalation (15 min/day). The CS+NAC was treated with NAC 600 mg/mL. The treatment started after damage stabilized (day 61). Mice were sacrificed 24 hours after the last day of treatment (day 120) and left lung was collected for histological processing for morphometric analyses through mean linear intercept (Lm). Right lung was collected to analyze: protein concentration, malondialdehyde (MDA), reactive oxygen species (ROS), superoxide dismutase activity (SOD) and catalase (CAT) activity. The statistical difference was considered to be $p < 0,05$. Results: The CS group showed lung injury and higher Lm (41,1 ± 1,0) when compared to the control group (25,6 ± 0,5) ($p<0,05$). On the other hand, the CS+NAC group showed a decrease in Lm compared to the CS group (28,9 ± 0,5) ($p<0,05$). Redox marker (ROS, MDA, SOD and CAT) showed an increase when compared to the control group ($p<0,05$). CS+NAC group showed reduction of MDA (211,6 ± 9,7; $p<0,0001$), ROS (15,6 ± 1,4; $p<0,05$), SOD (54,5 ± 4,3 $p<0,01$) and CAT (47,9 ± 3,4; $p<0,0001$) when compared to the CS group. Conclusion: N-acetylcysteine is able to promote lung repair in CS-induced emphysema and it is involved with the oxidative balance. **Keywords:** EMPHYSEMA;ANTIOXIDANT;LUNG.

IC - 112 - **Hypercorticoidism in BALB/c and C57Bl/6 mice infected with Leishmania infantum is related to up-regulation of ACTH receptor in the adrenal glands and high circulating levels of IL-6**

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Previously, we showed that *Leishmania infantum* increased cortisol levels in hamsters, a suitable experimental model for visceral leishmaniasis (VL), and that cortisol levels are closely associated with haematological and immunological parameters indicative of VL severity. Nevertheless, tools to investigate the immunological mechanism related to hypercortisolism in hamsters are limited. In this work, we investigated the putative influence of *L. infantum* infection on glucocorticoid production in mice. Mice were infected intraperitoneally with 2×10^7 *L. infantum* promastigotes, and analyses were performed 5, 30, and 120 days postinfection (dpi). *L. infantum*-infected C57Bl/6 mice presented increased spleen and liver parasite burden at 30 dpi but only liver presented a decrease at 120 dpi. Compared to noninfected mice, *L. infantum*-infected BALB/c mice presented increased plasma corticosterone levels up to 30 dpi. This increase paralleled a rise in the spleen parasite burden and adrenocorticotropic hormone (ACTH) receptor (MC2R) expression in the adrenals. Despite the elevated parasite burden at 120 dpi, a reduction in corticosterone levels along with a decrease in MC2R expression were observed. To test the hypothesis of adrenal exhaustion, mice were treated with ACTH in vivo. Corticosterone levels significantly increased in noninfected and infected mice at both 30 and 120 dpi, demonstrating that the functionality of the adrenal was preserved in *L. infantum*-infected mice. Indeed, similar to that observed for corticosterone, IL-6 production exhibited a bell-shaped profile with an increase at 30 dpi but a decrease at 120 dpi. MC2R expression as well as circulating IL-6 levels mimicked the plasma corticosterone pattern at both 30 dpi and 120 dpi. We plan to use knockout mice in future studies. Together, our data suggested that the induction of adrenal MC2R expression and IL-6 levels play roles in regulating hypercortisolism during the course of *L. infantum* infection. **Keywords:** Visceral Leishmaniasis;ACTH;IL-6.

IC - 113 - **NK cells for immunotherapy: phenotypic and functional characterization of CIML-NK cells and genetic engineering using PiggyBac Transposon System**

DA SILVA, L.H.R.; LIMA, M.P.O.; DE OLIVEIRA, T.G.M.; RAMOS, R.N.; ROCHA, V.G.; ALMEIDA, R.R.; COUTO, S.C.F.. LABORATÓRIO DE INVESTIGAÇÃO MÉDICA EM PATOGÊNESE E TERAPIA DIRIGIDA EM ONCO-IMUNO-HEMATOLOGIA (LIM-31), DEPARTAMENTO DE HEMATOLOGIA E TERAPIA CELULAR, HC - FMUSP, SÃO PAULO - SP - BRASIL.

Immunotherapy using chimeric antigen receptor-bearing NK cells (CAR-NK) has become prominent due to its safety, efficacy, and potential for allogeneic application. Non-viral genetic editing strategies for the CAR insertion was recently explored, with potential to overcome several challenges related to viral methods. This study aimed to investigate the feasibility of using the PiggyBac transposon system for integrative gene editing in cytokine-induced memory-like NK cells (CIML-NK). The experimental approach involved differentiating peripheral blood-derived $CD56^{+}CD3^{-}$ NK cells into CIML-NK cells (16h pre-activation with IL-12/15/18), followed by *ex vivo* expansion with IL-15 and feeder cells. Phenotypic and functional evaluations were conducted using flow cytometry. The PiggyBac transposon system's feasibility was evaluated by nucleofecting test plasmids accessing reporter gene expression (eGFP) and cell viability (7-AAD). CIML-NK cells were nucleofected with PBCAG-eGFP and pCAG-PBase plasmids; electroporated plasmid-lacking cells served as the control (Mock). Pre-activation of NK cells with IL-12/15/18 led to a high proliferative potential and culture conditions resulted in enrichment of NK cell purity (>97%). Phenotypic analysis revealed increased expression of NKp46 and CD69 activation markers and the NKG2A receptor in CIML-NK cells compared to non-activated NK cells. Functionally, CIML-NK cells demonstrated enhanced IFN-γ production and degranulation and performed cytotoxicity against $K562^{wt}$ cells. EGFP plasmid-expression was successful in a preliminary microscopy analysis, yet a reduced cell viability was noted. In summary, CIML-NK cells exhibited a high proliferative and functional capacity, highlighting their imunotherapeutic potential. The validation of reporter gene expression using the PiggyBac system was accomplished. However, further nucleofection optimization is necessary when aiming to improve viability and genetically modify NK cells for CAR expression. **Keywords:** Immunotherapy;NK Cells;PiggyBac.

IC - 114 - **EVALUATION OF LIPID PROFILE OF SYMPTOMATIC PATIENTS OF POST- ACUTE COVID-19 SYNDROME (PACS)**

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The post-acute COVID-19 syndrome (PACS) is characterized by the persistence of patients' clinical symptoms who are affected by acute COVID-19. This syndrome can have long-term effects on various organ systems, including the heart, lungs, kidneys, skin and brain, that contribute to the symptoms presented by patients. However, these symptoms are not pathognomonic, and it is necessary to identify new altered laboratory parameters in these patients to make a more accurate diagnosis and a more assertive course of treatment. Previous work by our group showed that lipid metabolism was altered in severe COVID-19 patients compared with healthy volunteers, and it is suggested that lipid reprogramming can be associated with the worst prognosis of the disease. In this context, we investigated the lipid profile of symptomatic patients with PACS. We prospectively included 22 moderate to severe COVID-19 survivors up to 6 months after hospital discharge and 19 healthy volunteers. Plasma was evaluated for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TGs). Low-density lipoprotein cholesterol (LDL-c) was calculated based on Friedewald's equation (LDL-c = TC - HDL-c - TG/5), and non-HDL cholesterol (non- HDL-c) was calculated by subtracting HDL-c from TC. We observed that TC, TGs and non- HDL-c did not significantly change their concentrations compared to healthy volunteers. However, HDL-c was lower in COVID-19 survivors than in healthy volunteers. Furthermore, the ratio of non-HDL-c/HDL-c suggests a predisposition for COVID-19 survivors to develop cardiovascular diseases. Our results indicate that lipid metabolism in PACS continues to be altered with low blood HDL concentrations. In addition, the ratio of non-HDL-c/HDL-c indicates that COVID-19 survivors may have an increased risk of developing cardiovascular diseases. Therefore, HDL-c can be an important parameter in the PACS prognosis, and its role in that syndrome needs to be better elucidated. **Keywords:** COVID-19;lipid metabolism;PACS.

IC - 115 - **THE ROLE OF NLRP3 INFLAMMASOME ACTIVATION-DEPENDENT PYROPTOSIS ON THE MODULATION OF CARCINOGENIC PARAMETERS OF HUMAN GASTRIC CANCER CELLS**

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Introduction: Pyroptosis is a type of programmed lytic cell death that was first discovered in immune cells, but it has garnered significant attention within the field of oncology. In tumor cells, cell death pathways are inhibited as an evasion mechanism. However, when pyroptosis is induced in cancer cells, dual phenomena can be observed in tumor progression. Thus, the aim of this work is to elucidate the role of NLRP3 inflammasome activation-dependent pyroptosis in human gastric cancer cells. **Methods:** AGS cells were stimulated with lipopolysaccharide (LPS) (1ug/mL) for 24h and nigericin for 2h and replaced with culture medium (standard stimulus (SS)), or continuously stimulated with LPS and nigericin (CS), according to the period of the analysis. The mitochondrial viability was assessed by MTT assay. The enzyme lactate dehydrogenase (LDH) release was evaluated by the CyQUANT™ kit. The cell death profile was assessed by annexin-V/propidium iodide (PI). The cell cycle, DNA fragmentation, and membrane pore formation were assessed by PI staining. Cell proliferation was assessed by CFSE staining. Cytokine levels were evaluated by ELISA. **Results:** It was observed that both SS and CS reduced cell viability in AGS cells, in addition to inducing an increase in lytic cell death, whose effect was most prominent in CS. Moreover, both stimuli reduced the AGS cell proliferation, which was intensified in SS. Both stimuli induced pore formation, but it was more notable in CS, which also showed augmented LDH release in AGS cells. **Conclusion**: Taken together, our data demonstrated that the induction of pyroptosis had an antitumor effect on carcinogenic parameters, including the reduction of proliferation and cell viability, increase in lytic cell death and LDH release, and induction of pore formation in human gastric cancer cells. These findings offer novel insights into potential therapeutic strategies against gastric cancer. **Keywords:** gastric cancer;pyroptosis;NLRP3 inflammasome.

IC - 116 - **Identification of SARS-CoV-2 proteins that modulate the expression of efferocytic genes in macrophages.**

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Every day, billions of cells die through various forms of cell death, including apoptosis. Their efficient removal, processing and degradation, a process known as efferocytosis, is pivotal to promote tissue renewal and protect against complications caused by the excessive accumulation of tissue damage. In general contexts, macrophages play an important role in the phagocytosis and clearance of these apoptotic cells. Conversely, efferocytosis is combined with other environmental clues to determine the functional reprogramming of macrophages towards an anti-inflammatory and tissue repair profile, which means that failures in this process can lead to aggravated inflammation. Recently, our group has demonstrated that phagocytosis of apoptotic cells infected with viable SARS-CoV-2, when compared to the phagocytosis of sterile apoptotic cells, reduces the expression of efferocytic receptors in macrophages, potentially interfering with the ability of these phagocytes to promote efficient tissue repair (eLife. 11:e74443, 2022). Based on this, our hypothesis is that protein effectors of SARS-CoV-2 negatively modulate the expression of genes encoding these efferocytic receptors, impairing the internalization of new apoptotic cells. According to this hypothesis, we are expressing each protein encoded on an open reading frame of SARS-CoV-2 genome in macrophages, using a lentiviral expression system to confirm their effect on the expression of efferocytic receptors by RT-qPCR. Furthermore, putative candidates are to be confirmed by directly assessing the capacity of macrophages to uptake apoptosis cells. We thus aim to associate the expression of viral components in macrophages with their failure in efferocytosis. **Keywords:** Efferocytosis;SARS-CoV-2;Macrophages.

IC - 117 - **Characterization of anti-checkpoint autoantibodies in autoimmune and tumor patients**

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The expression of cell surface proteins PD-1 (programmed cell death protein 1), PD-L1 (Programmed *Death* Ligand *1),* and CTLA-4 (cluster of differentiation 152) in immune cells constitute major immunosuppression mechanisms of the immune responses. Monoclonal antibodies that block the interaction of these proteins with their ligands are largely used as immunotherapy drugs. Antibodies anti-PD-1, CTLA4 or PD-L1 are used for the treatment of solid tumors. CTLA-4-Ig drugs are used for rheumatoid arthritis and transplant rejection. Each of these drugs has limited efficacy and adverse, off-target effects. We hypothesized that autoantibodies (AAbs) against these proteins can be produced in autoimmune patients, as anti-drug antibodies (ADAs) in CTLA-4-Ig treated patients. Moreover, a literature search revealed that little is known about the frequency, isotype, or affinity of naturally occurring anti-checkpoint AAbs in patients or healthy individuals. Few studies have tried to characterize their involvement in adverse effects or efficacy of anti-checkpoint monoclonals. This study aims to characterize anti-PD-1, anti-PD-L1 and anti-CTLA-4 autoantibodies in lung cancer patients, autoimmune patients, and healthy individuals. To do that, we recruited patients at Hospital de Santa Casa de Misericórdia de Porto Alegre after the approval of Research Ethics Committee and Plataforma Brasil (Approval Number: 5,523,051). Blood samples were collected, and centrifugated for plasma separation. Levels and isotypes of autoantibodies were analyzed by enzyme-linked immunosorbent assay (ELISA). Affinity and neutralizing potential are being characterized by in-house designed assays. Preliminary results indicate a high frequency of anti-checkpoint antibodies, especially of the IgM subclass. Our findings will contribute for the design of novel therapeutic approaches for cancer and autoimmune diseases. Support: Ministério da Saúde – PRONON; FAPERGS - Programa RITES. **Keywords:** autoantibodies;PD-1;CTLA-4.

IC - 118 - **Persistent immune activation following valvuloplasty for rheumatic heart disease is associate with restenosis and disease severity.**

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Background: Rheumatic heart disease (RHD) is characterized by an immune-mediated valvular damage resulting from infection with type A Streptococcus. Valvuloplasty (PMV) is a minimally invasive treatment for patients with significant valve stenosis. It often is successful, but in some cases restenosis may occur. To date there are no markers to predict disease severity or PMV effectiveness. Methods: We measured the plasma levels of immune molecules using the Bio-Plex Pro Human Cytokine Standard 27- plexKit (Biorad) and determined their involvement in signaling pathways (KEGG database). Patient groups were composed of RHD patients with severe disease who received PMV (n=93; 92% woman and 8% men) and those with less severe disease, who did not need PMV (n=64; 70 % women and 30 % men). A 5-year follow-up analysis was performed in 7 patients who underwent PMV and presented or not restenosis. **Results**: We found high plasma levels of cytokines (IL-1β, IL-1Ra, IFN-γ, IL-17. IL-2, IL-4, IL-6, G-CSF), chemokines (CCL2, CCL3, CCL4, CCL5, CXCL8, CXCL10, CCL11), and fibrotic factors (FGF-basic, PDGF) in RHD patients who underwent PMV compared to those who did not. Enriched pathway analysis indicated the participation of the upregulated molecules in cytokine-cytokine receptor and IL-17 signaling pathways, associating immune activation with PMV. Patients who developed restenosis after PMV had higher levels of inflammatory chemokine receptors and fibrotic factors as compared to those who did not need PMV. In addition, even after 5 years of PMV, an increase in IFN-γ, IL-9 and IL-1β was observed. **Conclusion**: A systemic immune activation was observed in RHD patients that underwent PMV and a persistency of inflammation was detected after 5 years, suggesting the use of inflammatory cytokines, chemokines and fibrotic factors as markers of severity in RHD. **Keywords:** Rheumatic Heart Disease (RHD);Valvuloplasty (PMV);Immune Activation.

IC - 119 - **PRODUCTION OF IgY (Yolk Immunoglobulin) FROM ZIKA VIRUS NS2B PROTEIN PRODUCED IN PLANT FOR USE IN IMMUNODIAGNOSTIC KIT**

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NS2B is a 14 kDa Zika virus (ZIKV) protein that functions as a cofactor for another protein, NS3, a serine protease. The ZIKV NS2B-NS3 complex catalyzes viral precursor polyprotein processing as an essential step during viral replication. Even with its underexplored potential, serological analyzes have brought the ZIKV NS2B protein as an alternative for diagnosis, showing high specificity and sensitivity. In addition, the recombinant NS2B protein produced in plants proved to be immunogenic, making it possible to obtain anti-ZIKV NS2B IgY antibodies from the egg yolk of immunized chickens. Based on the above, the objective of this study was to evaluate the immunogenicity of the ZIKV NS2B produced on a plant platform in the generation of low-cost antibodies of the IgY type. The NS2B protein fused to the hydrophobin I tail (HFBI) was transiently expressed in Nicotiana benthamiana, purified by ATPS separation followed by hydrophobic interaction chromatography (HIC) on a HiTrap Butyl FF column (Cytiva). Then, the NS2B-HFBI protein was used in the immunization of White Leghorn chickens, with 0.5 mL of the protein solution intramuscularly with incomplete Freund's adjuvant. IgY was isolated from the egg yolk of the immunized chicken and characterized by 12% polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and ELISA. The antibodies produced from immunization with the plant-produced NS2B-HFBI protein were able to recognize the recombinant protein, as well as the Zika virus itself in immunoblot and ELISA. The results obtained demonstrated that IgY anti NS2B Zika antibodies have the potential to be used as input in the development of diagnostic kits for Zika virus infection. **Keywords:** Zika virus;IgY;NS2B protein.

IC - 120 - **Immune Response in Mice with Tumor and Leishmania Infection: Insights into Tumor Extracellular Vesicle-Mediated Systemic Immune Response**

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Understanding the dynamic interplay between cancer and infectious diseases is crucial for developing effective therapeutic strategies. In this context, how the immune system burdened by tumor coordinates defense mechanisms against infections is still unsettled, especially because cancer is associated with immunosuppression and increased infection burden. Tumor extracellular vesicles (TEV) can affect immune cell function, cytokine production, and the recruitment of immune cells to the tumor and, possibly, infection site. TEV can be found abundantly in the blood with the possibility to modulate immune responses in distant sites. Our preliminary data using murine models have shown the presence of a tumor or i.v. TEV treatment can alter the immune response to Leishmania infection. Similarly, the immune response to Leishmania infection hampers the development of an effective anti-tumor response, leading to enhanced tumor progression. However, the specific mechanisms underlying this immunosuppressive crosstalk between the tumor and Leishmania infection needs to be investigated. Targeting tumor-derived extracellular factors that promote immune suppression or immune evasion could enhance anti-tumor immune responses and improve treatment outcomes. Additionally, modulating the immune response to infections in the presence of tumors may help reduce the negative impact of concurrent infections on cancer progression and patient outcomes. In conclusion, studying the immune response in mice with tumor and Leishmania infection is essential for unraveling the complex interactions between these pathologies and understanding the role of TEV tumor in systemic immune responses. This knowledge can inform the development of innovative therapeutic approaches that consider the complex immune landscape in cancer patients with concurrent infections, ultimately leading to improved treatment outcomes. **Keywords:** Leishmania;Tumor extracellular vesicles;Concurrent infections.

IC - 121 - **PHYSIOLOGICAL ACCUMULATION OF HEPATIC FAT IN THE POSTNATAL PERIOD**

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Introduction: The liver is a key organ for the body's immunometabolic functioning, essential for the evolution of a healthy individual. However, the dynamics of hepatic fat accumulation during the neonatal phase are unclear. Since accumulation of fat is associated with metabolic diseases, we aimed to describes the occurrence of fat accumulation in the liver of mice in the postnatal period and establish how long this phenomenon persists. Methods and results: 0 day; 4 days; 1-4 weeks and 8 weeks-old C57BL/6 mice were used. Confocal microscopy was used to visualize the hepatic fat accumulation at the different life stages of mice. The images showed hepatic deposition of lipid droplets from the 1st day of life, located in the hepatocytes cytoplasm, being even more expressive at both 4th day and 1st week, with a reduction perceived from the 2nd week and a drastic decrease by the 3rd and 4th weeks, temporal markings of the weaning process. Deposition stabilizes when adulthood is reached. The same protocol was used in embryos to investigate whether the accumulation could be congenital, however, an inexpressive presence of droplets was shown. Thus, the origin of fat seen in mices breastfed by the mother and in mices kept with foster mothers was investigated, with the deposition being significantly expressive in the biological mother group. In the absence of a precise literature description for the reason for lipid accumulation, a lipidomic was also performed – indicating a different profile of lipids when comparing adult animals with groups at neonatal phase and weaning period. Conclusion: Due to the hepatic dynamism, especially in the postnatal period, the authors of this work consider that the postnatal hepatic fat deposition is closely related to the maturation process of the animal’s immune response – a yet partially immature immune system. New experiments are being conducted to elucidate this intriguing question. **Keywords:** Fatty Liver;Newborn;Immunity.

IC - 122 - **Induction of Tertiary Lymphoid Structures through Lactobacillus delbrueckii UVF H2b20 Intranasal Treatment: A Potential Strategy to Prevent Lung Metastasis**

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Lung metastasis is a major cause of mortality in patients with several types of cancer. The formation of tertiary lymphoid structures (TLS) within the tumor microenvironment has been associated with improved anti-tumor immune responses and favorable clinical outcomes. In recent years, there has been growing interest in exploring novel approaches to induce TLS formation in the lungs to prevent or limit lung infections and inflammatory conditions. However, if this approach can be used to prevent lung metastasis still lacks evidence. We aim to discuss the potential of *L. delbrueckii* intranasal treatment in inducing TLS formation in the lungs and its implications in preventing lung metastasis. *L. delbrueckii*, a probiotic bacterium, has been demonstrated to possess immunomodulatory properties and the ability to enhance local immune responses. Our preliminary data utilizing murine models have shown that intranasal administration of *L. delbrueckii* can effectively promote the recruitment and organization of immune cells leading to the formation of TLS in the lungs. These induced TLS exhibit characteristics similar to secondary lymphoid organs, including the presence of B cell follicles, T cell zones, and specialized antigen-presenting cells. Consequently, the presence of TLS in the lungs could function as a barrier against the establishment and progression of metastatic tumor cells. In conclusion, the induction of TLS through *L. delbrueckii* intranasal supplementation could be a promising approach to prevent lung metastasis. By promoting local immune activation and the organization of lymphoid structures within the lungs, *L. delbrueckii* treatment holds the potential to enhance immune vigilance status in lungs, thus improve anti-tumor immune responses and provide a favorable microenvironment for immune cell-mediated tumor control. On going research is aimed to assess *L. delbrueckii*-based therapies and determine their efficacy and safety in preventing lung metastasis. **Keywords:** Lung metastasis;tertiary lymphoid structures;L; delbrueckii.

IC - 123 - **Activation of the kallikrein-kinin system after infection of endothelial cells with dengue virus**

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Dengue virus (DENV) infection is marked by enhanced inflammation, with increased vascular permeability and coagulation dysfunctions. Kallikrein-kinin system (KKS) is related to blood coagulation and inflammatory processes, which motivated us to investigate its role during dengue pathogenesis. KKS activation is initiated by the contact of factor XII (FXII) with negatively charged surfaces, such as polyphosphates and extracellular traps released by platelets and mast cells, and phosphatidylserine (PS) exposed by apoptotic cells. Activated FXII activates prekallikrein (PK), leading to the cleavage of kininogen (HK) and release of bradykinin (BK). BK modulates endothelial functions, inducing vascular permeability, edema and pain. We have previously evidenced KKS activation in plasmas from dengue patients and demonstrated that DENV infection of endothelial cells (HBMECs) upregulated BK receptors. Addition of BK to DENV-infected cells increased viral replication through modulating NO and delaying apoptosis. Here, we investigated whether infection of HBMECs with DENV promoted KKS activation. HBMECs were infected with DENV-2 and, at the peak of virus replication, cells were incubated with plasmas from healthy donors or purified HK and PK, with or without FXII. DENV-infected cells presented enhanced levels of cleaved HK on its surface, in comparison to cells cultured with mock or inactivated DENV, indicating that virus replication affects HBMECs phenotype and induces KKS activation. Assays performed with purified proenzymes showed that this event was dependent of FXII. We also observed that blocking PS with Annexin V reduced HK cleavage, suggesting that virus-driven apoptosis may fuel KKS activation. Our findings demonstrate that infection of endothelial cells with DENV may contribute to the KKS activation detected on patients' plasmas, and further studies are being performed to address whether this event is related to dengue-associated vascular permeability. **Keywords:** Bradykinin;DENV;HBMEC.

IC - 124 - **miR-193b, miR-671 and TREM-1 as molecular prognostic biomarkers in leishmaniasis caused by L. braziliensis**

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Background: Leishmaniasis are neglected tropical diseases caused by Leishmania, an important public health problem affecting 12 millon people worldwise. Cutaneous leishmaniasis (CL) is the most frequent form of this disease. It was demonstrated from gene expression analysis that miR-193b, miR-671 and TREM-1 in CL lesions correlate in patients with early cure (within 60 days), indicating that these molecules have potential use as prognostic bioindicators. **Methods**: The study is approved by the Research Ethics Committee. Samples (biopsy and blood) of 21 patients were collected in Corte de Pedra – Bahia. The blood was processed to obtain peripheral blood mononuclear cells. Total RNA was extracted, cDNA was obtained and gene expression was evaluated by RT-qPCR. **Results**: In these samples, it was possible to profile the expression of miR-193b and TREM-1 in comparison with healthy controls from a non-endemic area. miR193b expression was significantly reduced in patient biopsies compared to controls. Besides, TREM1 expression was significantly increased in biopsies when compared to the healthy control. But, when comparing patients who heal within 59 days of treatment with those who heal after 60 days, there was no statistical difference in the expression profile of miR-193b and TREM-1. **Conclusion**: Due to the complexity of the interaction between the host and the pathogen, the immune response can evoke different solutions for the establishment, survival and persistence of the parasite. Studying this diversity is important to define molecules that may indicate the early prognosis of a chronic disease, such as CL. The data reinforce the potential of miRNA and TREM-1 as possible biomarkers of CL patients with shorter healing time. Based on the results obtained, it will be possible to understand the potential of miR-193b, miR-671 and TREM-1 as promising candidates for application in clinical practice in patients with CL caused by L. braziliensis. **Keywords:** Leshimaniosis;miRNA;Biomarker.

IC - 125 - **Antileishmanial activity of diterpenes from Salvia procurrens on Leishmania amazonensis**

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Leishmaniasis is a widespread zoonotic disease with limited chemotherapy options. Additionally, disease control is hindered by misdiagnosis and poor treatment adherence. This study aimed to investigate the *in vitro* leishmanicidal activity of four diterpenes (fruticulin A, demethylfruticulin A, procurrenin A, and procurrenin B) from *Salvia procurrens* Benth. The diterpenes were isolated and characterized using spectroscopic and spectrophotometric techniques, with novel compounds pA and pB. The viability of *Leishmania amazonensis* was evaluated by incubating promastigote forms with fA, dfA, pA, and pB (1 to 50 µM). The effects on reactive oxygen species (ROS) generation, cell membrane integrity, mitochondrial membrane potential, lipid peroxidation of promastigotes, and action of diterpenes on intracellular amastigote forms were assessed. Additionally, cytotoxicity was evaluated on peripheral blood mononuclear cells (PBMCs) and human erythrocytes. The isolated products fA, dfA, pA, and pB exhibited IC_{50} values of 13.7 µM, 12.7 µM, 4.8 µM, and 16.26 µM, respectively. Moreover, all compounds induced loss of membrane integrity with propidium iodide labeling percentages of 52%, 47%, 40%, and 43%. All isolated products led to an approximately 100% increase in ROS generation and lipid peroxidation. Furthermore, they caused mitochondrial depolarization of 48%, 56%, 60%, and 74%, respectively. The tested diterpenes demonstrated low cytotoxicity towards PBMCs and human erythrocytes. The products fA, dfA, pA, and pB showed significant anti-amastigote activity at the IC_{50} concentration. These findings highlight the leishmanicidal activity of isolated diterpenes against promastigotes and amastigotes of *L. amazonensis*. Further research is needed to evaluate the activity of fA, dfA, pA, and pB *in vivo*. **Keywords:** Leishmaniasis;Oxidative stress;diterpenes.

IC - 126 - **Treg cells from mild dengue patients display improved phenotype when stimulated with SARS-COV-2-specific antigens.**

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Dengue and Covid-19 can manifest from asymptomatic to severe clinical presentation that can lead to death. Some works have shown that previous infections, related or not, can influence the clinical evolution of Covid-19 either with a protective or pathological effect. We have shown that Tregs are an important mechanism to control immune responses and avoid immunopathology caused by diseases such as Covid-19 and dengue. Tregs play an important role in controlling effector immune responses in several diseases, regulating the activation and proliferation of cells, suppressing the production of inflammatory cytokines, minimizing collateral damage in the tissues, and preventing exacerbated inflammation in acute or chronic infections. Our objective was to assess whether previous exposure to dengue may impact Covid-19-specific Tregs. PBMCs from DENV-positive patients were collected between the years 2013 and 2019 and frozen until use. Thawed PBMCs were cultured in the presence of Nucleocapsid, Spike, ORF and Membrane peptide libraries from SARS-COV-2 and evaluated by flow cytometry. Patients affected by mild dengue, during the defervescence phase and in the convalescence period, showed increased frequencies of Tregs when stimulated with the Spike, ORF and membrane peptide libraries of the SARS-COV-2 virus when compared to unstimulated cells. Tregs of dengue patients also expressed increased levels of GITR when stimulated with SARS-COV-2 antigens. Importantly, there was an increase in TNF production by Spike-specific Tregs from mild dengue during convalescence. Our results suggest that Tregs from patients with mild dengue display improved fitness when stimulated with SARS-COV-2 antigens. **Keywords:** Covid19;dengue;Tregs.

IC - 127 - **Soluble epoxide hydrolase inhibitor-mediated immunoregulation in a preclinical model of Chagas disease**

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Introduction: 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), a synthetic compound, proved to be an efficient inhibitor of the soluble epoxide hydrolase enzyme with promising and specific biological activity in terms of anti-inflammatory function and reduction of fibrosis formation. Chagas disease is mediated by an intense immune response dependent on the host-parasite relationship and that in the chronic phase there may be loss of heart function and other organs. Objective: Evaluate the effects of therapeutic intervention with TPPU in a preclinical study model of Chagas disease. Methods: The experimental protocol was previously approved by the Animal Use Ethics Committee (n = 403/UFTM). The “Gpower” program was used, which considered the losses, the effect sizes for the variables under study (it was selected based on the smallest effect size) and the power of inferences (minimum of 80%). Balb/c animals were used and the parasite strain was the “Colombian strain of T. cruzi”. Parasites such as parasitemia, mortality, water and food intake, weight, histometric parameters (inflammatory infiltrate, amastigote nests, collagen quantification), tissue cytokines, hematological and biochemical parameters (sodium, potassium, AST, ALT, CPK, nitric acid and CK-MB). Results: The intervention with the inhibitor was able to modulate the inflammatory response in the initial stages of the infection, with a reduction of pro-inflammatory cytokines (TNF-α, INF-γ) and infiltration in the cardiac tissue. There were no complications indicated by the liver and muscle teaching concentrations (p>0.05). Conclusions: However, this study indicated the possibility of drug intervention to reduce the harmful effects of the disease in the early stages and especially in the phase of functional loss of the disease. However, it is necessary to understand more parameters to enable better routes to achieve effective intervention in further clinical studies. **Keywords:** Chagas disease;TTPU;Endomyocardial fibrosis;.

IC - 128 - **Effect of the association of temozolomide and radiation on the induction of glioblastoma cell death and the impact on efferocytosis by dendritic cells.**

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Glioblastoma (GBM) is a malignant brain tumor whose treatment consists of surgical removal and theassociation of radiotherapy and oral administration of temozolomide (TMZ). The treatment regimens can drive immunogenic (ICD) and nonimmunogenic cell death (NICS). NICD inducers might promote the accumulation of apoptotic cells (ACs), and DCs play an important role during the clearance of those ACs, called efferocytosis, resulting in a tolerogenic phenotype. The tolerogenic phenotype of these phagocytes is characterized by a reduction in molecule expression as well as anti-inflammatory mediator production. Therefore, this study aimed to characterize the discrimination of nonimmunogenic vs. immunogenic forms of cell death induced by TMZ, UV-C, and the association with UV-C radiation and TMZ in the GBM cell line. The glioblastoma cell line (U87MG) was treated with different UV-C radiation doses (50-200mJ) in the presence or absence of different concentrations (100-500mM) of theTMZ. After treatment, the GBM cell line was stained with Viability Fixable Die and cleaved caspase-3 to evaluate the distinct cell death by flow cytometry. Our findings showed that 50 mJ resulted in~25%, 100 and 200 mJ resulted in 60% of apoptosis (cleaved caspase 3+); surprisingly, the treatment with different concentrations of TMZ didn’t show any effect on cell death, the cell viability was similar to untreated condition. The combination of UV-C (50, 100, and 200 mJ) and all different concentrationsof TMZ didn’t improve the percentage of apoptosis observed with radiation treatment alone (100 e 200mJ). Knowing thatTMZ is the gold standard treatment for GBM, new assays will be conducted by adjusting concentration, intensity, and time to generate the best possible source of ACs or other different cell death for assessing the impact of efferocytosis on DCs. **Keywords:** Glioblastoma;Cell Death;Efferocytosis.

ME - 010 - **Development of in-silico antibody design pipeline leveraging large scale experimental immunoglobin data analysis and high-fidelity CDR modelling**

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With the growing interest in the development of immunobiological drugs, the use of bioinformatics tools has become increasingly common in this field due to their reduced cost and development time compared to solely in vitro or in vivo methods. However, the currently available methods for antibody structure design generally do not account for the distinct characteristics of naive and memory antibodies, the most frequently observed mutations in nature, the relative diversity of each CDR (complementarity-determining regions), or the conformational changes caused by modifications in the CDRs. These aspects are important for the efficient design of structurally and chemically diverse antibodies. Therefore, in this study, millions of sequences of human naive and memory antibodies present in the Observed Antibody Space (OAS) database were analyzed, and the diversity of each CDR and each position within it was calculated. The collected data were incorporated into a Python-based pipeline that progressively optimizes an antibody by generating its three-dimensional model to assess the effect of modifications on the antibody-antigen (Ab/Ag) complex. Firstly, a selected CDR of the initial antibody model is modified by grafting a CDR from a different antibody. The probability of selecting one of the six CDRs for modification is based on the diversity of each one of them. Next, point mutations are chosen and tested according to the diversity of different positions within each CDR and the most common residues at those positions in the OAS sequences. This allows prioritizing the testing of the most likely modifications in the regions of greater importance in nature, thus increasing the efficiency of the pipeline. A large reduction in Ab/Ag binding energy was observed over 48 hours of computational testing, demonstrating the speed and effectiveness of the new pipeline. **Keywords:** Antibody design;Complementarity-determining region;In silico.

ME - 011 - **Effect of consumption of ultra-processed foods on the immune composition of maternal colostrum.**

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Due to the abundance of bioactive compounds and its dynamic composition, human milk represents the best source of nutrition for newborns. In the first week of life, newborns receive colostrum, which contains the highest concentrations of immune compounds essential for the maturation of the gastrointestinal tract and the infant immune system. Evidence already indicates that factors such as maternal obesity and the consumption of ultra-processed foods can interfere with the concentration of immune compounds in colostrum and, therefore, impact the development of the newborn's immune system. In light of this context, the objective of this study was to evaluate whether maternal consumption of ultra-processed foods and nutritional status interfere with the immune composition of colostrum. A retrospective cross-sectional study was conducted with three groups of women: 1) eutrophic mothers, 2) overweight mothers, and 3) obese mothers. Socioeconomic, health, and anthropometric data were collected, and colostrum samples were collected for the analysis of cytokines, chemokines, and growth factors using multiplex immunoassay. Our data show that the group of obese women consumes fewer fresh foods and more ultra-processed foods. Women with high consumption of ultra-processed foods have lower concentrations of VEGF and FGF-basic, but higher concentration of IL-10, whereas women with higher consumption of fresh foods have a higher concentration of IL-7. Overall, it was observed that women who consume more ultra-processed foods have a higher frequency of production of inflammatory cytokines and a lower frequency of anti-inflammatory cytokines and Th2. **Keywords:** colostrum;ultra-processed foods;cytokines;.

ME - 012 - **Characterization of the immune response and cell exhaustion in patients with Chromoblastomycosis**

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Chromoblastomycosis (CBM) is a neglected chronic mycosis caused by fungi of the order Chaetothyriales. After inoculation in the skin, the fungus is phagocytosed and differentiates into muriform cells. Patients with severe CBM exhibit a Th2 and/or Treg immune response while resistant patients produce a Th1 response; however, even in the presence of IFN-γ the fungus can persist, and it is not well understood. To clarify the factors that lead to the chronicity of CBM, the present study aimed to determine the polarization of the cellular immune response and quantify the cell exhaustion that occurs in the inflammatory infiltrate of the skin of CBM patients. Immunohistochemical reactions were performed to quantify positive cells for iNOS, IFN-γ, IL-4 and IL-10, as well as the expression of the cell exhaustion markers PD-1 and PD-L1 in the skin of CBM patients. Skin biopsies of healthy individuals were used as controls. Fungal density was measured on histological sections stained with hematoxylin and eosin. The data obtained allowed us to divide patients into groups with high and low fungal burden; however, the intensity of the immune response in these patients was independent of the mycotic load. In relation to the control, a significant increase was observed for all immunological markers, however the density of IL-10+ cells was higher than that of IL-4+, IFN-γ+ and iNOS+ cells ($p<0.05$). Furthermore, the cell exhaustion markers PD-1 and PD-L1 increased in the inflammatory infiltrate of patients with CBM compared to the skin of the control group ($p<0.05$). The results suggest that in CBM there is a mixed inflammatory response that does not correlate with the intensity of the fungal load, also, the predominance of IL-10+ cells and cell exhaustion mediated by the PD-1 and PD-L1 pathway may be factors associated with the maintenance of the fungus in the skin and disease progression. Supported by FAPESP (2021/13772-8) and Lim50-FMUSP. **Keywords:** Cell exhaustion;Pro- and anti-inflammatory cytokines;Melanized fungi.

ME - 013 - **Brazilian Brown Propolis immunomodulates neutrophil response in experimental paracoccidioidomycosis and has direct antifungal effect on Paracoccidioides brasiliensis**

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Paracoccidioidomycosis (PCM) is a mycosis caused by fungi of the *Paracoccidioides brasiliensis*. Persistence of the inflammatory infiltrate is characteristic of PCM. The treatment of PCM is long. Brazilian Brown Propolis (BBP) has great economic and medicinal importance. Therefore, we evaluate whether BBP improves PMNs activation state and antifungal activity against *Paracoccidioides brasiliensis* (Pb18). Antifungal activity of BBP at a concentration of 500mg/mL was tested against a virulent *P. brasiliensis* isolate (Pb18). The antimicrobial activity was evaluated using the macrodilution technique. Mice were inoculated via subcutaneous (sc) air-pouch with Pb18. On the fifth day of infection, treatment with 500 mg/mL BBP for sc via was initiated for 3 days until the collection of the neutrophils (PMNs), at 8 days of infection and treatment. The following parameters were analyzed: absolute number of cells at the air-pouch, mitochondrial activity, ROS, total proteins production, as well as the number of viable fungi. *In vitro* experiments showed remarkable direct antifungal activity of BBP. Reduced the number of viable fungi in relation to the original inoculum after 72 h of incubation. *Ex vivo* experiments showed BBP caused a decrease in the influx of PMNs in relation to control. Mitochondrial activity was higher in mice treated with BBP, that control group (Pb18). ROS production was lower in mice treated with BBP. BBP was able to reduce the number of viable Pb18 in treated mice. Our results suggest that BBP has a direct antifungal effect and is able to prevent fungal growth and increase PMNs activation. This data allows us to suggest that BBP can be a new natural alternative the therapeutic options in PCM treatment. Grants: CNPq-309917/2020-4, FAPEMIG-PPM-00497-18 and FAPEMIG-BPD-00341-22. L.A.Santos a scholarship from CNPq within the Young Doctors Fixation in Brazil Support Program. T.D.Andrade and J.C.Dutra recipient CAPES scholarships and E.M.Picoli of CNPq-PIBIC. **Keywords:** Brazilian Brown Propolis;Paracoccidioides brasiliensis;Neutrophil response.

ME - 014 - **Brazilian Green Propolis has stimulatory effect on the innate immunity and also inhibits Paracoccidioides growth**

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Paracoccidiodomycosis (PCM) is a severe systemic, granulomatous mycosis caused by fungi to the genus *Paracoccidioides* spp. PCM presents an intense inflammatory response and serious clinical consequences that require more extensive therapeutic options than those presently available. Therefore there is a need for coadjuvant therapies with drugs already in clinical use. Brazilian Green Propolis (BGP) was reported to present anti-inflammatory, antimicrobial and immunomodulatory activities. Our objective was to study the effect of BGP on the neutrophil activity as well as its direct effect against *Paracoccidioides brasiliensis* (Pb18) and *Paracoccidioides lutzii* (Pl). Mice were inoculated via air-pouch with Pb18 or Pl and on the 5th day after infection, subcutaneous treatment with 500 mg/mL BGP was initiated and maintained until the 7th day. Collection of polymorphonuclear (PMNs) was performed on the 8th day of infection and treatment. The following parameters were analyzed: concentration of nitric oxide (NO), proteins, catalase, peroxidase and cytokines, mitochondrial activity, and number of viable neutrophils and fungi. BGP treatment increased the viability of neutrophils, the production of proteins, catalase, peroxidase, and of IFN-y, IL-6 and GMC-SF cytokines as well as mitochondrial activity of mice infected with either PB18 and Pl. NO production was not significant in BGP- treated mice with. We found a marked reduction in the number of viable fungi in mice treated with BPG, when compared to the control groups, only infected with Pb18 or Pl. Therefore, our results allow us to suggest that BGP has a double effect, both increasing the production of oxygen metabolites and of IFN-y, IL-6 and GMC-SF cytokines, thus stimulating the host's immune response and also exerting a direct antifungal effect on both Pb18 and Pl. **Keywords:** Paracoccidioides brasiliensis;Neutrophil response;Brazilian Green Propolis.

ME - 015 - **Investigation of the Effects of Aging on the Dynamics of Macrophage Populations in the Course of Bone Regeneration**

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Bones fully restore their shape and function after a fracture. However, because of aging, this scenario changes in the elderly population, which becomes more prone to consolidation failures. Considering that the available treatments are not optimized to overcome these regenerative deficiencies and the increasing rates of populational aging, innovative therapies for this public are urgently needed, what in turn demands a better understanding of the molecular and cellular aspects of fracture healing. Evidence indicate that macrophage activity is key for successful consolidation, with these cells instructing both bone formation and revascularization. However, it is not known how this instruction happens, in molecular terms. So, the aim of this study is to map the macrophage populations acting during the different stages of fracture healing, verifying the effects of aging on this dynamic. To this end, we established a murine model of femural fracture in young (10-12 weeks) and middle-aged Balb/c mice (48-50 weeks), and validated it by radiography, computed microtomography, and histology. We observed the development of fracture calluses until day 14, when it reached peaked volume and started bridging the bone cortices, an event that was completed by day 21. In elderly mice, however, this occurred much less prominently, with lower amounts of cartilaginous and bone tissue formed in the fractured area. Next, we isolated macrophages from fracture calluses collected at the inflammatory, at the tissue neoformation, and at the remodeling phases, and evaluated their immunophenotypic profile. Preliminary results showed a progressive increase of the F4/80+ CD206+ Mac2+ population in both groups. Further experiments will be performed to compare the transcriptomic profiles of these macrophages, in the young and elderly groups, to evaluate whether their function is altered by aging, an information that can base the development of macrophage-oriented therapies for bone fractures. **Keywords:** Fracture Healing;Macrophage;Ageing.

ME - 016 - **Role of GDF11-signalling in myogenesis: development of a computational approach for the design of GDF11-based peptides**

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Muscle regeneration is a highly coordinated process that depends mainly on satellite cells (SCs) and immune cells. Macrophages, for example, produce molecules capable of stimulating the proliferation of SCs, and the differentiation and fusion of these cells into new myofibers. Members of the transforming growth factor-beta (TGF-β) superfamily are produced by macrophages and muscle cells and are involved with myogenesis. Myostatin, for example, is known to be an important negative regulator of muscle mass, while GDF11 (growth and differentiation factor 11) has been implicated in the rejuvenation of cardiac muscle and skeletal muscle. We found that treatment with recombinant GDF11 (rGDF11) induced myoblast proliferation and inhibited cell differentiation when compared to the untreated control, suggesting that GDF11 has distinct functions at different stages of myogenesis. Therefore, we aim to design a modified protein inspired by the structure of GDF11 that is able to stimulate muscle regeneration. We selected the best quality three-dimensional structures of the proteins involved in GDF11 signaling, with GDF11 itself and its receptors (ALK4, ALK5, ACTR-IIB, and ACTR-IIA) deposited in the PDB, with the exception of ALK7 in which it was necessary to perform the comparative modeling stage, in which a good quality model was obtained through Swiss-Model. After obtaining the structures, we identified the amino acid residues that are part of the interaction between GDF11 with ALK5 and GDF11 with ACTR-IIB through a good-quality structure that had already been solved experimentally. Thus, based on the three-dimensional structures of the GDF11 pathway components, we intend to continue molecular docking simulations in order to identify the residues that interact between GDF11 and its receptors with subsequent analysis of alanine scanning to propose substitutions of amino acid residues that improve the function, specificity, and affinity of the ligand. **Keywords:** GDF11;Structural bioinformatics;Muscle regeneration.

ME - 017 - **Enhanced Effect of VIP against Inactivated SARS-CoV-2 Pro-inflammatory Stimulus through CD10 and CD26 Inhibition**

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The neuropeptide VIP is endowed with several homeostatic functions, including regulation of the inflammatory response. In previous studies, we showed that VIP exerts anti-inflammatory and antiviral activity on HIV-1 replication through the production of β-chemokines, IL-10, and inhibition of NF-kB in macrophages and lymphocytes. In addition, we observed that VIP was able to control the inflammatory response and viral replication in SARS-CoV-2-infected monocytes. Like others peptides, VIP activity can be modulated through degradation by peptidases, which are pointed as therapeutic targets for various inflammatory diseases. Here, we aimed to study the involvement of these peptidases on the immunomodulatory effects of VIP in PBMCs exposed to inactivated SARS-CoV-2 (inCoV-2), as potential enhancers of its antiinflammatory actions. We initially observed that PBMCs exposed to inCoV-2 exhibit a 5 to 20-fold increase in the production of pro inflammatory cytokines TNF-alpha, IL-6 and IL-1 beta and of the anti-inflammatory mediator IL-10 as well, and that VIP neutralized this effect. Furthermore, the VIP ability to modulate inflammatory reaction was potentiated by 40% when pan-peptidase inhibitors, as well as specific inhibitors targeting CD10 and CD26, were added to cells in combination with VIP. We will next evaluate the level of expression of these peptidases in resting and in inCoV-2-activated PBMCs. Our data suggest that VIP immunomodulatory effect can be improved through inhibition of peptidases, mainly CD10 and CD26. **Keywords:** PBMCs; neuropeptide; peptidases.

ME - 018 - **Evaluation of the role of M1, M2 and regulatory macrophages on 5- fluorouracil induced intestinal mucositis**

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5-fluorouracil (5-FU) has been used as an effective chemotherapeutic agent for more than 40 years, but is considered a controversial drug because it causes severe side effects such as oral and intestinal mucositis. Mucositis is the inflammation of mucosa and it is associated with life-threatening complications which leads to high impact in morbidity, mortality and cost of therapy. Prevention of mucositis is important to improve the success of chemotherapy, but currently available treatments usually focus on symptoms and not on preventing desease's development. Macrophages are abundant cells in the gut, with the ability to respond to microenvironment and change their polarization towards different profiles. As macrophages exacerbate inflammatory response in an inflammatory microenvironment, we investigated whether the reprogramming of macrophages to a regulatory profile can attenuate intestinal mucositis. Simutaneously, we also evaluated the role of other macrophage's subpopulations, such as M1 and M2 macrophages. Regulatory macrophages are obtained by the stimuli with high-density-immuno-complexes in the presence of TLR4 agonist, and it is marked by strong upregulation of immunomodulatory cytokines. M1 and M2 macrophages were obtained in the presence of LPS and IL-4 respectevely. Our results evaluated clinical effects in C57BL/6 mice challenged by 450 mg/kg of 5-FU, by parameters such as the loss of body mass, water and food intake. Small intestines were collected to perform cytokines quantification and histological evaluation. The histological score incluiding mucosal erosion, ulceration and inflammation were used to measure the impact of macrophages on preventing tissue damage. Data shows that macrophages actively participate in mucositis pathogenesis, exarcerbating inflammation induced by 5-fluorouracil. This research has been aproved by Etics Cometee (CEUA) from Universidade Federal de Minas Gerais under the protocol 144/22 and has CAPES as financial support. **Keywords:** macrophage;mucositis;5-fluorouracil.

ME - 019 - **Effects of Adipose Mesenchymal Stromal Cells Extracellular vesicles in experimental infection from Leishmania amazonensis**

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Leishmaniasis is a neglected disease caused by protozoa of the genus *Leishmania*. One of its characteristics is the imbalance of the host's immune response to favor the survival of the parasite, generating inflammatory processes that do not promote parasite control and induce tissue injury. In this context, the use of mesenchymal stromal cells (MSCs) and some of their biological products such as their Extracellular Vesicles (MSC-EVs) may be a viable therapeutic strategy, given the already described immunomodulatory potential they present. Our group demonstrated that treatment with MSCs from adipose tissue contributes to lesion control in C57BL/6 mice infected with *Leishmania amazonensis.* As the paracrine effect is one of the main mechanisms of efficacy of treatments with MSCs, this project aims to investigate whether extracellular vesicles these cells secrete have the property of controlling the lesion similar to full cell treatment. With this, the project aims to understand the effects of treatment with MSC-EVs from adipose tissue of C57BL/6 mice in the model of experimental leishmaniasis induced by *L. amazonensis*. The first step it's the purification and expansion of MSC cells from adipose tissue and extraction and characterization of it's extracellular vesicles. Next, we evaluate the *in vitro* effect of extracellular vesicles. Infected C57BL/6 macrophages were treated with MSC-EVs and the parasite load of these macrophages were evaluated. Finally, we evaluate the ability of MSC-EVs to control injury *in vivo* in infected C57BL/6 animals. The size of the lesions was evaluated by pachymetry, the parasite load by limiting dilution technique and the immune response by RT-PCR and ELISA. As preliminary results, MSC-EVs reduced lesion size without affect parasite load. MSC-EVs could be an alternative therapy for the treatment of leishmaniasis lesion. **Keywords:** Cellular Therapy;Extracellular Vesicles;Cellular Immunology.

ME - 020 - **Extracellular Vesicles from macrophages treated with the neuropeptides VIP and PACAP promote anti-inflammatory and antiviral effects in SARS-CoV-2-infected monocytes**

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Extracellular vesicles (EVs) are cellular structures released of endosomes or membrane budding. Classified according to their size, origin, and density, EVs can be divided into larger vesicles (>200 nm) and smaller vesicles (<150 nm). Studies with immune cell derived EVs show functions, such as antimicrobial activities and regulation of the inflammatory response. Previous studies have demonstrated that EVs secreted by different cellular sources play important roles during SARS-CoV-2 infection, leading to both stimulatory and inhibitory activities. In this study, we used macrophages derived from circulating monocytes of healthy donors to obtain EVs. In previous studies, we observed that VIP and PACAP, two endogenous neuropeptides, could inhibit SARS-CoV-2 replication in monocytes, as well as reduce the production of proinflammatory mediators in virus-infected cells. Therefore, we investigated whether EVs obtained from macrophages treated with VIP or PACAP could control the effects related to SARS-CoV-2 infection in primary monocytes. The EVs derived from macrophages were isolated by differential centrifugation of conditioned medium collected from primary cells cultured for 24 hours. We observed that EVs could transmit antiviral effects to virus-infected monocytes, as evidenced by the decrease in SARS-CoV-2 RNA synthesis/replication in human monocytes, protected these cells from virus-induced cytopathic effects, and reduced the production of proinflammatory mediators, in the reduction of levels of IL-6, IL-8, and TNF-α. We further observed that EVs prevented the SARS-CoV-2-induced NF-kB activation in monocytes, which is critically involved in the production of inflammatory mediators. Our findings suggest that EVs are endowed with immunoregulatory properties that might contribute to the antiviral response against SARS-CoV-2-infected monocytes and expand our knowledge of the regulation and effects of extracellular vesicles during COVID-19 pathogenesis. **Keywords:** Extracelular Vesicles;Macrophages;Anti-inflammatory.

ME - 021 - **Neutrophil Extracellular Traps activate primary human lymphocytes and inhibit HIV-1 replication**

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Neutrophils exert their immune activities by different mechanisms, such as degranulation, phagocytosis and release of Neutrophil Extracellular Traps (NETs). NETs consists of decondensed chromatin associated with granular, nuclear and cytoplasmic molecules, like neutrophil elastase and myeloperoxidase. NETs can interact with and activate other immune cells, since it has already been described that this complex may induce T CD4+ lymphocyte activation. It is known that HIV-1 induces NET release and, in previous studies, we showed that these structures decrease viral replication in in vitro HIV-1-infected macrophages. Now, we describe that NETs inhibit HIV-1 replication in HIV-1-infected peripheral blood mononuclear cells (PBMCs) from healthy donors in a dose-dependent fashion. This effect was observed on PBMCs exposed to NETs 2 hours before or 2 hours after the infection. We also observed that NETs increased the proinflammatory response as they induced high levels of cytokine and chemokine release, some of then known for the anti-HIV-1 role, like IL-10 and the β-chemokines CCL3, CCL4 e CCL5. The NET proteins neutrophil elastase and myeloperoxidase did not change the HIV-1 replication, suggesting that the NET anti-HIV-1 effects on PBMCs depend on the integrity of its molecular structure. We are now investigating other molecular mechanisms possibly involved in NET-mediated HIV-1 inhibition in PBMCs. **Keywords:** Neutrophil Extracellular Traps ;HIV-1;Lymphocytes .

ME - 022 - **Flavonoid rutin reduces viability and miR-125b expression of human glioblastoma cells and modulates microglial phenotype.**

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INTRODUCTION: Glioblastoma (GBM) is the most aggressive and treatment-resistant brain tumor. In the GBM microenvironment, the interaction with microglia is associated with dysregulation of cytokines, chemokines, and miRNAs, including miR-125b, which contribute angiogenesis, proliferation, and anti-apoptosis and chemoresistance. Understanding the role of miRNA on mechanisms underlying GBM/microglia interactions and its modulation is important to develop adjuvant therapies. Flavonoid rutin has been shown to be able to induce inhibition of glioma cells growth associated with microglial activation and production of proinflammatory mediators. OBJECTIVE: This study aimed to characterize the effects of rutin on expression of miRNAs by human GBM cells and impact of its secretome on microglia phenotype. METHODOLOGY: Cell viability was analyzed by MTT test on human GL15 GBM and C20 microglia cells treated or not with rutin (1-50 μM) for 24 h. miR-125b expression in GL15 cells and in secretoma was analyzed by RT-qPCR 24 h after treatment or not with rutin (30 μM). Additionally, microglia morphology was analyzed by interference microscopy after exposure to the secretome of GBM cells treated or not with rutin. RESULTS: Rutin (30-50 μM) reduced the viability of GBM cells, not affecting the viability of microglia cells. Moreover, treatment of GBM cells with rutin reduced significantly miR-125b expression. Furthermore, microglia subjected to the conditioned medium from GBM cells treated with rutin (30 μM) presented morphological changes suggesting reactivity. CONCLUSION: Rutin possesses specific antitumor properties in GBM cells, modulating miRNA-125b expression that could be involved in the interaction with microglial and modulation of its inflammatory response. SUPPORT: CNPq, INCT-Translational Neuroscience, CAPES. **Keywords:** miR-125b;GLIOBLASTOMA;RUTIN.

ME - 023 - **The dual role of Axl signaling during pneumococcal pneumonia**

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Despite the availability of a vaccination, *Streptococcus pneumoniae*-related pneumonia remains a public health concern. Pneumonia is characterized by a massive influx of immune cells and apoptosis in the lung parenchyma, which any deficiency in efferocytosis can be detrimental to the host. TAM receptor-mediated efferocytosis (Axl and MerTk) also plays a crucial role in inflammation regulation since its activation suppresses Toll receptors and pro-inflammatory cytokine signaling pathways. Thus, we hypothesize that TAM receptor efferocytosis has a key role in controlling the magnitude of inflammation as well as parenchymal damage during pneumococcal pneumonia. **Material and methods:** C57BL/6 wild-type (WT), MerTk deficient mice ($MerTk^{-/-}$) and Axl deficient mice ($Axl^{-/-}$) were infected intratracheally with S. pneumoniae (ATCC 49619) whose dose was 10^6 CFU. On the fourth day, we performed a bronchoalveolar lavage and the immune cells populations were analyzed by flow cytometry. Also, the first bronchoalveolar fluid (BALF) was used to measure Colony forming units (CFU), nitric oxide levels, cytokines and protein levels. **Results and conclusion:** We found that $MerTk^{-/-}$ and WT mice recruited more inflammatory monocytes than $Axl^{-/-}$ animals. Furthermore, $MerTk^{-/-}$ BALFs had a higher number of neutrophils and interstitial macrophages, whereas WT BALFs had a higher number of AMs, both compared to $Axl^{-/-}$ BALFs. Despite the recruitment of more inflammatory cells, we found higher bacterial units in the BALFs of $MerTk^{-/-}$ and WT mice compared to $Axl^{-/-}$ mice. Our findings suggest that the Axl receptor is important for recruiting monocytes, which are likely susceptible to *S. pneumoniae* infection. **Keywords:** TAM receptors;Streptococcus pneumoniae;Macrophages.

ME - 024 - **EVALUATION OF THE IMMUNOMODULATORY POTENTIAL OF CHALCONE (E)-1-BENZO[D][1,3]DIOXOL-5-YL)-3-(3-BROMO-4-ETHOXY-5-METHOXYPHENYL)PROP-2-EN-1-ONE IN MURINE MACROPHAGES AND LYMPHOCYTES**

UZÊDA, L.F.A.[1]; SANTOS, F.D.S.[1]; DOS SANTOS, B.C.[1]; CAMPOS, C.A.M.[1]; SILVA, G.R.[2]; FERREIRA, F.L.[2]; GUERRA, F.S.[2]; RODRIGUES, L.C.[2]; MAGALHÃES, H.I.F.[2]; MENDONÇA JUNIOR, F.J.B.[2]; SOARES, M.B.P.[3]; GUIMARÃES, E.T.[1]. 1. UNIVERSIDADE DO ESTADO DA BAHIA, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE FEDERAL DA PARAÍBA, JOÃO PESSOA - PB - BRASIL; 3. FUNDAÇÃO OSWALDO CRUZ, SALVADOR - BA - BRASIL.

Introduction: The immune system plays a crucial role in the body's homeostasis and disturbances in its function can result in immune-mediated diseases. Treatment for these diseases is often associated with severe side effects. The high dosages used, the length of the treatment, and the potential for opportunistic pathogen infections make it challenging for patients to adhere to drug therapy. Therefore, the search for new drugs with a more effective immunomodulatory action and fewer side effects becomes necessary. **Objectives**: Evaluate the immunomodulatory potential of chalcone (E)-1-BENZO[D][1,3]DIOXOL-5-YL)-3-(3-BROMO-4-ETHOXY-5-METHOXYPHENYL)PROP-2-EN-1-ONE (FERAI) in activated murine macrophages and lymphocytes *in vitro*. **Materials and Methods:** FERAI was synthesized in the Organic Chemistry Laboratory of UFPB and kindly provided to our research group for testing. Cytotoxicity was assessed in murine macrophages using the AlamarBlue method. The cytokine and NO dosages were determined in the supernatant of macrophages, stimulated or not with lipopolysaccharide (LPS), by ELISA and the Griess reaction, respectively. Lymphoproliferation was evaluated in murine lymphocytes, stimulated or not with concanavalin A, using ATP test. **Results**: FERAI showed low cytotoxicity (CC_{50} = 66 ± 0.12 µM) and reduced the production of pro-inflammatory cytokines IL-6 and IL-1b, and NO after 24 and 48 hours of treatment. As expected, macrophages stimulated with LPS and treated with dexamethasone also decreased the production of these cytokines and NO. Additionally, FERAI significantly reduced the production of IL-10 at all tested concentrations. Moreover, FERAI inhibited lymphocyte proliferation in a concentration-dependent fashion. **Final Considerations:** The results suggest that FERAI modulates the production of nitric oxide and cytokines by macrophages and reduces lymphocyte proliferation *in vitro*. **Keywords:** Macrophages;Chalcones;Immunomodulation.

ME - 025 - **MMR vaccination increases protection against severe forms of COVID-19. Serum evaluation before SARS-CoV2 specific vaccine introduction**

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Introduction: COVID-19 is the world's serious health emergency since 2019. The possible cross-reactivity of antibodies of MMR immunization and SARS-CoV2 antigens may be an alternative and SARS-CoV2 specific vaccine complement. Objective: To investigate the apparent cross-reactivity of MMR antibodies with SARS-CoV2 antigens. Methodology: This cross-sectional study that recruit 284 donors, presenting positive and/or negative serology for SARS-CoV2 and who have or do not have MMR vaccination serologic signature. The study was develop using as a database professionals from HUPES-UFBA and community. Sera tested to determine SARS-CoV2 IgM and/or IgG as well MMR IgG antibodies. Results: Subjects vaccinated with MMR had less chance to contracting SARS-CoV2, presenting a lower production of IgG class antibodies against S1-RBD and N-protein compared MMR unvaccinated subjects. MMR vaccinated group was asymptomatic to covid-19 compared to MMR unvaccinated group. Conclusion: MMR vaccination have some degree of protection against SARS-CoV2 infection or leads milder symptoms of covid-19. **Keywords:** SARS-CoV-2;cross-reactivity;MMR vacine.

ME - 026 - **Mast cells release DNA Extracellular Traps (DETs) upon activation by SARS-CoV-2**

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Different pathogens activate mast cells to release DNA Extracellular Traps (DETs) composed of chromatin associated with granular proteins like tryptase and LL-37. Activated mast cells also secrete pro-inflammatory cytokines and can induce vascular permeability, facilitating inflammation. In the present work, we evaluated whether SARS-CoV-2 could induce the release of DETs by mast cells. Thus, HMC-1 human mast cell line was stimulated with SARS-CoV-2 in distinct MOIs for 4 h, and DETs quantified by Picogreen and visualized by microscopy. Our results showed DETs production by HMC-1 in all different MOIs tested. We evaluated signaling pathways involved in DET production, such as ROS, calcium, and tryptase activity pre-treating HMC-1 with inhibitors (NAC, DPI, BAPTA, and Nafamostat) before incubation with SARS-CoV-2, and Picogreen measured DET production after 4 h. Our results showed that all inhibitors significantly reduced DET production, suggesting that DET release was ROS, calcium, and tryptase dependent. We also evaluated DETs toxicity to pulmonary cell lines A549 and Calu-3, treating these cells with different concentrations of DETs (50, 100, 200, and 400 ng/ml) for 24 h and measuring LDH release after this period. We observed significant cell death in both cell lines treated with DETs, as indicated by the release of LDH. To summarize, our findings reveal that SARS-CoV-2 induces DET production by mast cells dependent upon ROS, calcium, and tryptase activity and those DETs are toxic to pulmonary cells, possibly contributing to COVID-19 pathology. **Keywords:** MAST CELL;DETs;SARS-CoV-2.

ME - 027 - **Immunomodulation of the immune response in acute murine gouty arthritis through macrophage reprogramming**

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Gouty arthritis is characterized by the deposition of MSU crystals in joints. In the context of gouty inflammation, macrophages become the primary cells involved in the pathogenesis. Macrophages adapt their activation profiles based on the microenvironment and contribute to physiological and pathological processes to achieve homeostasis. The aim of this study was to investigate new therapeutic strategies for gouty arthritis by targeting the regulation of macrophage activity and reducing the acute clinical effects of the disease. The study was approved by the Animal Ethics Committee at the Universidade Federal de Minas Gerais under research protocol number 330/2019. In vitro and in vivo studies were conducted using bone marrow-derived macrophages (BMDMs) and animal models of gout. BMDMs were polarized into different activation profiles, and the expression of markers and cytokines was evaluated, along with the response of macrophages to stimulation with MSU crystals. Initially, MSU crystals were injected into the tibiofemoral joints of female C57BL/6 mice, resulting in tissue inflammation associated with a significant influx of leukocytes, particularly neutrophils. Differentiation of macrophages into a regulatory profile led to a remarkable reduction in IL-12 release and an increase in IL-10 release compared to other macrophage subtypes. In vitro, co-incubation of regulatory macrophages with naive macrophages resulted in a reduction in IL-1β release, a key cytokine in gout inflammation. These preliminary results will guide further investigation into whether regulatory macrophages also reduce inflammation in vivo models of gout involving joint and peritoneal cavities, as well as their ability to reduce activation of the NLRP3 inflammasome pathway. The study highlights macrophages as potential therapeutic targets in gouty arthritis, and these findings may contribute to the development of innovative therapeutic strategies for the condition. **Keywords:** NLRP3;Macrophages;IL-1β.

ME - 028 - **EVALUATION OF IFN-γ AND IL-10 LEVELS INDUCED BY Mycobacterium bovis BCG Moreau NONPOLAR LIPIDS COMPARED TO Mycobacterium tuberculosis**

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), remains one of the main challenges to public health with were 1.6 million deaths and 10.6 million new cases in 2021. Bacillus Calmette-Guérrin (BCG) is the only licensed vaccine against TB, despite its variable efficacy (0-80%) against the pulmonary from of the disease in adults. Recent clinical trials indicate the importance of exploring diverse classes of antigens to increase the vaccine protection already induced by BCG. Thus, lipid antigens are promising, considering their high concentration in the mycobacterial cell wall and central role in host-pathogen interactions. Here, the levels of IFN-γ and IL-10 induced by nonpolar lipids of BCG Moreau and *Mtb* in the supernatant of peripheral blood mononuclear cells (PBMC) cultures from healthy individuals were compared. Nonpolar lipid extracts were obtained from planktonic cultures with the addition of petroleum ether and methanol with 0.3% NaCl (1:10) for cell culture plates sensitization. Culture supernatants were collected to measure IFN-γ and IL-10 levels by ELISA after 24_h, 48_h and 72_h of culture. *Mtb* nonpolar lipid extract induced higher concentrations of IFN-γ and IL-10, unlike BCG Moreau's lipid extract. After 24_h and 48_h, *Mtb* lipid extract induced a higher concentration of IFN-γ, when compared to BCG Moreau (*p<0.05*) and unmarked control (NCS) (*p<0.001*). Furthermore, nonpolar lipids from *Mtb* induced greater productions of IL-10 in all times-points of cultures evaluated, when compared to NCS (*p<0.05* and *p<0.0001*). BCG's lipid extract induced only an increase of IL-10 levels after 72_h (*p<0.0001*). The further characterization of the cellular response induced by mycobacterial nonpolar lipids extracts might facilitate future identification of isolated lipids with greater antigenic potential that could be considered as adjuvants in new vaccine schemes against TB. **Keywords:** BCG Moreau;Tuberculosis;Lipids.

ME - 029 - **Evaluation of the humoral and cellular immune response of the different anti-SARS-CoV-2 vaccine platforms**

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Neutralizing antibodies (nAb) to SARS-CoV-2 induced by vaccines and/or natural infection decline over time, and there is insufficient knowledge of the cellular immune response induced by different vaccine platforms. This study compared humoral and cellular immune responses in individuals vaccinated with different SARS-CoV-2 vaccines. Participants (>18 years old) received two doses of CoronaVac (n=7), ChAdOx1 (n=8), or BNT162b2 (n=21) up to 6 months prior. Percent inhibition (PI) of neutralizing antibodies (nAb) was assessed using the ECO F Covid nAb kit. Interferon-gamma-producing T cells in response to spike protein peptides mega pools (MP) from the original SARS-CoV-2 sequence, as well as the Gamma and Delta variants, were quantified using ELISPOT. Results were expressed as cells forming spots (CFS)/10^6 cells. The mean age in the ChAdOx1 group (37 years) was higher than in the Coronavac (19 years) and BNT162b2 groups (22 years) (p=0.001 and 0.03, respectively). The time between vaccination and enrollment was 6 months for ChAdOx1, 5 months for CoronaVac, and 4 months for BNT162b2 (p=0.01). nAb was detected in 57% of CoronaVac participants and in all ChAdOx1 and BNT162b2 participants (p<0.0001). The median PI in the CoronaVac group (59%, 0-99%) was significantly lower than in the ChAdOx1 (99%, 90-100%; p=0.03) and BNT162b2 (99%, 42-100%; p=0.01) groups. Cellular immune response rates to the original sequence, Gamma and Delta variants were similar between groups (Wuhan – CoronaVac: 59 CFS/10^6 cells; ChAdOx1: 223 CFS/10^6 cells; BNT162b2: 582 CFS/10^6 cells. Gamma variant – CoronaVac: 199 CFS/10^6 cells; ChAdOx1: 163 CFS/10^6 cells; BNT162b2: 722 CFS/10^6 cells. Delta variant – CoronaVac: 83 CFS/10^6 cells; ChAdOx1: 278 CFS/10^6 cells; BNT162b2: 710 CFS/10^6 cells). In conclusion, nAb PI was higher in individuals vaccinated with BNT162b2 and ChAdOx1 and cellular immune response elicited by vaccines against SAR-CoV-2 was similar. **Keywords:** Sars-cov-2;Vaccines;Cellular immune response.

ME - 030 - **Evaluation of the persistence of cellular and humoral response six months after the administration of the SARS-CoV-2 vaccine booster**

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This study assessed the long-term persistence of the cellular (CD8+ T-cell) and humoral response six months after SARS-CoV-2 vaccine booster. Samples were collected from vaccinated (CoronaVac or ChAdOx1) healthcare professionals (n=25) 6 months after the 1st booster (BNT162b2) and up to 3 months after the 2nd booster (BNT162b2 or Ad26.COV2.S) (n=18). IFN-gproducing T-cells in response to mega peptides pools of spike protein (S-MP) from the original sequence and the gamma, delta, omicron, omicron BA.4-5, and omicron XBB.1 variants was quantified using ELISPOT and expressed as spot-forming cells (SFC)/10^6 cells. Percent inhibition (PI) of neutralizing antibodies (nAbs) was quantified using the ECOF COVID nAb kit. To date, nine participants with 1st boosters (CoronaVac+BNT162b2, n=6, and ChAdOx1+BNT162b2, n=3) and thirteen participants with 2nd boosters were evaluated by ELISPOT. The frequency of responders to S-MP was 33% in the CoronaVac+BNT162b2 group and 67% in the ChAdOx1+BNT162b2 group. The CoronaVac+BNT162b2 responded more for gamma (50%), delta (50%), omicron (50%), omicron BA.4-5 (67%), and XBB.1 (83%) variants. In the ChAdOx1+BNT162b2 group, all participants responded to Omicron XBB.1, and the frequency of responders to other variants was similar (67%). The mean SFC in response to MP of the original sequence was higher in the ChAdOx1+BNT162b2 (415.8 SFC/10^6 cells, SD 591.1) than in the CoronaVac+BNT162b2 group (294.2 SFC/10^6 cells, SD 470.5). Mean SFC between groups in response to variants was similar. There was no significant increase in SFC after the 2nd booster. The PI of nAb after the 1st booster was higher in the CoronaVac+BNT162b2 (90%, SD 24) than in the ChAdOx1+BNT162b2 group (71%, SD 27). PI increased after the 2nd booster (83%, SD 27 to 99%, SD 2; $p<0.05$). In conclusion, cellular and nAb responses persisted 6 months after vaccination, regardless of the primary vaccination schedule. The 2nd booster enhanced the PI, but not the cellular response. **Keywords:** Cellular response;Humoral response;Sars-CoV-2.

ME - 031 - **Distribution, activation and development of dendritic cell subsets in sickle cell disease: a comprehensive analysis**

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Sickle cell disease (SCD) results from a beta globin gene mutation, causing abnormal hemoglobin (HbS) and sickling of red blood cells. Prior observations noted alterations in circulating dendritic cells (DCs) in patients, which contribute to systemic inflammation and skewed T cell response to Th17. Our study aims to characterize the development and migration of DC subsets in SCD. We employed a SCD mouse model (Townes - SS) and WT littermates with normal Hb (AA) as controls. Data reveal increased DC numbers in SS mice's spleen and lymph nodes, with decreased levels in the bone marrow, suggesting DC migration. Subset analysis indicates higher levels of type 1 conventional dendritic cells (cDC1) in all three organs of SS mice compared to WT mice. In contrast, plasmacytoid dendritic cells (pDCs) are higher in lymph nodes but lower in the bone marrow of SS mice compared to WT mice. Additionally, SS mice's pDCs exhibit a more mature phenotype in all three organs, with increased Sca-1 expression, while cDC1s display an immature phenotype, evident from lower MHC-II and CD86 expression. Progenitor cell analysis in the bone marrow demonstrates an increased monocyte and DC progenitor (MDP) ratio and a decreased common DC progenitor (CDP) ratio in SS mice, indicating changes in the DC compartment during their developmental stage. In vitro differentiation of DCs from bone marrow cells shows no differences in subset ratios between SS and WT mice, implying that the altered DC development in SS mice is due to environmental factors rather than intrinsic factors. These findings enhance our understanding of DC dynamics in SCD, including the development and migration of distinct subsets, which may significantly impact the observed dysfunction in adaptive immune response during SCD. **Keywords:** Dendritic Cells;Antigen-presenting cells;Sickle Cell Disease.

ME - 032 - **CHRONIC PRODUCTION OF IFN-I IN SICKLE CELL ANEMIA: CELLULAR SOURCE AND PATHOLOGICAL EFFECT**

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Sickle cell anemia (SCA) is a hereditary disease characterized by the sickling of red blood cells that occurs due to the homozygosis of a point mutation on β-globin gene, resulting in the generation of hemoglobin S (HbS). Studies show that SCA patients have an important signature of IFN-I inducing genes in PBMCs. We aimed to investigate the role of plasmacytoid dendritic cells (pDCs) in this process, as they are professional producers of IFN-I and the impact of chronic production of IFN-I in the susceptibility to develop autoimmune disease. We used mice model of SCA, which express HbS and collected blood from HbSS patients to analyze serum and PBMCs. Our data show a significant increase of IFN-α in serum from HbSS patients when compared to controls and a higher production of IFN-α and IFN-β by spleen and lymph nodes cells from HbSS mice upon stimulation. However, cells from bone marrow of HbSS mice showed a reduced production of both cytokines. When HbSS mice were stimulated *in vivo* with CpGA for 4h to induce IFN-I by pDCs, we observed significant inhibition of IFN-I production in HbSS mice compared to HbAA. The amount of pDCs in different organs of HbSS mice was reduced and they showed an activated phenotype even in steady state, as observed by higher Sca-1 expression. These suggest that pDCs are being chronically activated in SCA to produce IFN-I. To investigate whether chronic IFN-I can change the susceptibility of HbSS mice to autoimmunity, we induced experimental autoimmune encephalomyelitis. HbSS mice showed higher weight loss and clinical score than HbAA mice, suggesting higher susceptibility to autoimmune disease in SCA. The levels of IFN-α in serum from HbSS patients correlated with levels of proinflammatory cytokines. HbSS patients have higher percentage of transitional and naive B cells and significative raise in anti-nuclear antibodies, corroborating the hypothesis that chronic production of IFN-I in SCA might turn them prone to autoimmune diseases. **Keywords:** SICKLE CELL ANEMIA;IFN-I IN;pDCs.

ME - 033 - **The role of annexin-A1 in regulating the inflammatory response associated with Graft-versus-Host Disease (GVHD)**

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Introduction: The resolution of inflammation is a crucial process that is governed by endogenous resolving mediators, such as annexin-A1 (ANXA1).These mediators play a crucial role in restoring tissue homeostasis by inducing the cessation of pro-inflammatory mediators, promoting apoptosis, and facilitating efferocytosis. Failure under these conditions can lead to the progression of inflammatory diseases, such as graft versus host disease (GVHD). GVHD is a systemic inflammatory condition secondary to allogeneic bone marrow transplantation, with high morbidity and mortality rate in affected individuals. In this context, our objective is to investigate the role of annexin-A1 in regulating the inflammatory response associated with GVHD. **Methods:** Balb/c recipient WT and ANXA1-/- were lethally irradiated with 7Gys and injected i.v with $1x10^7$ bone marrow cells + $1x10^7$ splenocytes from C57BL/6. The control group received isogenic cells from Balb/c WT mice. Following the transplantation, the recipients were clinically evaluated with a standard scoring system. In another experiment, animals received daily treatment with annexin-A1 mimetic peptide, ac2-26, via intraperitoneal injection (i.p.) at a dose of 150 µg from day 0 to day 22 of the experiment. The target organs were collected at the onset of mortality, and further analyzes were conducted. **Number CEUA:** 22/2020. **Results:** The deficiency of annexin-A1 in the recipient mice led to accelerated mortality and worsened GVHD-related morbidity. Additionally, these mice exhibited elevated intestinal damage, characterized by increased levels of chemokines and neutrophil infiltration in the organ. In contrast, GVHD mice treated with ac2-26 improved survival and clinical score. However, when the treatment was initiated at the onset of clinical signs, the mice were not protected. **Conclusion:** Our data clearly demonstrate an important contribution of annexin-A1 in regulating GVHD inflammation. **Keywords:** RESOLUTION OF INFLAMMATION;GVHD;ANNEXIN A1 .

ME - 034 - **TLR-4 SIGNALING IN HEPATOCYTES, BUT NOT IN IMMUNE SYSTEM, IS THE MAIN DRIVER FOR HEPATIC METABOLIC ALTERATIONS DURING ENDOTOXEMIA**

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INTRODUCTION: The liver is the main body metabolic organ, and hepatocytes – functional units responsible for these hepatic metabolism – constitute about 80% of the cell population present in this organ. However, the liver also harbors a vast population of immune cells that are in constant activity, and how this immune environment can interfere with hepatic metabolic functions is still elusive. Taking advantage of the existence of a common receptor to these two hepatic compartments, called Toll like receptor 4 (TLR-4), we studied their interaction in the hepatic microenvironment. **OBJECTIVE:** To dissect the differential contribution of TLR-4 activation in hepatocyte versus immune system on the hepatic metabolic function. **METHODS AND RESULTS:** C57/BL6 male mice (WT) between 14-16 weeks of age were stimulated by LPS, and 24 hours later had their hepatocytes isolated for evaluation of main hepatic metabolic pathways gene expression. Our data shows that LPS reduces the expression of key genes on hepatic lipid metabolism. Furthermore, the liver of these mice was analyzed by Intravital Microscopy through the fluorescent labeling of lipid droplets by Bodipy. Thus, it was seen that LPS induces hepatic lipid droplets accumulation after 24 hours of stimulation. Lastly, WT mice were chimerized and the following groups were formed: chimerized mice WT>TLR-4-/- and TLR-4-/->WT. These mice were stimulated by LPS, and after 24 hours their hepatocytes were isolated for the same WT analysis. Results showed that only TLR-4-/->WT group reproduces the hepatic lipid metabolic change seen in WT mice. **CONCLUSION:** After inflammatory stimulation via LPS, the liver presents a significant metabolic alteration represented by a reduction in the expression of hepatic lipid metabolic genes and a hepatic lipid droplets accumulation. Still, these modifications appear to be controlled independently of immune system activation, that is, hepatocytes are the protagonists of these modifications. **Keywords:** hepatocytes;TLR-4;lipids.

ME - 035 - **Impact of MR1-blockage on the functional profile of circulating neutrophils and monocytes in Visceral Leishmaniasis**

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Visceral Leishmaniasis (VL) is an infectious disease caused by the Leishmania protozoan. The clinical presentation varies from asymptomatic infection (AS) to classical (VL), reflecting the balance between parasite multiplication in phagocytes and the host immune response. Cytokines from Type 1 activates macrophages, promoting a pro-inflammatory and antiparasitic phenotype, while cytokine from Type 2 favors a modulatory phenotype in macrophages and the success of infection. Therefore, in the infection microenvironment, the interactions among parasites, phagocytes, and lymphocytes are determinants for disease progression. Recently, there has been growing interest in populations of "innate-like" T cells, including MAIT cells, which are significantly found in peripheral blood and the liver. The hypothesis that MR1-restricted MAIT cells recognize Leishmania antigens and presented ability to produce a clinically relevant immune response in VL, underscores the importance of characterizing *in vitro,* through flow cytometry, the impact of MR1 blockade on *L. infantum* internalization, functional profile, and nitric oxide (NO) production by circulating neutrophils and monocytes in individuals with AS and classical VL, before (VL-BT) and after (VL-AT) treatment. In summary, the results indicated that VL-BT and VL-AT patients showed reduced internalization of *L. infantum* by activated, and non-activated neutrophils, and monocytes. Conversely, increased NO production was observed in non-activated neutrophils from the AS and VL groups when stimulated only with *L. infantum*. Activated and non-activated neutrophils from the AS group exhibited decreased TNF-a and TGF-b production, respectively, while monocytes in the AS and VL groups showed increased TNF-a and IL-10 production. Nevertheless, we believe that the MR1 molecule is important for the production and secretion of modulatory and Th17 cytokines, highlighting the protective role of MAIT cells in *L. infantum* infections. **Keywords:** Visceral Leishmaniasis;Fhogocytes;MAIT cells.

ME - 036 - **The adjuvant effect of Coumaric acid and derivatives in iNKT-based immunotherapy**

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Although the activation of invariant Natural Killer T cells (iNKT) by alpha-galactosylceramide (aGC) administration potentializes NK cells and CD8 T lymphocyte cytotoxic activity, the efficacy of aGC-based immunotherapy remains underwhelming. Because we previously demonstrated that the esterification of coumaric acid (p-CA), an abundant phenolic acid found in nature, increased its natural antitumor effect, we aimed to determine the potential of the ethyl and butyl derivatives as adjuvants to increase the efficacy of iNKT-induced cytotoxic responses. First, we treated B16F10 melanoma cells for 16h with p-CA and its derivatives to determine a dose with a low cytotoxic effect and high immunogenic activity. We found that at the concentration of 0.1mM, the treatment with the butyl ester induced the expression of CD1d molecule in B16F10 cells and increased the expression of Fas molecules on their surface. The co-culture of B16F10 cells treated with butyl ester with splenocytes from the Nur77gfp mice revealed a significant increase in the levels of Nur77 in NK cells, indicating that they probably responded to the alteration in Fas expression within the melanoma cells. For notice, the B16F10 cells do not express CD1d molecules on their surface, and the antitumor activity provided by aGC treatment resides in the cascade of events triggered by iNKT activation. Although further experiments are needed to determine the functional activity of iNKT against the treated B16F10, it is correct to suppose that the induction of CD1d molecules in their membrane surface makes them able to present aGC, becoming a direct target for iNKT-mediated cytotoxicity. In conclusion, our data support the notion that the butyl ester coumarate is a potential adjuvant to iNKT-based immunotherapy. **Keywords:** Coumaric acid;Tumor;iNKT-based immunotherapy.

ME - 037 - **INFLUENCE OF ENTEROENDOCRINE HORMONES RECEPTORS ON THE MACROPHAGE POLARIZATION**

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Introduction: Enteroendocrine cells reside in the epithelial layer of the gastrointestinal tract and activate from the presence of nutrients. The enteroendocrine hormones (EEH) can act on immune cells of the intestinal lamina propria or reach the bloodstream. Evidence suggests that some of the EEH have immunoregulatory properties. We hypothesize that cells of the immune system can undergo modulation of their differentiation through the EEH. The aim is to evaluate the expression of EEH receptors GIPR (Gastric Inhibitory Polypeptide), GLP-1R (Glucagon-like Polypeptide 1), and Somatostatin (SST) receptors in M1 and M2 macrophages (MØ). **Methods**: Bioinformatic analyses were used to investigate the EEH receptors differentially expressed between the M1 and M2 MØ, with the GSE57614 dataset. Genes were considered differentially expressed when the adjusted p-value was less than 0.05 and the relative expression logarithm value was greater than 1 or less than -1. Bone-marrow-derived MØ were generated with 20% of L929-M-CSF conditioned medium in RPMI for six days (M0). For M1 polarization, M0 was stimulated with interferon-gamma (10 ng/mL) and lipopolysaccharide (100 ng/mL), and for M2, with interleukin 4 (IL-4, 10 ng/mL) and IL-13 (10 ng/mL) for 24 hours. Treatment with sitagliptin (200 µM), a drug that prolongs the action of GLP-1 and GIP, was done during the polarization. **Results**: The *in silico* analyses revealed higher levels of the SST receptors 2 and 5 in M1 than in M2 MØ, suggesting a modulation of these receptors during the macrophage polarization. The expression of GLP-1R and GIPR did not change comparing the phenotypes. The *in vitro* treatment with sitagliptin revealed an increase in the frequency of CD86+ and CD206+ cells in the M2 group, despite no changes in the CD26+ cells, a molecule target of the sitagliptin drug. **Conclusion**: M1 MØ increased SST receptors, and treatment with sitagliptin modulated M2 MØ polarization. **Funding:** FAPESP 2019/14755-0; 2021/10908-6. **Keywords:** Enteroendocrine hormones;macrophages;incretins.

ME - 038 - **ROLE OF THE ENDOSOMAL TOLL-7 (TLR7) RECEPTOR IN MODULATION OF THE IMMUNE RESPONSE DURING EXPERIMENTAL LEISHMANIA INFANTUM INFECTION**

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Introduction: During parasites infection, besides inciting the innate immunity, TOLL-like receptors (TLRs) also manager different aspects of the adaptive immune response, influencing the chronic inflammation. Depending on the TLR-signaling pathway triggered, the inflammatory response may be permissive, leading to the dissemination of *Leishmania infantum*, the parasite that causes visceral leishmaniasis (VL) in target-organs (i.e. TLR4), or it contributes to control of parasite replication (i.e. TLR2/TLR9). **Aim:** We aims to determine the TLR7 role during experimental VL, since this receptor shares several molecules with other TLRs, promoting susceptibility or resistance to infection. **Method:** TLR7 expression was evaluated during *in vivo* and *in vitro* L. *infantum infection* in myeloid cells. TLR7 role upon the innate and adaptive immune response modulation was determined in an experimental model using $TLR7^{-/-}$ and WT mice at different weeks of infection (wpi). **Results:** TLR7 was preferentially expressed in neutrophils, macrophages and conventional DCs (cDC) on target-organs of infected WT mice during kinetic assay, comparing to uninfected animal. The data were confirmed by *in vitro* infected bone-marrow-derived dendritic cells (BMDCs) and BM-isolated neutrophils, compared to control group. Interestingly, the TLR7 absence increased Th1-infflammation and $GZB^{+}Perf^{+}CD8^{+}$T lymphocytes, reflecting an enhanced frequency of $iNOS^{+}CD11b^{+}$ cells. **Conclusion**: Altogether, we demonstrated that TLR7 subverts the effector response of Th1 and $CD8^{+}$T lymphocytes during parasite infection. We will focus on the role of TLR7-induced IFN-I as adaptive immunity regulator. **Keywords:** Visceral leishmaniasis;Inflammation;TLR7.

ME - 039 - **INFLUENCE OF BCG (BACILLUS CALMETTE-GUÉRIN) ON THE MATURATION OF HUMAN DENDRITIC CELL (DCs) DIFFERENTIATED FROM MONOCYTES**

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INTRODUCTION AND OBJECTIVES:DCs are obtained in vitro through monocyte differentiation, previous studies show the maturation of DCs by live BCG, but for models targeting immunosuppressed patients, the maturation of DCs by dead BCG needs to be investigated.In this work we evaluated the phenotypic and functional characteristics of human DCs in vitro stimulated by dead BCG.**METHODOLOGY:**DCs were obtained from the isolation of monocytes from the blood of healthy donors.After the isolation step,monocytes were cultured with IL-4 and GM-CSF for differentiation into immature DCs.For terminal maturation,TNF-α and IFN-α(DC-TNF) or killed BCG(DC-BCG) were added.After maturation,DC-TNF and DC-BCG were co-cultured for 5 days with autologous and allogeneic lymphocytes previously stained with CFSE fluorochrome.To phenotypically characterize DCs, after their terminal maturation, some markers were investigated.The strain used was the lyophilized Moreau-RJ.**RESULTS:**DC-TNF and DC-BCG cultures showed rates of cells expressing HLA-DR:98% and 98%;CD1A:69% and 72.37%,CD86:97% and 98%,CD80:39% and 36%,CD40:21% and 15%,PD-1:18% and 14% and CD83:55% and 47%, respectively.There was a significant difference in the expression of percentage values only for CD80.MFI(mean fluorescence intensity) levels in DC-TNF and DC-BCG were high in HLA-DR:9251 and 6867, in CD86:12975 and 10772,and in CD1A:6242 and 5694, respectively.DC-TNF and DC-BCG had MFI for PD-1:755 and 1156; CD40:877 and 745, in CD80:753 and 598, and in CD83:624 and 1529, respectively.There was a significant difference in HLA-DR MFI levels.In lymphocyte proliferation DC-BCG showed greater proliferation of autologous lymphocytes:11% and 21% allogeneic, while DC-TNF cells:5% autologous and 16% allogeneic. **CONCLUSION:**The use of dead BCG promotes the maturation of DCs at levels equivalent to TNF.But BCG potentiates lymphocyte proliferation at a higher rate. To interpret the results obtained,a study of cytokine production will be performed. **Keywords:** dendritic cell;BCG ;lymphocyte proliferation .

ME - 040 - **Evaluation of the role of NRLP12 and miR-294 in macrophage activation and susceptibility to infection with Leishmania amazonensis**

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Leishmaniasis is a set of parasitic diseases caused by protozoa of the genus *Leishmania spp*. As a clinical manifestation, leishmaniasis can cause cutaneous, mucocutaneous and visceral lesions. *Leishmania amazonensis* causes cutaneous leishmaniasis. These parasites mostly infect macrophages and grow within the phagolysosome, altering the pro-inflammatory signaling, such as inflammasome formation by directly inactivating NOD-like-receptors, caspase-1 and cytokines. Another mechanism is the transcription of microRNAs (miRNAs), small noncoding RNAs, which can decrease gene expression by pairing its seed with the target 3'UTR of mRNA. Previously, we observed a differential expression of miR-294 family in murine macrophages infected with *L. amazonensis.* The miR-294 family is transcribed from the antisense region of the *NLR family gene, pyrin domain containing 12 (Nlrp12 or Nalp12)* in *Mus musculus.* Here, we aim to evaluate the function of NALP12 and microRNA-294 in regulating the response of macrophages infected with *L. amazonensis.* We analyzed the expression of *Nalp12*, *Nalp3*, premiR-294, miR-294 and *Il-1b* in macrophages from BALB/c mice infected with *L. amazonensis* or *L. infantum* (MOI 5:1) or stimulated with 100ng/uL of lipopolysaccharide (LPS) by RT-qPCR. We observed that infection of macrophages with *L. amazonensis* and *L. infantum* induced *Nalp*12, premiR-294 and miR-294. However, we did not observe a significant modulation of *Il-1b*, *Il-18* and *Nalp3* in infected macrophages. As for macrophages stimulated with LPS, the expression of *Il-1b* was significantly increased at 4 and 24 hours. Curiously, the expression of *Nalp12*, premiR-294 and miR-294 was not modulated in LPS stimulation.Our data suggest that infection with *L. amazonensis* regulates the expression of NALP12 and miR-294 in a different way compared to observed with LPS stimulation, suggesting that they are targets of the subversion mechanism of the immune response. **Keywords:** Leishmania;microRNA;NALP12.

ME - 041 - **Measles, Rubella and Mumps particles are capable of inducing B cells proliferation from SARS-CoV2 convalescent or vaccinated individuals**

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Introduction: Cross-reactivity caused by similar viral antigens leading antibody production are observe in several pathologies including SARS-CoV2 infection. This seems to occur with the measles, rubella and mumps viruses contained in the MMR vaccine. The cross-reactivity MMR immunization within SARS-CoV2 antigens may be an alternative vaccine complement to specific vaccines for SARS-CoV2. Objective: To investigate the ability of MMR antigens to induce the proliferation of B cells able to produce antibodies against SARS-CoV2 antigens in convalescent Covid-19 patients and vaccinated subjects. Methodology: Cross-sectional study that has recruited 24 donors so far, with positive and/or negative SARS-CoV2 serology and who have or do not serological MMR signature. PBMC cultures were labeled using MitoTracker™ (Invitrogen), upon different MMR concentration. Herein, cell proliferation by FACS and S1, S2 and N-protein of SARS-CoV2 antibodies measured by ELISA. Results: B cells from SARS-CoV2 vaccinated and/or convalescent individuals for undergo proliferation when stimulated with 25 and 50 IU/mL of MMR. These cells produce antibodies of the IgG class against S2 and N-protein of SARS-CoV2. MMR (+) Covid19(-) control group showed proliferation with production of anti-SARS-CoV2 antibodies. Conclusion: MMR antigens induces B cells proliferation and antibodies that present cross-reactivity against SARS-CoV2 S2 and N-protein. **Keywords:** SARS-CoV-2;B cells;MMR.

ME - 042 - **Evaluation of DENV replication in a platelet-monocyte interaction model.**

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Dengue virus (DENV) and host immune factors are responsible for dengue disease, which vary from asymptomatic infection to severe haemorrhagic fever. DENV can infect various cells, such as monocytes, macrophages and endothelial cells. DENV replication in monocytes is amplified in antibody-enhanced dengue infection. Platelets from dengue-infected patients are activated, apoptotic and secret inflammatory mediators. These signals modulate platelet-monocyte interaction regulating monocyte inflammatory response. Platelets sustain DENV genome translation and replication, but not a productive cycle. We aimed to establish a protocol for DENV replication in monocytes and to evaluate if platelets modulate virus replication by transferring newly synthesized viral RNA to monocytes or by regulating monocyte permissiveness *in vitro*. Primary human monocytes isolated from healthy donors through plate adhesion were cultured with human serum (HS) or foetal bovine serum (FBS) and infected with DENV-2 without specific antibodies. DENV RNA copies were measured in cell lysate and supernatant by qRT-PCR and infectious viral particles by plaque forming units (PFU). DENV replication and infectious viral particles were higher in HS-cultured monocytes and increased linearly overtime. Platelets were infected with DENV-2 and, after washing out the unbound viruses, cocultured with monocytes. After 24, 48 and 72h of interaction we quantified DENV RNA and PFU. DENV RNA levels were increased in infected-platelets after 24 and 48h, but reduced in 72 h. However, only basal levels of viral RNA were observed in monocytes after coculture. No infectious viral particles were detected in platelet or coculture supernatants. Our data demonstrate that platelets do not transfer DENV genome to monocytes in a way they are able to continue the viral replication. We will now assess if platelets modulate the monocytes` permissiveness to DENV infection and, if so, which pathways participate in this modulation. **Keywords:** Platelets;Monocytes;Dengue virus.

ME - 043 - **T LYMPHOCYTE EXHAUSTION DURING IN VIVO LEISHMANIA INFANTUM INFECTION**

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Introduction: The inhibitory receptors expression is important for controlling self-tolerance and preventing immunopathologies development. During a chronic inflammatory process, frequent exposure to antigen induces leucocytes to express inhibitor molecules (CTLA-4, PD1, TIGIT, TIM-3) promoting an exhaustion process, making them unresponsive. Patients with visceral leishmaniasis (VL) have defective cellular immune response and high expression of inhibitory receptors, suggesting that exhaustion occurs during the disease progression. **Objective**: We aim to determine the role of PD-1 and CTLA-4 in the exhaustion of T lymphocytes during experimental VL. **Method:** The kinetic of inhibitory receptors expression was determined in different leukocytes present in target-organs during experimental VL in C57/BL6 mice. Results: Parasite infection significantly increased both $CD4^{+}$T and $CD8^{+}$T expressing CTLA-4 and PD1 in the spleen at the 4^{th} weeks post-infection (wpi) and at 8^{th} wpi into liver. Other inhibitory receptors were also positively expressed on lymphocytes along the disease (i.e. TIGIT on both lymphocytes subsets) in target organs. Among the myeloid cells phenotyped, PDL1/PDL2 expression was mostly detected in neutrophils, macrophages and cDCs. **Conclusion:** Altogether, we concluded the inhibitory receptors involved in the exhaustion of T lymphocytes are positively modulated during chronic inflammatory process on VL disease. The inhibitory molecules role will be determined by *in vivo* treatment with neutralizing antibodies to PD1, CTLA-4 or in association. **Keywords:** Leishmania;Immunology;inhibitory receptors.

ME - 044 - **EVALUATION OF CORONAVAC IMMUNOGENICITY IN MICE GENETICALLY SELECTED FOR HIGH OR LOW ANTIBODY PRODUCTION**

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CoronaVac is an inactivated SARS-CoV-2 virus vaccine, developed to control the COVID-19 infection. Most vaccines confer protection by the production of neutralizing antibodies (nAbs), however, there is a high heterogeneity of the immune response among the population. Mice phenotypically selected for High (H_{III}) or Low (L_{III}) antibody production were obtained by bidirectional genetic selection; they are heterogeneous strains that represent a population with different patterns of immune response. The objective is to evaluate the mechanisms involved in the adaptive immune response to CoronaVac in these strains of mice. Mice were immunized with two doses of CoronaVac or aluminum hydroxide, a booster was administered on the 21st day and serum samples were collected on the 7th, 28th, and 35th day. ELISA measured IgG and subclasses antibody titers against RBD protein. H_{III} and L_{III} mice secreted high IgG levels after immunization and no antibody was detected in the control group. A similar increase was observed with IgG1 and IgG2a, however, H_{III} mice produced more IgG2a than L_{III}. The IgG antibody binding strength to the RBD was measured by avidity assay, both vaccinated strains demonstrated a high antibody avidity after the booster. These results corroborate with the quantification of nAbs by pseudovirus assay, both strains showed high nAbs titer. Cellular immunity was analyzed by the phenotype of lymphocytes ($CD4^+$, $CD8^+$, and $B220^+$) in lymph nodes and spleen at different time points. Preliminary results showed differences in the cell types between the strains, the number of cells in H_{III} mice was higher than in L_{III} mice in the lymph node and no difference was observed in the spleen. Our results indicated the ability of CoronaVac to induce antibody responses in L_{III} mice since the levels of IgG and IgG1 were similar to H_{III} mice. CoronaVac was effective in inducing anti-RBD antibodies with high avidity and neutralizing capacity, even in low-responder mice. **Keywords:** mice genetically selected;CoronaVac;antibody.

ME - 045 - **ROLE OF miRNAs IN THE ALTERATION OF CYTOKINE PRODUCTION IN MESENCHYMAL STEM CELLS INDUCED BY SEPSIS**

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells with a great capacity for proliferation, self-renewal and differentiation. They are easy to obtain and due to their immunomodulatory and antimicrobial characteristics, they are strong candidates as new therapeutic instruments to treat sepsis. In this context, the interaction of sepsis with bone marrow mesenchymal stem cells (BMSCs) was investigated. BMSCs were obtained from femurs of Balb/c male mice with moderate sepsis, induced by 8 hours of cecal ligation and puncture (CLP) using a 18G gauge needle and 2 punctures. Sham operated group was used as control. Sepsis did not cause morphological changes in BMSCs and did not alter cell cycle profile (proliferation and death). However, cytokine and chemokine expressions from septic BMSCs were decreased when compared to control BMSCs. Among the 32 proteins investigated, 28 had decreased expression in septic cells. Notably, there was a substantial reduction in the expression of pro-inflammatory cytokines like TNF (±66%), IFN-γ (±65%), and IL-1β (±66%) ($p \leq 0.001$ for TNF and IFN-γ; $p \leq 0.01$ for IL-1β). In order to investigate the mechanisms involved we evaluated the participation of miRNA in the modulation of cytokine production in septic BMSCs. Using PCRArray technique, we analyzed 84 miRNAs related to inflammatory response and autoimmunity. The results revealed decreased expression of miR-140-5p (28%) and miR-26b-5p (12%), and an increase in miR-9-5p (18%) in septic BMSCs when compared to control. These findings suggest that miRNAs are involved in the regulation of cytokine expression in BMSCs obtained from animals with moderate sepsis. In order to validate the hypothesis, further analyses using inhibitors of these specific miRNAs will be conducted. These experiments aim to provide additional evidence of the role of miRNAs in modulating the immune response of BMSCs, thus shedding light on potential avenues for manipulating these cells in sepsis treatment. **Keywords:** sepsis;mesenchymal stem cells;epigenetics.

ME - 046 - **TAM receptor-mediated efferocytosis regulates lung inflammation during the early stages of cigarette smoke exposure in mice**

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Cigarette smoking (CS) causes lung inflammation, cell death, and tissue damage, and it is the leading cause of chronic obstructive pulmonary disease (COPD). Alveolar macrophages (AMs) play a key role in CS-induced inflammation by phagocyting cigarette-derived particles, microorganisms, and apoptotic cells. The TAM receptor family (Tyro3, Axl, and MerTk) mediates efferocytosis and inhibits proinflammatory pathways by binding to phosphatidylserine on apoptotic cells via Gas6 or Protein S, making it one of the most important regulatory mechanisms for regulating inflammation. Indeed, individuals exposed to CS have lower Axl expression, which correlates with the severity of COPD. Here, we investigated the role of efferocytosis mediated by Axl and MerTk receptors in the establishment of pulmonary inflammation by CS (acute model). We first found that CS extract (CSE-10%) increased nitric oxide production and induced apoptosis and necrosis in AMJ2-C11 cell line compared to the controls *in vitro*. For *in vivo* short-term CS exposure, WT, Axl-/-, and MerTk-/- mice were exposed to nine Malboro cigarettes or filtered air (control) per day for four days. We found that CS exposure led to higher levels of cells in the BALFs of Axl-/- mice compared to MerTk-/- or WT mice. Furthermore, we found that CS increased the number of AMs, necrotic and apoptotic AMs, IMs, and neutrophils in both Axl-/- and MerTk-/- mice compared to WT mice. Finally, we found more AMs CD11b+Ly6C+ in Axl-/- mice than in MerTk-/- or WT mice, indicating that this group recruits more monocytes that differentiate into AMs during CS exposure. Our preliminary data suggests that TAM receptor-mediated efferocytosis is important for controlling inflammatory responses during the early stages of CS exposure. **Keywords:** TAM receptor;Efferocytosis;Cigarette smoke.

ME - 047 - **MUTATIONS IN THE AUTOIMMUNE REGULATOR (AIRE) PHD-1 DOMAIN AFFECT MEDULLARY THYMIC EPITHELIAL CELL MORPHOLOGY, PROLIFERATION AND EXPRESSION OF MHCII AND CD80**

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The diversity of T cell receptors (TCRs) is generated in the thymus, conferring a variety of T cell clones recognizing a wide range of pathogens. However, T cell clones expressing TCRs that recognize self-antigens are inevitably generated. In the thymic stroma, the medullary thymic epithelial cells (mTECs) express the autoimmune regulator protein (AIRE), which is the main transcriptional controller of peripheral-tissue antigens (PTAs). The PTA expression and its presentation by mTECs allow the negative selection of autoreactive T cells that recognize PTAs (Trends in Immunology. 38:805-816, 2017). The plant homeodomain 1 (PHD-1) is an AIRE protein domain that plays a critical role during the interaction of AIRE with the chromatin of mTECs. It preferentially recognizes regions associated with silenced PTA genes, allowing for their accessibility and regulation. Mutations in the AIRE PHD-1 domain found in many autoimmune disease patients demonstrate the crucial role of this domain (Immunobiology 224:728-733, 2019). To evaluate the effects of AIRE PHD-1 mutations on mTEC function, clones of mTECs presenting mutations in the AIRE PHD-1 domain were generated using the CRISPR-Cas9 system. Two clones with distinct base pair deletions were selected for further analysis. One of the mutant mTEC clones express a truncated AIRE protein (two base pair deletion at position 5.676 and 5.677), while the other had a frameshift mutation (one base pair deletion at position 5.675). Our *in vitro* studies showed that these mutations affect the mTEC cell morphology and decrease cell proliferation rate. Furthermore, cytometry analyses showed a significant decrease in cells expressing major histocompatibility complex class II (MHC II) and CD80 in both mutant clones. A reduction of MHCII or CD80 was pronounced when mTECs were co-cultured with thymocytes, suggesting that mutations in the AIRE PHD-1 domain may harm mTEC self-antigen presentation and generation of self-tolerance. **Keywords:** Aire;PHD-1 domain;mTECs.

ME - 048 - **Characterization of Corynebacterium diphtheriae DIP0733 adhesion protein, a vaccine antigen candidate**

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Corynebacterium diphtheriae is the etiological agent of diphtheria, a vaccine preventable respiratory disease. Despite the reduction of disease cases since the introduction of vaccination using bacterial toxoid, the isolation of the bacteria from several infections persists, with an increase in the number of cases. Besides, the atoxigenic bacteria has also been isolated, which encourages the study of other bacterial virulence factors as an alternative or complement for vaccine against the disease. Therefore, we aimed to clone and purify the surface adhesin (hemagglutinin) DIP0733, and investigate some preliminary biological effects of this protein. For this purpose, the extracellular portion of the DIP0733 protein sequence, with the addition of a histidine TAG sequence (HisTag), was cloned into plasmid Pet28a and the expression performed in ***Escherichia coli*** BL21(DE3) cells. Then, the protein extraction was performed in a potassium phosphate buffer (pH: 7.2), with sonication at an amplitude of 40%. Afterwards, the material was purified in a liquid chromatography system (AKTA pure) using HisTrap HP columns. Yield and quality of purification were evaluated by SDS-PAGE and Western blot for His-Tag. Preliminary biological assays with purified DIP0733 were (i) cytotoxicity test with lineage cells (VERO, AMJ2C11, RAW and A549) using MTT, (ii) RAW macrophage activation assay, for nitric oxide (NO) detection and (iii) hemagglutination assay with B and AB human erythrocyte. Cloning and purification was successfully performed as shown by Western blot analysis. No significant cell cytotoxicity effects, using DIP0733 concentrations ranging from 10 to 100µg/mL was detected.Purified DIP0733 induced NO production by RAW macrophages and was not able to hemagglutinate the human erythrocytes tested. In conclusion, the DIP0733 protein was successfully cloned and purified and will be further used as a vaccine antigen for mouse immunization tests. **Keywords:** Vaccine; Diphtheria;DIP0733.

ME - 049 - **Assessing the impact of Favipiravir on Zika virus in Aedes aegypti**

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The high error rate replication of RNA-dependent RNA polymerase (RdRp) leads to the generation of a diverse viral population known as quasispecies. The high diversity of quasispecies is crucial for arboviruses, enabling their adaptation to different environments and facilitating the alternation between mammalian and arthropod hosts, as well as their interaction with the respective immune systems. Drugs that interact with RdRp, such as favipiravir, a purine analogue, induce higher mutation rates and alter the composition of the viral quasispecies. Consequently, this increases the ratio between infectious and defective particles, ultimately increasing the amount of available PAMPs (RNA) and reducing the virus's ability to evade the immune system. Defective viral particles cannot infect new cells, but their genomes are recognized by Dicer-2 and activate the RNAi pathway in the mosquito host, which can impair infection. Therefore, we tested the effect of favipiravir on Zika vírus (ZIKV) during *Aedes aegypti* mosquito infection. For this, we incubated ZIKV with favipiravir in Vero cells and used the resulting virus to infect *A. aegypti*. ZIKV incubated in the presence of favipiravir (ZIKVfav) replicated about ten times less compared to the control group. When the mosquitoes were infected with ZIKVfav, a lower prevalence of infection was observed, with nearly half the number of infected animals compared to the control group. Among mosquitoes that were infected, there was no significant difference in viral load. Therefore, exposure of ZIKV to favipiravir reduced the prevalence of infection but not the viral titer in infected mosquitoes. This indicates that altering the quasispecies impairs the fitness of the virus, however, after establishing the infection this barrier is overcome. To further understand the relationship between the observed phenotype of ZIKVfav and the immune system, we aim to investigate the role of the RNAi pathway in mosquitoes. **Keywords:** Quasispecies;Innate immunity;RNAi.

ME - 050 - **The role of IL-17 and IL-22 cytokines in the gut-liver axis during autoimmune hepatitis**

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Autoimmune hepatitis (AIH) is an inflammatory multifactorial disease with unclear etiology. AIH physiopathology is characterized by exacerbated inflammation mediated by innate and adaptive immune cells, which ultimately induces hepatocyte death and liver failure. Although the mechanisms driving the inflammatory responses remain lacking clarity, a growing body of evidence supports the idea that gut-liver axis alterations contribute to tissue damage. Because the role of IL-17 in hepatitis appears to be controversial and IL-22 has been associated with bacterial control, we aim to investigate their participation in a model of AIH induced by concanavalin A (ConA) administration. C57BL6/J mice from wild type (WT), IL-17A/F-/-, IL-17R-/- and IL-22-/- strains received a non-lethal dose of ConA (14mg/Kg) intravenously, and the liver function, characterized by ALT and AST levels in serum, was determined 12h later. Although the WT and IL-22-/- mice exhibited similar levels of liver injury upon ConA administration, the absence of IL-17 signaling, both in the IL-17A/F-/- and IL-17R-/- mice, resulted in exacerbated tissue damage, with higher levels of ALT and AST levels compared to observed in the other mice strains. The colony-forming assay revealed that although IL-22-/- mice exhibited the highest number of liver resident bacteria at the steady state, it was unrelated to liver dysfunction. In contrast, following ConA administration, the number of hepatic colony-forming units was significantly lower in animals lacking IL-17 signaling compared to the other mice strains, suggesting a higher microbicidal activity. Considering the relationship between immune response and inflammation, we hypothesize that IL-17 mitigates the inflammatory response triggered by ConA administration. Although the mechanisms leading to these discrepant responses observed among these three mice strains remain to be elucidated, our data corroborate a protective role for IL-17 during AIH. **Keywords:** autoimmune hepatitis;IL-17;IL-22.

ME - 051 - **Generation of allogenic 19bbz car-t cells with crispr**

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INTRODUCTION AND OBJECTIVES: The use of autologous T cells bearing CAR, synthetic constructs that redirect lymphocyte specificity to tumor membrane antigens shows good results, although it presents some limitations. To circumvent these, allogenic CAR-T cells can be made. In order to avoid GVDH, genes encoding the TCR can be knockout (Ko) and the CAR transgene be delivered through a viral approach, transposons, such as Sleeping Beauty (SB), or donor DNA (dDNA). The use of SB can optimize the production of CAR-T since it is cheaper and less laborious compared to viral assembly and delivery. The use of dDNA can improve the cells generated by knock-in (KI) of the transgene in specific genetic sites. The objective is the generation of allo 19BBz CAR-T cells KO for TCR via CRISPR and CAR+ via SB or KI. **RESULTS AND CONCLUSION**: The editing system was optimized and tested in Jurkat, which achieved 41 days post-electroporation 70% of KO, with better results with a reason of 1:3 (Cas9/gRNA). Using PBMC as starting material, the rate of CD3 negative (CD3-) cells was 38%, and after the expansion, the rate of KO in T cells was 20%. The SB transposon carrying CAR was co-delivered with the RNP achieving 20% of CD3- cells. The editing system alone shows 9% KO, but when we used it along with SB the KO rate was 21%, with higher CAR expression in the CD3- subpopulation. We used a mock donor DNA sequence that impaired CAR expression to test the KI and compare it with KO rates. This condition with a donor DNA generated a stable population of 60% of CD3 negative cells throughout the expansion. We can conclude that the editing system works in the Jurkat, PBMCs, and CD3 purified population. It was possible to generate allo CAR-T cells with the SB system, CD3- population showing an advantage in the expression of the CAR molecule. The mock KI promoted higher KO rates, stabilizing the CD3- population during the expansion. **Keywords:** CRISPR;CAR-T;CANCER.

ME - 052 - **Immunomodulatory role of purified capsular polysaccharides from Cryptococcus neoformans on macrophages infected with Leishmania major.**

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Cryptococcus neoformans is an encapsulated yeast that causes infection in humans and animals. Its capsule, an important survival factor in the host, is mainly composed of two polysaccharides, glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal). Different types of cells are targeted by GXM and GXMGal, such as macrophages, with GXM being capable of inducing an immunosuppressive response, stimulating the secretion of anti-inflammatory cytokines, such as IL-10, while GXMGal, which is immunoactivating, promotes the increase in pro-inflammatory cytokines. For this reason, we considered evaluating the role of these polysaccharides in macrophages infected by *Leishmania major (L. major*), since the macrophage is the main host cell of these parasites and the response profile of these cells is important for the resolution or maintenance of the disease infection. The first objective was to analyze the parasite burden of the extracellular form of *L. major* (promastigotes) in macrophages that were treated with GXM or GXMGal in the presence or not of the IFN-γ. As a result, a reduction in parasite load was observed in infected cells treated with GXMGal, differently of infected cells treated with GXM that induced a higher parasite release. From this, we evaluated the profile of these macrophages. We measured nitrite, IL-10, TNF-α, TGF-β and PGE2. Our data revealed that the GXMGal favors the activation of infected macrophages, controlling the infection by *L. major*. **Keywords:** Capsular Polysaccharides;Leishmania major;Macrophahes.

ME - 053 - **GENERATION OF CAR-T CELLS AND CAR-MYELOID CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSC)**

CATHARINO, L.C.C.[1]; RIBEIRO, P.D.A.C.[1]; EVANGELISTA, G.C.M.[1]; ALMEIDA, R.R.[2]; ROCHA, V.G.[2]; OLIVEIRA, T.G.M.[2]; RAMOS, R.N.[2]; BARBUTO, J.A.M.[1]. 1. INSTITUTE OF BIOMEDICAL SCIENCES OF THE UNIVERSITY OF SÃO PAULO, INSTITUTE OF BIOMEDICAL SCIENCES OF THE UNIVERSITY OF SÃO PAULO SÃO PAULO - SP - BRASIL; 2. LIM-31 / HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, LIM-31 / HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO SÃO PAULO - SP - BRASIL.

Among immunotherapies, the Chimeric Antigen Receptor (CAR)-T cell therapy has been standing out in the past few years, and, more recently, other promising CAR-modified cells are also being tested in the clinic. The use of autologous cell sources for the generation of the CAR-modified cells present limitations, specially manufacturing ones, such as the high cost and extensive production time, the presence of dysfunctions and limited number of these cells in the peripheral blood of the patient, and the possibly restricted proliferative potential of these cells. The use of human Induced Pluripotent Stem Cells (hiPSCs) for the generation of the CAR-modified cells is an interesting alternative, since they can proliferate with indefinite potential, differentiate to various cell types and also have good susceptibility to genetic manipulation. Our project aims to generate a platform for the production of CAR-modified immune cells from hiPSC to treat cancer. We have successfully expanded a hiPSC clone and differentiated it into CD34+ hematopoietic progenitor cells through the formation of embryoid bodies, using ultra-low attachment plates and StemPro-34 SFM medium, supplemented with GlutaMAX, 2-mercaptoethanol, NEAA, L-Ascorbic acid and the cytokines BMP4, FGF2, VEGF, SCF, FLT3l and IL3. At differentiation day 9, the EBs were dissociated and the resultant cells were evaluated by flow cytometry. The same hiPSC clone has also been successfully transduced with a GFP encoding plasmid using both lipofection or electroporation methods. Our preliminary data show that the differentiation process led to the generation of about 7,86% (± 3,4) of CD34+ cells and that the transduced plasmid is able to integrate in the cell genome, presenting stable expression after 15 days. In future steps, the hiPSC will be transduced with a CAR encoding plasmid, and the differentiated CD34+ cells will be sorted, expanded and used for further differentiation into T lymphocytes or myeloid cells. **Keywords:** Human Induced Pluripotent Stem Cells (hiPSC);CAR-T cells;Immunotherapy.

ME - 054 - **EFFECT OF Aedes aegypti SALIVARY COMPONENTS ON NEUTROPHIL BIOLOGY: PRELIMINARY RESULTS**

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Introduction: *Aedes aegypti* is a known vector of arboviruses such as dengue, yellow fever, Zika and chikungunya. The transmission of such diseases occurs during the feeding of female mosquitoes which is dependent on salivary molecules presenting immunomodulatory, anti-hemostatic and anti-inflammatory properties. Considering this immunomodulatory potential identified in saliva of *Ae. aegypti*, the goal of this work was to evaluate the effect of its salivary gland extract (SGE) on parameters of neutrophil biology. **Methods:** Neutrophils were isolated from mouse bone marrow by Percoll gradient and immunophenotyped by flow cytometry. The obtained cells were cultured in the presence or absence of *Ae. aegypti* SGE for 1 hour followed by stimulation with inflammatory stimuli. The cultures were maintained for 4 and 24 hours, when the cells were harvested for flow cytometric analysis and the supernatant was collected for IL-10, TNF-α, IL-12 and IL-1β determination by ELISA. **Results:** The purity of neutrophil isolation was around ~80%, confirmed by flow cytometry on the $Ly6G^{+}/CD11b^{+}$ cell population. The median of fluorescence intensity (MFI) of CD11b and CD62L markers were compared in the different groups and an increase in CD11b MIF and decrease in CD62L was observed under stimulation, when compared to the unstimulated control, with no significant differences regarding the SGE preincubation. Likewise, no significant differences were observed in the cytokine production in the presence of absence of *Ae. aegypti* SGE. **Conclusion:** *Ae. aegypti* salivary components do not seem to affect the expression of markers associated with neutrophil activation. The results obtained are still preliminary and new experiments will be performed to evaluate the SGE activity on other parameters of neutrophil biology such as reactive oxygen species, neutrophil extracellular traps and other cytokines. Financial support: CAPES, CNPq and FAPESP. **Keywords:** Aedes aegypti;Saliva;Immunomodulation.

ME - 055 - **Evaluation of reactive oxygen species and nitric oxide production in macrophages from patients with american tegumentary leishmaniasis after infection with different isolates of Leishmania (Viannia) braziliensis**

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INTRODUCTION: American Tegumentary Leishmaniasis (ATL) is a non-contagious infectious disease caused by *Leishmania*. These parasites can cause a wide spectrum of diseases in humans, such as cutaneous (CL) and disseminated (DL) leishmaniasis. Studies have shown that the immune response during ATL varies among different clinical forms of the disease with differences in oxidative response. The hypothesis tested was that the production of reactive oxygen species (ROS) and nitric oxide (NO) in macrophages from DL patients differs from that observed in macrophages from CL patients. **OBJECTIVE:** To evaluate the production of ROS and NO after infection with isolates of *L. (V.) braziliensis*, obtained from different clinical forms, in macrophages from CL and DL patients, as well as healthy individuals. **MATERIALS AND METHODS:** The production of ROS and NO in macrophages from CL and DL patients, as well as healthy individuals, was evaluated. The results were obtained using Flow Cytometry and Confocal Microscopy, employing fluorescent probes to quantify ROS, including superoxide (O_2^-) and hydrogen peroxide (H_2O_2), as well as NO. **RESULTS:** The evaluation of ROS production over 2, 4, and 8 hours of infection revealed a similar behavior between macrophages from CL and DL patients. However, a distinct behavior was observed in the macrophages from CL patients compared to macrophages from healthy individuals during the first 2 and 4 hours of infection with the DL isolate. As for NO production, a similar behavior was observed between macrophages from CL, DL patients, and healthy individuals. **CONCLUSIONS:** The production of ROS and NO in macrophages from DL patients showed no significant differences compared to macrophages from CL patients, even after infection with isolates of CL and DL. However, statistically significant differences in ROS production were found between macrophages from CL patients and those from healthy individuals after infection with DL isolates. **Keywords:** LEISHMANIASIS;REACTIVE OXYGEN SPECIES;NITRIC OXIDE.

ME - 056 - **CARBONIC ANHYDRASE IX INHIBITION REGULATES IMMUNE CHECKPOINT AND TUMORIGENESIS MEDIATORS**

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About 95% of clear cell renal cell carcinoma (ccRCC) cases have a constitutive expression of an enzyme called carbonic anhydrase IX (CAIX) responsible for pH control and subsequent tumor development. Moreover, a well-known tumorigenic enzyme called cyclooxygenase -2 (COX-2) is expressed in about half of the ccRCC patients. These renal tumors also present a complex and immunosuppressive microenvironment. About 56% of ccRCC cases overexpress the programmed cell death receptor-1 (PD-L1), which interacts with the programmed cell death receptor-1 (PD-1), located mainly on T cells, favoring the exhaustion of these cells, which becomes incapable of curbing tumor development. To determine if there was a regulatory relationship among these molecules, we promoted CAIX inhibition to check their effects on PD-L1 and COX2 in ccRCC. Two renal tumor cell lines, SKRC 52 and SKRC 59, both positive for CAIX, COX2, and PD-L1, were tested. The CAIX, PD-L1, and COX-2 expression was assessed through immunofluorescence and flow cytometry of these cells before and after the treatment with two different anti-CAIX monoclonal antibodies in variable doses. The blockade of CAIX engendered a dose-dependent diminution in the PD-L1 and COX-2 expression levels in both ccRCC cell lines, potentially hampering T cell exhaustion and tumorigenesis, avoiding subsequent tumor progression. These results indicate that individuals harboring CAIX, PD-L1, and COX2-positive ccRCC could reap potential additional therapeutic benefits from therapies with CAIX inhibitors. The institutional review board approved this project. **Keywords:** CAIX;COX-2;PD-L1.

ME - 057 - **Anti-carbonic anhydrase IX CAR T cells releasing programmed cell death ligand 1 antibodies for the treatment of glioblastoma**

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Glioblastoma (GBM) is the most prevalent of all brain tumors, leading to the death of most patients within 15 months after the diagnosis. Hypoxia is a frequent feature of GBM that leads to the overexpression of several proteins, including carbonic anhydrase IX (CAIX). CAIX is a transmembrane metalloenzyme responsible for the reversible hydration of carbon dioxide, expressed in more than 60% of GBM cases. Solid tumors have a microenvironment that promotes the progressive loss of T cell effector functions, a process known as T cell exhaustion or immune checkpoint, which favors tumor development. A member of the immune checkpoint group called the programmed cell death ligand-1 (PD-L1) is overexpressed in 88% of GBM cases. PD-L1 interacts with programmed cell death receptor 1 (PD-1) on T cells, favoring T cell exhaustion. Since GBM often overexpresses CAIX and PD-L1, they become excellent targets for combined therapies against this type of tumor. A new therapeutic strategy has been highlighted in the fight against cancer: T cells armed with chimeric antigen receptors (CAR T cells) designed against a tumor antigen. This project aims to evaluate the in vitro efficacy of anti-CAIX CAR T cells releasing anti-PD-L1 antibodies in the tumor microenvironment, in combating different GBM cell lines in vitro, and in an orthotopic model to be established in NSG mice. Results indicate that only U251 cell lines express CAIX and PD-L1 significantly, A172 cells do not present CAIX, not even inducing hypoxia with CoCl2 treatment. All anti-CAIX CAR T cells were able to induce U251 (CAIX+/PD-L1+) cytotoxicity, and the anti-CAIX CAR T cells secreting anti-PD-L1 are slightly more cytotoxic for these cells than the anti-CAIX CAR T cells only and secrete more IL-2 and IFNγ, demonstrating the CAIX dependent activation of these CAR T cells. Project approved by the animal ethics committee FAB/ AC Camargo Cancer Center (088/21) and institutional ethics board. **Keywords:** CAR T cells;glioblastoma;solid tumors.

ME - 058 - **Metabolic syndrome favors expansion of activated eosinophils associated with plasma leptin levels and production of Th2 and Th17-related cytokines**

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It is estimated that almost 1 billion individuals suffer from metabolic syndrome (MetS), the vast majority presenting visceral obesity, thus increasing the risk of inflammatory disorders. Although MetS has been associated with elevated proportion of circulating M1-type monocytes, in humans, few reports have linked MetS with granulocytes. In the present study, plasma, PBMC and granulocytes were obtained from peripheral blood from the control group (obese subjects without MetS) and MetS individuals. The PBMC cultures were activated with PHA. The cells were analyzed by cytometry, and adipokines (leptin and adiponectin) and cytokines were measured by ELISA and Multiplex, respectively. Here, as compared to the control group, MetS individuals showed a higher frequency of activated eosinophils ($CD69^{+}CCR3^{+}$). No difference was observed in neutrophil subsets between the two experimental groups. The percentages $CCR3^{+}$ and $CCR3^{+}CD69^{+}$ eosinophils were directly correlated with plasma leptin levels. Furthermore, plasma levels of IL-5, IL-6 and IL-17 directly correlated with the frequency of these granulocytes. Also, the IL-5, IL-6, IL-17 and GM-CSF levels produced by polyclonally-activated T cells directly correlated with the percentage of $CCR3^{+}CD69^{+}$ eosinophils. In contrast, circulating levels of adiponectin inversely correlated with the percentage of $CCR3^{+}$ granulocytes. Although preliminary, our findings suggest that MetS is associated with expansion of activated eosinophils with elevated capacity to migrate to the tissues. In addition, these cells were associated with the *in vitro* production of cytokines related to Th2 and Th17 cells, both cell phenotypes widely implicated in hypersensitivity reactions. **Keywords:** Metabolic syndrome;eosinophils;cytokines.

ME - 059 - **Investigating the role of mRNA modification m6A during Leishmania amazonensis infection**

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Leishmaniasis is an infectious disease caused by parasites of the genus Leishmania, which during its life cycle shifts from an invertebrate to a vertebrate host. In the vertebrate host, Leishmania is an obligate intracellular parasite, infecting mainly macrophages. During evolution, the parasite developed several mechanisms to subvert the normal function of these cells to survive, including alterations in the host cell gene expression. Chemical modifications of specific nucleotides have been described as important regulatory posttranscriptional mechanisms of mRNAs life, and N6-methyladenosine (m6A) was found as one of the most abundant.The m6A levels in the host cell have been implicated in the immune response against intracellular pathogens, and are regulated by different enzymes: writers (METTL3 and METTL14), which add the m6A; erasers (FTO and ALKBH5) that removes, and the readers (YTHDC1-2 and YTHDF1-3), which recognize the modification. Considering the studies that demonstrate the role of m6A in the immune response, in this work we begin to investigate the role of m6A during the Leishmania infection. Analysis of publicly available RNAseq data showed increased METTL3 expression and decreased ALKBH5 expression in patients with cutaneous and visceral leishmaniasis compared to healthy individuals. Also, we assessed m6A levels of samples derived from macrophage infection collected at 2, 4, 8, 24 and 48 hours after infection. There is an increase in m6A in the initial times of infection followed by a reduction in later times. In parallel, ALKBH5 knockout macrophages were obtained using the CRISPR/Cas9 and will be used in further in vitro infection assays, to investigate the impact on parasite infection. In the end, with the experiments in progress, we intend to investigate the possible involvement of m6A during Leishmania-macrophage infection. **Keywords:** Leishmania;m6A;epitranscriptome.

ME - 060 - **EVALUATION OF SERUM LEVELS OF VCAM-1 AND L-SELECTIN IN PEOPLE LIVING WITH HIV**

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Introduction: In search of new biomarkers to understand the pathogenesis of Human Immunodeficiency Virus (HIV) and its progression to Acquired Immunodeficiency Syndrome (AIDS), soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble L-selectin (sCD62L) have emerged as promising molecules in HIV infection, particularly for the exploration of new therapies. Therefore, this study aimed to compare the serum levels of sVCAM-1 and sCD62L in people living with HIV (PLHIV). **Methods:** Serum samples from 118 PLHIV drug naïve and 40 uninfected individuals (Control Group) were collected. PLHIV were classified according to $CD4^+$ T lymphocyte levels into two groups: 1) AIDS Group (N=56, CD4<350) and 2) no-AIDS Group (N=62, CD4>350). Serum levels of sVCAM-1 and sCD62L were measured using Cytometric Bead Array (CBA). Differences between groups were calculated using the Wilcoxon and Mann-Whitney tests, Spearman's test was used for correlation analyses, and results with $p<0.05$ were considered statistically significant. **Results:** We observed that both groups of PLHIV had lower levels of sVCAM-1 compared to the Control Group (no-AIDS, $p=0.05$; AIDS, $p<0.0001$). Furthermore, sVCAM-1 levels in the AIDS group were lower than those in the no-AIDS group ($p<0.0001$). Correlation analyses showed that sVCAM-1 exhibited a weak negative correlation with viral load ($r=-0.39$, $p<0.0001$), a moderate positive correlation with $CD4^+$ T lymphocyte levels ($r=0.54$, $p<0.0001$), and a weak positive correlation with the CD4:CD8 ratio ($r=0.45$, $p<0.0001$). Comparisons between groups did not show any statistically significant variation for sCD62L. **Conclusion:** Our findings show that sVCAM-1 molecule levels are low in people with severe immunological impairment. This shows that sVCAM-1 has the potential to be used as a biomarker for HIV clinical progression. **Keywords:** HIV;VCAM-1;L-selectin.

ME - 061 - **Discarded leukodepletion filters: a new source of mononuclear cells for in vitro research.**

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BACKGROUND: Advancements in immuno-oncology have heightened the demand for obtaining mononuclear cells for in vitro assays. However, obtaining cells from healthy donors remains complex due to challenges in donor recruitment and the requirement of large blood volumes (Hematol Transfus Cell Ther. 43:494-498, 2021). Therefore, it is essential to develop methodologies that allow for the isolation of mononuclear cells from healthy donors in large quantities and purity. **OBJECTIVE**: To standardize the isolation of peripheral blood mononuclear cells (PBMC) from leukodepletion filters used in the Hemonucleus of Barretos Cancer Hospital. **METHODS**: PBMCs were successfully recovered from leukodepletion filters, which were originally utilized during the donation of whole blood and erythrocytes by apheresis, employing centrifugation with a concentration gradient facilitated by Ficoll. Cell viability was analyzed by MTS and flow cytometry. Additionally, flow cytometry was employed to analyze the presence of the following subpopulations: $CD4^+$, $CD8^+$ and $CD19^+$ lymphocytes, as well as $CD11b^+$ and $CD14^+$ monocytes. **RESULTS**: There was a higher yield of PBMCs from whole blood filters compared to erythrocytes by apheresis, and greater viability in fresh samples compared to frozen ones. By standardizing different methodologies, it was possible to isolate mononuclear cells such as $CD4^+$ helper T lymphocytes, $CD8^+$ cytotoxic T lymphocytes, $CD19^+$ B lymphocytes, and $CD14^+$ and $CD11b^+$ monocytes. **CONCLUSION**: Leukodepletion filters offer a cost-effective, efficient, and pratical source of mononuclear cells from healthy blood donors at blood centers. These cells can be utilized for in vitro assays. **Keywords:** Peripheral blood mononuclear cells;Ficoll density gradient;Immunophenotyping.

ME - 062 - **Interleukin-10 deficiency evokes perivascular adipose tissue conversion and vascular remodeling**

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O tecido adiposo perivascular (PVAT) é uma camada adiposa, envolvendo os vasos sanguíneos, com um papel modulador local. A interleucina-10 (IL-10) demonstrou modular o tecido vascular. Este estudo teve como objetivo caracterizar o papel endógeno da IL-10 na remodelação vascular e na fenotipagem do PVAT. Foram usados segmentos aórticos torácicos de camundongos machos controle (C57BL/6J) e nocaute para IL-10 (IL-10 $^{-/-}$).Foram realizadas análises da morfometria da aorta/PVAT e deposição de elastina, colágeno e reticulina. A proteína de desacoplamento de tecido 1 (UCP1) foi acessada por Western blotting. A ausência endógena de IL-10 acompanhou a área total do PVAT (p=0,0310) e a relação parede/lúmen (p=0,0024), enquanto aumentou a área e a espessura vascular (p<0,0001). A deposição total de colágeno foi aumentada em IL-10 $^{-/-}$, mas sob luz polarizada, foi encontrada redução do colágeno-I (p=0,0075) e aumento do colágeno-III (p=0,0055), simultaneamente com redução da deposição de fibras elásticas (p=0,0282) e aumento da deposição de fibras reticulares (p<0,0001). A área dos adipócitos aumentou na ausência de IL-10 (p=0,0225) e a expressão de UCP1 foi reduzida (p=0,0420). Além disso, a frequência relativa de células adiposas brancas e tecido conjuntivo foi aumentada em IL-10 $^{-/-}$ (p<0,0001), somada a uma redução de células adiposas marrons (p<0,0001). Juntos, esses dados caracterizam o PVAT da aorta de IL-10 $^{-/-}$como um fenótipo de adipócito semelhante ao branco. A IL-10 endógena previne a remodelação vascular e favorece um fenótipo de adipócito marrom, sugerindo um papel modulador para a IL-10 na plasticidade do PVAT. **Keywords:** IL-10;PVAT;vasculature.

ME - 063 - **EVALUATION OF MOLECULAR MARKERS FOR PULMONARY TUBERCULOSIS DIAGNOSIS**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains an important public health problem being one of the top ten causes of death worldwide. In many countries where TB is endemic, the diagnosis of pulmonary TB is mostly dependent of sputum smear microscopy by Mtb acid-fast staining or culture, which present low sensitivity or is time-consuming. Therefore, the World Health Organization (WHO) identified the need of a non-sputum and a rapid biomarker-based test as a high-priority for TB diagnosis. In a previous study, our research group applied an immune-based gene expression profile by NanoString platform and identified 23 genes associated with inflammatory mechanisms. The expression of these genes was able to distinguish, with high sensitivity and specificity, individuals with TB from those with other pulmonary diseases (OPD). Thus, in this study, we applied the RT-qPCR assay to evaluate the diagnostic power of the genes *FCGR1A, GBP5, IRAK3, PDCD1LG2, MAPK14, CD274, CD59, ICAM1, IFITM1, PML, C1QA* and *CR1* in whole blood-based samples from nine healthy individuals (HC) and ten individuals with pulmonary TB. Preliminary analysis showed differences in the expression levels of *FCGR1A, GBP5, IRAK3*, and *PDCD1LG2* genes between TB and HC groups. Receiver Operating Characteristic (ROC) curve analysis revealed that the area under the curve (AUC), sensitivities and specificities were, respectively, 0.93 (95% CI 0.81–1.00), 88.89% (56,50-99,43), and 90% (59,58–99,49) for *FCGR1A* and 0.83 (95% CI 0.64–1.00), 66,67% (35,42–87,94) and 90% (59,58–99,49) for *GBP5. FCGR1A* and *GBP5* genes stood out as potential markers for TB diagnosis as they were able to discriminate individuals with TB from HC. **Keywords:** Tuberculosis;gene expression;molecular immunology.

ME - 064 - **IL-1α protects obese mice against pulmonary inflammation and the susceptibility to M. tuberculosis infection**

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Tuberculosis (TB) is the second infectious disease that causes the great number of deaths, 1.6 million, worldwide annually. The hallmark of severe cases of TB is an exacerbated pulmonary inflammation induced by $CD4^+$ $IFN\text{-}\gamma^+$ cells. Obesity is a comorbidity that induces severe TB, and increases lung IFN-γ concentrations. We hypothesized that the alarmin IL-1α exacerbates the pulmonary inflammation and aggravates the progression of infection in the comorbidity obesity and TB. IL-1α deficient mice ($IL\text{-}1\alpha^{-/-}$) were fed with High Fat Diet (HFD) for 9 weeks, treated with recombinant murine IL-1α (rIL-1α) before and during infection with *Mycobacterium tuberculosis* (Mtb), and their lungs were evaluated 3 weeks post infection (12 weeks HFD feeding). Obesity accentuated pulmonary inflammation and aggravated the susceptibility to Mtb infection. Different from our hypothesis, obese animals treated with rIL-1α (HFD+rIL-1α group) exhibited decreased lung bacterial load and pulmonary inflammation compared to untreated obese mice (untreated HFD group). HFD+rIL-1α group also showed reduction in lung interstitial macrophages ($CD11b^+$ $SiglecF^-$ cells) and alveolar macrophages ($CD11b^-$ $SiglecF^+$ cells) expressing iNOS and monocytes ($CD11b^+$ $Ly6C^+$ and $CD11b^+$ $Ly6C^+$ $CCR2^+$ cells), and $CD4^+$ $IFN\text{-}\gamma^+$ cells, suggesting that higher myeloid cell influx and increased expression of iNOS are related to immunopathology along with Th1 cells. However, HFD+rIL-1α group exhibited higher frequency of $CD4^+$ IL 17^+ cells than untreated HFD group. $CD4^+$ IL 17^+ cells negatively regulate HIF-1α, and we found that as higher mRNA expression of RORγt, lower mRNA expression of HIF-1α in the lungs. Our findings show a protective role for IL-1α in the comorbidity and suggest that the HIF-1α-iNOS axis is inhibited by $CD4^+$ IL 17^+ cells. Further experiments are undergoing to investigate the deleterious role of HIF-1α-iNOS axis in the comorbidity obesity and TB.
Financial Support: FAPESP grants 2017/21629 5; 2022/03974 5. **Keywords:** IL-1α;Th17;HIF-1α.

ME - 065 - **Heme and hemozoin induce platelet death partially through ferroptosis during vivax malaria**

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Malaria is an infectious disease present in tropical and subtropical areas and caused by protozoans from the Plasmodium genus, being the infection with P. vivax the most frequent in Brazilian Amazon. During the infection, the parasite digests hemoglobin and releases free heme, which is transformed into hemozoin (Hz), a less toxic form. Heme and Hz are both released in the circulation during hemolysis. Anaemia and thrombocytopenia are major hematological alterations in vivax malaria patients. We hypothesized that heme and Hz support thrombocytopenia in vivax malaria by inducing platelet death. Preliminary data from our group has shown platelet transcriptomic alterations related to multiple pathways of cell death. We now observed that increased free heme levels are positively correlated with thrombocytopenia in vivax malaria patients. Moreover, plasma from patients induced platelet death ex vivo, which was inhibited by the heme chelator hemopexin. To explore the pathways involved in platelets death, we stimulated platelets from health donors with heme or Hz in vitro. We observed that heme is a stronger stimulus, as it induces degradation of Bcl-xL depending on calpain activity and earlier platelet death, while Hz induces later cell death. However, calpain inhibition did not prevent heme and Hz-induced platelet death. We then investigated necroptosis, canonical and non-canonical pyroptosis through RIP1, caspase-1 and caspase-4 inhibition, which did not prevent platelet death. Importantly, inhibiting ferroptosis with ferrostatin-1 partially prevented Hz-induced cell death, while the lipophilic antioxidant quercetin completely prevented platelet death under Hz stimulation. These data indicate a partial contribution of ferroptosis to Hz-induced death, in addition to other pathways also depending on ROS production. We propose that cell death induced by heme and Hz occurs through multiple pathways at once, and we are now evaluating the inhibition of combined pathways. **Keywords:** platelets;malaria;cell death.

ME - 066 - **Immunomodulatory potential of nanostructured lipid carriers (NLC) LOADED WITH red sacaca essential oil**

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INTRODUCTION: Red sacaca essential oil (RSO) derived from Amazonian tree is associated to the anti-inflammatory, antioxidant and antitumor activity. An alternative for lipophilic drug delivery is the use of nanostructured lipid carriers due their toxicity reduction and effective delivery. This study aimed to evaluate the immunomodulatory potential of red sacaca essential oil and the efficiency of its delivery through nanostructured lipid carriers *in vitro*. **METHODS:** We performed the oil characterization by gas chromatography coupled to a mass spectrometer (GC-MS). The cell viability profile, and the 50% inhibitory concentration (IC50) values, were accessed through the resazurin metabolization assay in A549 and BEAS-2B cells, and the THP-1 differentiated into macrophages. We measured the reactive oxygen species (ROS) levels by fluorescent probe 2', 7'-dichlorofluorescein diacetate. Cytokine levels were evaluated by flow cytometry using the Cytometric kit Bead Array. **RESULTS:** GC-MS analysis identified the presence of 33 RSO components, and the majority of metabolites were linalool and 7-hydroxy-calamene. RSO showed greater cytotoxicity when compared to NLC_RSO at the same concentrations. NLC_RSO tracked the levels of ROS in A549, while RSO increased the levels of ROS in BEAS-2B. The A549 cell line was more resistant to RSO treatment than the BEAS-2B. NLC_RSO reduced the inflammatory profile of THP-1 cells differentiated into macrophages showing elevation in interleukin 4 levels and decreasing pro-inflammatory cytokines like interleukin 6 and Tumor Necrosis Factor-alpha. **CONCLUSION:** RSO encapsulation confers oil protection and extended-release effect suggesting the approach of NLC_RSO as a potential delivery system for RSO. In addition, an expressive diversity of secondary metabolites in RSO associated with diverse biological functions support the potentiality of developing an RSO-based drug. **Keywords:** Croton cajucara BENTH;Sacaca vermelha;Carreadores lipidicos nanoestruturados.

ME - 067 - **AKT1 variants are associated with worse outcome of COVID-19**

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Background: The COVID-19 pandemic, caused by the SARS-CoV-2 virus, is a global crisis. Brazil is the second country with the highest number of deaths due to COVID-19 in the world, 701,494 deaths. Many factors can determine the severity of COVID-19, including viral load, genetic factors, presence of comorbidities, age, gender, and uncontrolled inflammation. It is estimated that approximately 5% of cases develop severe acute respiratory distress syndrome due to cytokine storm. A cell signaling pathway that may be involved in this process is Akt/PI3K/mTOR. Studies demonstrate that AKT inhibition can potentially suppress pathologic inflammation, cytokine storm, fibroproliferation, and platelet activation associated with COVID-19. **Objectives:** To investigate the association between AKT1 gene variants and the severity of COVID-19. **Methods:** Peripheral blood samples and sociodemographic data were collected from 502 individuals with COVID-19, 213 mild cases and 289 severe cases, from April 2020 to April 2021. Plasma cytokine concentrations were measured by ELISA. Genotyping of the SNPs, rs1130214 and rs2494746, and AKT1 gene expression were performed using Thermo Fisher kits and analyzed by qRT-PCR in the QuantStudio 12K (Applied Biosystems). **Results and Conclusions:** The rs2494746-C allele was associated with severity, ICU admission, and death from COVID-19. Meanwhile, the C allele of rs1130214 was associated with elevated TNF-α levels. In addition, variants demonstrated a cumulative risk associated with severity, criticality, and death from COVID-19. In the predictive analysis, the rs2494746 obtained an accuracy of 71%, suggesting a high probability of the test determining the severity of the disease. Therefore, the present study contributes to understanding the influence of the AKT1 gene and its variants on immune damage in individuals infected with SARS-CoV-2, which may be useful in the future to help predict a worse outcome of COVID-19. **Keywords:** AKT1;SNPs;COVID-19.

ME - 068 - **IN SILICO AND IN VITRO ANALYSES OF HEPATITIS C VIRUS IMMUNODOMINANT PEPTIDES RESTRICTED TO HLA-A*02:01**

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Hepatitis C is an inflammatory liver disease caused by *Hepacivirus hominis* (HCV) that can progress to cases of fibrosis and hepatocellular carcinoma. HCV is a blood-borne virus and currently over 71 million people are estimated to be chronically infected worldwide, constituting a major global health problem due to high morbidity and mortality rates. Furthermore, there are no vaccines against the disease and treatment with direct-acting antivirals (DAAs), although efficient, is costly and does not prevent reinfections. Considering that the process of peptide presentation by MHC-I to CD8+ T cells is crucial to trigger an effective antiviral cellular response against HCV, this project aims to present an overview of HCV-derived immunodominant epitopes restricted to HLA-A*02:01 using *in silico* and *in vitro* tools. For the *in silico* studies, 59 polyprotein sequences from 7 genotypes of the virus were obtained from NCBI nucleotide and applied for peptide prediction with NetCTL 2.0 software. A total of 2708 peptides with potential to activate CD8+ T cells were identified and kept for analysis of physicochemical properties and cytokine induction, with the identification of a clear bias towards IL-4 induction. For *in vitro* evaluation of peptide-MHC-I affinity, a peptide microarray was performed employing the peptides derived from the 7 HCV genotypes and a recombinant HLA-A*02:01 molecule. The results allowed the identification of peptides with high reactivity to HLA-A*02:01, mainly in the p7, NS2 and NS5B proteins. Moreover, the amino acid patterns observed in the reactive peptides highlighted the prevalence of leucine and tryptophan residues, which contributes to the binding of the epitope to the TCR and, consequently, to the activation of CD8+ T lymphocytes. Overall, the HCV-derived and HLA-A2*02:01-restricted peptide patterns observed in this work provide important information for the development of a multi-epitope vaccine that is effective against several HCV genotypes. **Keywords:** Hepatitis C;peptide microarray;CD8+ T cell response.

ME - 069 - **Evaluation of the effects of a calcineurin inhibitor in an animal model of cocaine-induced addiction**

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INTRODUCTION: There is evidence that the immune system contributes to the addiction process, as it regulates important events such as neuroplasticity and memory. FK506, an immunosuppressive calcineurin inhibitor, has been associated with important neural effects, including synaptic plasticity and learning. However, its role in neuroinflammation involved in the addiction process is not fully established. **OBJECTIVE:** To evaluate the effects of FK506 on behavior and mediators associated with inflammation and neuroplasticity in an animal model of cocaine addiction. **METHODOLOGY**: Male C57Bl/6 mice, aged 9- 12 weeks were used. FK506 (5 mg/kg, s.c.) was administered 90 min before cocaine (15 mg/kg, i.p.). Cocaine was administered immediately before each behavioral test. The behavioral sensitization test was used to evaluate locomotor activity, and the conditioned place preference test was used. The hippocampus and striatum were removed for BDNF, GDNF, NGF, TNF, IL-6, IL-10, and CX3CL1 measurement using ELISA. The analysis was performed using two-way ANOVA followed by Tukey's post-test. The difference between groups was considered statistically significant when $P<0.05$. **RESULTS:** FK506 significantly attenuated cocaine-induced locomotor sensitization in males starting from the fourth day ($p<0.05$). In the cocaine-treated groups, FK506 reduced the concentrations of GDNF ($p=0.0130$), TNF ($p=0.0397$), and IL-10 ($p=0.0259$) in the hippocampus of males compared to the groups treated with cocaine alone. There was also an increase in IL-10 in the striatum of the cocaine-treated group compared to the control group ($p=0.0180$). There were no changes in the other parameters in the other analyzed groups. **CONCLUSION:** Our results suggest the involvement of the calcineurin pathway in the locomotor effects caused by cocaine in males. This effect could be related to alterations in the concentration of neurotrophic factors and inflammatory mediators. Acknowledges to CAPES, CNPq, and FAPEMIG. **Keywords:** neuroinflammation;addiction;neuroplasticity.

ME - 070 - **Chemerin adipokine plays inflammatory role that dampen the control of Mycobacterium tuberculosis infection**

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Tuberculosis (TB) is a chronic disease caused by Mycobacterium tuberculosis (Mtb) that kills more than 1 million people worldwide annually. Diabetes, which increases three times the risk for TB development, induces chemerin secretion, an adipokine that may play pro or anti-inflammatory role. We hypothesized that chemerin negatively affects the microbicidal capacity and exacerbates the inflammatory response of macrophages. Bone marrow-derived macrophages (BMDM) from C57BL/6 mice were cultured in hyperglycemic (glucose) or normoglycemic (mannitol) medium followed or not by M. tuberculosis-infection (MOI 1) for 24 hours. BMDM cultured with glucose medium showed reduced bacterial load and secreted higher TNF and IL-10 levels compared with those in mannitol medium. The addition of chemerin in hyperglycemic culture increased bacterial load and IL-6 secretion. However, the addition of chemerin in Mtb-infected BMDM cultured in mannitol medium neither affected bacterial load nor cytokine secretion. BMDM from mice fed with High Fat Diet (obese, non-diabetic mice) controlled better the bacterial growth and secreted higher levels of TNF, IL-6 and IL-10 than BMDM from lean animals. BMDM from mice deficient for leptin receptor (db/db, obese and diabetic mice) showed no differences in bacterial growth and cytokine secretion compared to BMDM from control group (db/+ mice). In attempt to confirm our findings in vivo, db/db and db/+ mice were infected with Mtb. Lungs of infected db/db mice showed higher bacterial load, exacerbated inflammation, increase of alveolar macrophages and reduction of interstitial macrophages compared to infected db/+ group. However, the treatment in vivo of db/db mice with chemerin receptor antagonist (CCX823) did not affect bacterial load compared to non-treated group. Our findings show that the chemerin plays an inflammatory role, although it does notdampen the control of Mtb infection in vivo. Financial support: FAPESP grants 2017/216295; 2019/23446-0. **Keywords:** tuberculosis;chemerin;macrophage.

ME - 071 - **Exploring Extracellular Vesicles and Inflammatory Cytokines in Oral Cavity Cancer Patients and Cancer-Free Individuals: Towards Biomarker Discovery and Characterization**

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Introduction: Certain soluble molecules derived from tumors have a significant correlation with tumor development and progression. Notably, cytokines, which are intricately linked to inflammation, are among these molecules and have the potential to undergo dysregulation in cancer patients (Semin Immunol. 26(1):54-74, 2014). Extracellular vesicles (EVs) are an additional crucial component in understanding the mechanisms behind inflammation and tumor metastasis. These vesicles play a vital role in the transportation of essential substances for cellular communication (Oral Dis. 26(1): 173-181, 2020). Consequently, a comprehensive investigation into cytokines and extracellular vesicles can shed light on their significance in the progression, diagnosis, and prognosis of patients with oral cavity tumors. **Objective:** To identify and characterize extracellular vesicles and inflammatory cytokines that may predict diagnosis, prognosis, and/or be involved in the progression of oral cavity cancer. **Methods:** Plasma samples from patients with oral cavity tumors and control individuals were collected at the Barretos Cancer Hospital. The Cytometric Beads Array (CBA) technique will be used to evaluate the cytokines: IL-2, IL-4, IL-6, IL-10, IL-17A, TNF, and IFN-γ. For extracellular vesicle isolation, the characterization process will involve sequential centrifugation and ultracentrifugation technique, followed by a transmission electron microscopy, nanoparticle analysis, and western blot for EV proteins (CD63, Alix, Flotillin) and PD-L1. The molecular findings will be associated with clinical and pathological data of the patients using the statistical program SPSS 23.0. **Keywords:** Oral Cavity Cancer;Cytokines;Extracellular Vesicles.

ME - 072 - **IL-33 activates eosinophil lipid body-related LTC4 synthesizing machinery**

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As resident cells, eosinophils respond to tissular stress signals, such as the alarmin IL-33. Eosinophils express IL-33 receptor ST2, which induces eosinophil differentiation, migration, and cytokine release; however, little is known about IL-33 effects on their lipid mediator synthesis. Here we investigate IL-33's ability to orchestrate differential eicosanoid synthesis both *in vivo* and *in vitro* by eosinophils. *In vivo*, eosinophilic inflammation induced by IL-33 was achieved in sensitized BALB/c mice [ovalbumin plus Al(OH)3] followed by intrapleural injection of IL-33 (0.1 µg/cavity). IL-33 in sensitized animals induced pleural eosinophil accumulation (from virtually none to $1.2 \pm 0.3 \times 10^6$ cells/cavity within 24 h; n = 12, $p \leq 0.05$), without alteration of pleural population of mononuclear cells. In contrast, IL-33 in non-sensitized mice failed to trigger pleural eosinophilia ($0.25 \pm 0.1 \times 10^6$ eosinophils/cavity; n = 9 – similar to saline injected mice). IL-33-injected sensitized mice showed increased lipid body content within eosinophils (15.5 ± 1.4 lipid bodies/eosinophil; n = 12; $p \leq 0.05$) when compared to lipid body numbers found in pleural eosinophils from IL-33-injected non-sensitized mice (8.5 ± 1.1 lipid bodies/cell; n = 9). Of note, pleural mononuclear cells of IL-33-injected sensitized or non-sensitized mice did not show altered lipid body content. Parallel to IL-33-elicited eosinophil activation, higher levels of pleural PGD_2 and LTC_4, but not RVD_1 were found. *In vitro*, human eosinophils were stimulated for 1 h with IL-33 (100 ng/mL); IL-33 was able to directly trigger lipid body biogenesis within human eosinophils (from 8.5 ± 1.7 to 15.7 ± 2.4 lipid bodies/eosinophil; n = 3, $p \leq 0.05$), which displayed enhanced ability to synthesize and secrete LTC_4 (but not RVD_1). These initial findings unveil that IL-33 is capable of eliciting eosinophil lipid body-driven eicosanoid synthesizing machinery in an acute and differential fashion. **Keywords:** eosinophils;alarmin IL-33;eicosanoid synthesis.

ME - 073 - **GENERATION OF HUMAN MACROPHAGE EXPRESSING CHIMERIC ANTIGEN RECEPTORS (CAR) DERIVED OF CD34+ CELLS FROM UMBILICAL CORD BLOOD FOR THE TREATMENT OF SOLID TUMORS**

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Introduction: T lymphocytes expressing CAR have been shown to be less efficient for solid tumors, due to the tumor microenvironment involving: heterogeneity of tumor antigens, difficulty in maintaining of immune responses, the immunosuppressive milieu, and the dense extracellular matrix. Thereby, macrophages emerge as an exceptional option for the insertion of CAR (CAR-Mac), given their capacity to infiltrate the tumor matrix, to phagocyte, present antigens and secrete cytokines. Thus, we aim to generate CAR-Mac from umbilical cord blood CD34+ progenitor cells to treat solid tumors. **Methods:** CD34+ cells were isolated, expanded in vitro for 07 days and differentiated into macrophages using StemSpan™SFEMII (SP) or RPMI1640 (RP) media with different cytokines (GM-CSF, M-CSF and IL-3) for 10 days. Then, the cells were phenotypically characterized by flow cytometry and morphology and evaluated for their phagocytic capacity after co-culture with Nalm6 leukemic cell line. Subsequently, to set-up a cell transformation protocol we used the piggyBac transposon system (PB) to insert the green fluorescent protein (GFP) via electroporation on CD34+ cells. **Results:** Data revealed that 93.3% of the CD34+ differentiated cells were CD64+CD14+CD1clowCD163+CD16+CD86lowCD80lowHLA-DR+ and their morphology indicate a typical macrophage phenotype, compared to monocyte-derived macrophages. The phagocytosis assay showed a percentage of 20,1% and 16,2% when cells were differentiated in SP and RP, respectively. Furthermore, 26.8% of CD34+ cells expressed GFP within 24 hours after electroporation with a viability of 60.7%. **Conclusion:** Cells differentiated showed features consistent with human macrophages and the PB is an interesting protocol for the insertion of CAR in CD34+ cells. Our next steps are the differentiation of CAR-expressing CD34+ cells into CAR-Mac for their functional characterization. **Financial support:** FAPESP#2022/11481-9, CNPQ#442676/2020-4 and PRONON#25000.027785/2021-21. **Keywords:** CAR;Macrophage;Cell Therapy.

ME - 074 - **Use of adjuvant therapies and their antitumor effects: Bacillus Calmette-Guérin and the effect of splenectomy on the regulation of B16F0 melanoma in C57BL/6**

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INTRODUCTION: Melanoma, a highly metastatic tumor, is known for its propensity to spread. Studies indicate that the spleen plays a role in creating a tumor microenvironment enriched with factors that promote tumor growth and regulatory cells. Consequently, investigating the impact of splenectomy and combining it with adjuvant treatment using Bacillus Calmette Guérin (BCG) becomes intriguing. **OBJECTIVE:** This project primarily aims to examine cells with regulatory potential and comprehend the composition and functional activity of infiltrated cells within the tumor following splenectomy or BCG vaccination. **METHODS:** C57BL/6 mice will be injected with $5x10^4$ B16F0 tumor cells directly into the pinna of the ear. BCG, consisting of $1x10^7$ organisms, will be administered locally or systemically ten days later. Some mice will undergo splenectomy, while others will receive BCG vaccination. Histological analysis will be conducted on tumor-containing sections of the ear, stained with HE for qualitative and quantitative evaluation. **RESULTS:** Animals with BCG-treated tumors (B16F0+BCG) exhibited increased infiltration of inflammatory cells, reduced necrosis, diminished desmoplastic reaction, and fewer tumor cells infiltrating the muscle. Furthermore, they demonstrated improved survival and better containment of the tumor at the injection site. Preliminary findings also suggest enhanced survival in animals that underwent splenectomy, with smaller tumor sizes observed in this group. Additionally, we observed an increase in spleen size as the tumor established itself. **CONCLUSIONS:** BCG treatment or splenectomy resulted in increased survival rates. In experimental melanoma, BCG vaccination improved inflammation, reducing necrosis, desmoplastic reaction, and muscular tumor infiltration. Our laboratory is currently conducting flow cytometry analysis on T lymphocytes infiltrating the tumor cells in the ear's pinna. **Supported by:** CAPES, CNPq and FIOCRUZ. **Keywords:** melanoma;immunoregulation;BCG.

ME - 075 - **Performance of Immunological Cells and Molecules as Potential Biomarkers of Therapeutic Response in B-Cell Acute Lymphoblastic Leukemia**

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Bone marrow is the main site affected by hematological malignancy, however, the evaluation of laboratory markers in the peripheral blood can be useful tools for the follow-up and monitoring of the evolution of the disease. Here, we investigated the performance of cell populations and immunological mediators as prognostic biomarkers in children with B-cell acute lymphoblastic leukemia (B-ALL) undergoing remission induction therapy. Samples from 28 children without B-ALL were collected and Receiver-operating Characteristic (ROC) curve analysis were constructed based on five laboratory parameters of classification of response to induction therapy, referred as: absolute neutrophil counts (ANC) at diagnosis (D0) and at day 8 (D8); absolute lymphocyte counts (ALC) and platelet counts (PC) at day 35 (D35). Performance of immunological cells (NK and NKT cells, Treg, $CD4^+$ and $CD8^+$ T cells) and Molecules (CXCL8, CCL2, CXCL9, CCL5, CXCL10, IL-6, TNF, IFN-γ, IL-17A, IL-4, IL-10 and IL-2) selected ($p<0.05$) were calculated at specific cut-off and the area under the curve (AUC), sensitivity, specificity and likelihood ratio as indicator of global accuracy. Several biomarkers showed high performance in classifying patients. For ANC at D0, five potential biomarkers were identified, two of which are early biomarkers, $TCD8^+$ at D0 and TNF at D8. In parallel, for ANC at D8, 17 potential biomarkers were identified, being CCL2 and IFN-γ at D0, and CXCL8, CCL2, CXCL9, CXCL10 and IL-10 at D8. For ALC at D35, 7 biomarkers were identified, CXCL9 on D0, and $TCD4^+$, IL-17A and IL-4 on D8. Finally, for PC at D35, 19 potential biomarkers were identified, $TCD8^+$, CCL5, TNF and IL-17A at D0 and Treg, CCL5 and TNF at D8. The results demonstrated that immunological cells and molecules in peripheral blood showed high performance in classifying the response to induction therapy at an early stage and can be used as an alternative for follow-up and monitoring of the evolution of the patients. **Keywords:** B-ALL;Biomarkers;Follow-up.

ME - 076 - **Characterization of the inflammatory response in experimental skin transplantation in mice with deletion for the XPC gene**

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Introduction: Burns represent a pathological condition of high clinical and epidemiological relevance, both nationally and worldwide. In cases of severe burns, a possible therapeutic approach is the use of allogeneic skin transplantation, a palliative therapy. The phenotypic and functional characterization of the inflammatory infiltrate and Treg cells present in the skin graft will be evaluated by comparing the absence and presence of the nucleotide excision repair (NER) mechanism influenced by the XPC gene, using wild-type mice and XPC knockout mice. Objective:For this reason, the objective of this work is to evaluate the role of the absence of the XPC gene in semi-allogeneic transplantation and its modulation in immunological responses, evaluating the characteristics of the inflammatory process in the absence of the gene in the global genome repair system (GG -NER) and transcription coupling repair (TC-NER) and CG-NER pathway, where consequently there will be no adequate recognition of lesions in DNA regions. The hypothesis of this project is that due to the absence of the xpc gene, transplant collection will be greater. Methods: Male C57BL-6 WT and XPCKO mice will undergo skin transplant surgery from a donor derived from crosses of Balb/c and C57 wt mice , the generated process will be evaluated, in addition to the possible access or exclusion of the graft. Afterwards, the skin of the graft and adjacente regions, draining lymph nodes and spleen will be collected to perform the following techniques: Induction of in vitro differentiation of iTregs, Suppression assay of Tregs in vitro, Quantification of cytokines by Enzyme-Linked Immunosorbent Assay, Immunophenotyping
of cells derived from skin transplantation, RT-qPCR and flow cytometry to analyze the inflammatory and systemic process between the two groups.**Keywords:** Skin Transplant;DNA repair; XPC gene;Regulatory T cells.

ME - 077 - **Characterization of the granulocyte population in the MMTV-PyMT C57BL/6 transgenic mice model of spontaneous breast cancer**

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Introduction: Neutrophils are the most abundant type of granulocytes in the blood, being classified into different subsets based on their differential effects on cancer initiation, development, and progression. Here, we show the identification of neutrophil and eosinophil granulocytes in tumor samples untreated, treated with anthracycline (FAC), or treated with taxane chemotherapy in the transgenic murine model MMTV-PyMT background C57BL/6 (delayed tumor, 15 weeks) of spontaneous breast cancer, closer to the clinics. **Methods and Results:** Female C57BL/6 mice (15-25 weeks) and MMTV-PyMT C57BL/6 transgenic female mice (14 weeks) were used. Mice tumors were treated when reached between 800-1300 mm^3 (tumor stage of late carcinoma). The chemotherapy drugs used in this study were: 5-fluorouracil, doxorubicin and cyclophosphamide composing the anthracycline group (FAC) and docetaxel composing the taxane group (T). Tumor cells were dissociated from the tumor mass and labeled for flow cytometry analyses using a previously validated protocol. The granulocyte panel was composed of CCR3, Gr-1, Siglec F and CD16/32 markers. When analyzing the population of neutrophils, we found the following frequencies: Control: 2.72% average; 0.67% mean deviation; Taxane: 1.31% average; 0.73% mean deviation; FAC: 2.98% average, 2.65% mean deviation and eosinophil population: Control: 0.60% average; 0.42% mean deviation; Taxane: 0.84% average, 0.51% mean deviation; FAC 0.19% average; 0.13% mean deviation. **Conclusion:** We found no significant differences in granulocyte frequencies between the studied groups. Further studies are needed to understand the role of these cells in the microenvironment of late breast carcinoma, and after treatment with chemotherapy, seeking the use of standardized markers to describe these cell populations. **Keywords:** Breast cancer;Granulocytes;Chemotherapy.

ME - 078 - **STUDY OF THE INFLAMMATORY PROCESS INDUCED BY BOTHROPSTOXIN-I IN MICE SELECTED FOR HIGH AND LOW ACUTE INFLAMMATORY RESPONSE - AIRMAX AND AIRMIN**

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Introduction: The envenomation by Bothrops jararacussu snake results in tissue myonecrosis and intense inflammatory. Bothropstoxin-I (BthTX-I), a PLA2-Lys49 isolated from this venom, exerts myotoxic activity and is involved in the inflammatory process. Innate immunity plays an important role in the local inflammation, as well as tissue repair process. Several immune sensors are involved in triggering the inflammatory response such as the inflammasome NLRP3. Mice genetically selected for maximum (AIRmax) and minimum (AIRmin) acute inflammatory response have been a useful tool to study the inflammatory process. **Objectives:** Evaluate the inflammatory profile and muscle regeneration induced by BthTX-I in AIRmax and AIRmin mice and the role of the NLRP3 in this process. **Methods and Results:** Firstly, it was analyzed the effect of BthTX-I in muscle damage, then C2C12 muscle cells were incubated with the toxin for 6h and 24h. The data showed that BthTX-I was able to induce LDH release by C2C12 culture indicating cytotoxicity in the muscle fibers. The in vivo myotoxic effect of BthTX-I was evaluated in AIRmax and AIRmin mice by intramuscular injection. After 4h, it was verified increased levels of CK on serum samples of AirMax and AirMin mice-groups injected with BthTX-I corroborating the in vitro results. The ability of BthTX-I to activate macrophages from AirMax and AirMin in vitro was also evaluated. The data demonstrated higher IL1β production by macrophages from AIRmax mice incubated with LPS/BthTX-I than macrophages from AIRmin incubated at the same condition. However, it was verified higher IL-16 production in macrophage cultures from AIRmin compared with culture cells from AirMax mice. Therefore, the data suggest that BthTX-I induces different inflammatory effect in AirMax and AirMin suggesting a role of NLRP3 in this process. **Keywords:** bothropstoxin-I;acute inflammatory response;inflammasome.

ME - 079 - **GALECTIN-3 PARTICIPATES IN THE MECHANISMS OF INFECTION BY Toxoplasma gondii IN HUMAN VILLOUS TROPHOBLASTIC CELLS (BeWo) AND IN THIRD-TRIMESTER HUMAN PLACENTAL VILLI EXPLANTS**

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Congenital toxoplasmosis is a disease caused by transplacental transmission of the obligatory intracellular parasitic protozoan *Toxoplasma gondii*, which can lead to miscarriage and fetal malformation. Galectin-3 is an abundant lectin in the placental microenvironment and is related to the control of infection by different pathogens. However, there are few studies showing the functional role of Gal-3 during *T. gondii* infection at the human maternal-fetal interface. In this context, we have promoted Gal-3 knockdown in human villous trophoblast cell lines (BeWo) using human siRNA lentiviral particles and confirmed by Western Blotting analysis. Next, we assessed the *T. gondii* intracellular proliferation after 24h using the β-galactosidase assay. We observed that the Gal-3 knockdown had an increase in parasitism, suggesting that Gal-3 appears to be a key protein involved in the congenital transmission of *T. gondii*.
To corroborate with our *in vitro* results, we used third-trimester human placental villous explants as an *ex vivo* model. To impair the Gal-3 activity in the tissue, we used β-lactose, a sugar that has a high affinity for the lectin's binding site. Firstly, villi explants were treated with β-lactose (30, 50, 100 and 150mM) for 2h, and tissue viability measured by MTT, LDH, and histology assays. Afterwards, we evaluated the parasite proliferation for 24h by β-galactosidase activity, and illustrated them through immunohistochemistry technique. Curiously, we observed that β-lactose-mediated Gal-3 blockade, with non-toxic concentrations, reduced *T. gondii* proliferation. Taken together, our preliminary findings highlight an intriguing multifaceted activity of Gal-3 in different experimental models. However, future studies are necessary to gain insights into the roles of Gal-3 during *T. gondii* infection at the maternal-fetal interface, which can contribute to the future establishment of new strategies to combat congenital infection by *T. gondii*. **Keywords:** Galectin-3;Toxoplasma gondii;Placental.

ME - 080 - **Evaluation of the infection percentage of macrophages by Leishmania infantum through treatment with Hantzsch Adducts**

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Visceral Leishmaniasis (VL) is a disease caused by obligate intracellular parasites of the genus *Leishmania*. One of the major challenges to controlling the disease is the search for new therapeutic strategies, and synthetic compounds and their derivatives represent a strong trend in the development of new drugs. In this sense, by a flow cytometry approach, the present work evaluated the *in vitro* leishmanicidal potential of immortalized canine macrophage strains (DH82) infected with *L. infantum* transfected with GFP reporter gene and treated with synthetic compounds from the group of Hantzsch Adducts (HA). Complementarily, the physicochemical, pharmacokinetic and toxicological properties of these compounds were predicted using *in silico* ADMET predictions using the pkCSM program. The percentage of infection was evaluated after 24, 48 and 72 hours of treatment using 5 compounds from the AH group (8A2, 8A3, 8A4, 8B5 and 8B6). As treatment control (TC) the drug Amphotericin B was used. The compounds 8B5 (26.2%) and 8B6 (25.5%) significantly reduced macrophage infection after 24 hours of treatment compared to control (32.35%). After 48 hours, the compounds 8A4 (22.65%) and 8B6 (19.5%), together with the CT group (11.15%), showed a significant reduction in the infection rate compared to the control (33%). After 72 hours, the compounds 8A2 (22.95%) and 8B6 (28.05%), in addition to the TC group (11.85%), also demonstrated a significant reduction in the infection rate compared to the control (36.8 %). Compounds 8A4 and 8B6 demonstrated good prediction results with a better safety profile, potential for oral use, high biodistribution, excellent metabolism and excretion profile. The dataset of the present work ensures that, among the HA compounds evaluated, the compound 8B6 was the one that presented the most promising results regarding leishmanicidal activity as well as *in silico* predictions of ADMET properties. Suported by FAPEMIG, CNPq,CAPES, UFOP,UFMG, Fiocruz Minas, INCT-DT. **Keywords:** Visceral Leishmaniasis;macrophages;Hantzsch Adducts.

ME - 081 - **BETANIN ATTENUATES ACID-INDUCED ACUTE LUNG INJURY BY OXIDATIVE STRESS REDUCTION IN RATS**

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Introduction: Acute lung injury (ALI) can be induced by aspiration of gastric contents, which could result in considerable damage to the lungs followed by important oxidative damage. Betanin is a natural antioxidant, with well-known anti-inflammatory activity. Thus, we hypothesized that betanin may modulate the lung injury in ALI by modulate the oxidative damage. **Materials and methods:** Male Wistar rats (300-420 g) were divided into four groups (n=6). The ALI was induced by intranasal installation of single dose (0.5 ml/kg) HCl (0.1M, pH 1,25). The ALI group was treated with saline (ALI), betanin 10 mg/kg (ALI+10) or 50 mg/kg (ALI+50). The control group was treated with saline. All animals were sacrificed 24 hours after the last treatment. The lungs and bronchoalveolar lavage fluid (BALF) were collected to be used in oxidative and morphological analyzes. Significative diferences was considered when p<0,05. **Results:** Histopathological alterations were found in the ALI group, including presence of edema ($p<0.05$), intra-alveolar and interstitial hemorrhages ($p<0.001$) and cell infiltration ($p<0.01$) when compared to control group. Betanin reduced the morphological damages, showing a decrease in edema ($p<0.05$), hemorrhages ($p<0.05$) and cell infiltration ($p<0.05$) in ALI+50 group. Redox markers MDA ($p<0.001$), CAT ($p<0.0001$) and ROS ($p<0.0001$) increased in ALI group. ALI+10 showed a significant decrease of CAT ($p<0.05$) when compared to ALI group. ALI+50 showed a decrease in MDA ($p<0.001$), CAT ($p<0.01$) and ROS ($p<0.05$). **Conclusion:** Collectively, our results indicate that betanin attenuates lung damage acid-induced ALI by reducing the exaggerated inflammatory response. Thus, the potential for using betanin as an auxiliary therapy for ALI should be further explored. **Keywords:** Lung injury;Betanin;Oxidative stress.

ME - 082 - **Immunological tests for the diagnosis of canine visceral leishmaniasis officially adopted in Brazil: an investigation about the cross-reactivity to other canine infections and seroconversion by vaccination**

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Dogs are the main reservoir of the causative agent of Visceral Leishmaniasis (VL) and, especially in Brazil, the serological investigation of positive animals is part of the strategy listed in the Visceral Leishmaniasis Control and Surveillance Program (VLCSP). In the past, this program determined the serological screening by ELISA and confirmation by IFAT. Over the last ten years, an alteration in dog's diagnosis was instituted, and IFAT was replaced by the rapid test Dual-Path Platform (DPP). Besides this substitution, in turn, the serology by ELISA was relocated for confirmative test and DPP for screening. In this context, the aim of the present study was verify if this change was advantageous regarding the diagnosis specificity. A set of 198 sera samples were assessed with these three Brazilian official tests: DPP (DPP-LVC®), ELISA (EIE-LVC®), and IFAT (IFI-LVC®). The sera samples comprises nonendemic controls (n=30); endemic controls (n=40); dogs with other diseases (n=88) including Leishmania braziliensis, Trypanosoma cruzi, Babesia canis, and Ehrlichia canis; and vaccinated dogs (n=40) using Leishmune®, Leish-Tec® or LBSap. Concerning this panel of samples, the overall specificity were 99.4%, 77.2% and 69.0% regarding DPP, ELISA and IFAT respectively. The implementation of DPP showed a positive impact in the diagnostic specificity and directly contributes to the improvement of Brazilian VLCSP. **Keywords:** Leishmania infantum;Visceral Leishmaniasis;Immunodiagnosis.

ME - 083 - **Inhibitory capacity from calpeptin in renal cell carcinoma cells and extracellular vesicle synthesis**

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Cancer is considered one of the major public health problems. Clear cell renal cell carcinoma (RCC) is the most frequent among subtypes of RCC, displaying a high degree of malignancy. Extracellular vesicles (EVs) appear as important roles in cellular communication. In the tumor microenvironment (TME), cancer cells secrete EVs, which can modulate several processes, such as immune response, invasion, metastasis, and resistance to therapies. Calpains is a signaling pathway associated with a myriad of cellular mechanisms, including cancer progression and EVs formation. The goal of this study was to investigate the influence of calpain inhibition using Calpeptin, an inhibitor of EVs production, in 769-P cell. The concentration of calpeptin was determined using MTT assay in 24 and 48 hour. EVs were isolated from 769-P cell treated with calpeptin and characterized by Nanotracking analysis. While the expression of proteins that are associated with the EVs synthesis were evaluated by Western Blot. Our data suggests that calpeptin reduced the 769-P cell viability by MTT analysis. The calpetin concentrations of 10, 20, and 30 µM showed influence in the EVs formation, reducing the overall EVs concentration, especially the 20 µM. However, the calpeptin did not changed the expression of the proteins SMPD2, RAB27A, and Alix in the tested concentrations. In conclusion, calpeptin reduced the cell viability and has decreased the EVs formation after 48 hour exposure. On the other hand, calpeptin showed no influence in the expression of proteins associated with EV production. Take togheter, our results suggests that calpain signaling pathway are involved in crucial mechanisms for RCC development and should be explored as important therapeutic target. **Keywords:** exosomes;kidney cancer ; EV inhibitors.

ME - 084 - **Treg Cells and IL-10 profile in peripheral blood samples of B-Cell Acute Lymphoblastic Leukemia Patients Undergoing Induction Therapy**

DA SILVA, F.S.[1]; MAGALHÃES-GAMA, F.[2]; CARVALHO, M.D.P.S.S.[3]; NEVES, J.C.F.[4]; CATÃO, C.L.D.S.[5]; BARROS, M.D.S.[1]; HANNA, F.S.A.[1]; DE ARAÚJO, N.D.[3]; MALHEIRO, A.[3]; MARTINS-FILHO, O.A.[2]; CARVALHO, A.T.[2]; DA COSTA, A.G.[6]. 1. PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA, UNIVERSIDADE FEDERAL DO AMAZONAS, PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA, UNIVERSIDADE FEDERAL DO AMAZONAS MANAUS - AM - BRASIL; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE, INSTITUTO RENÉ RACHOU - FUNDAÇÃO OSWALDO CRUZ, PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE, INSTITUTO RENÉ RACHOU - FUNDAÇÃO OSWALDO CRUZ BELO HORIZONTE - MG - BRASIL; 3. DIRETORIA DE ENSINO E PESQUISA, FUNDAÇÃO HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO AMAZONAS, DIRETORIA DE ENSINO E PESQUISA, FUNDAÇÃO HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO AMAZONAS MANAUS - AM - BRASIL; 4. . PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL, UNIVERSIDADE DO ESTADO DO AMAZONAS, . PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL, UNIVERSIDADE DO ESTADO DO AMAZONAS MANAUS - AM - BRASIL; 5. PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS APLICADAS À HEMATOLOGIA, UNIVERSIDADE DO ESTADO DO AMAZONAS, PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS APLICADAS À HEMATOLOGIA, UNIVERSIDADE DO ESTADO DO AMAZONAS MANAUS - AM - BRASIL; 6. ESCOLA DE ENFERMAGEM DE MANAUS, UNIVERSIDADE FEDERAL DO AMAZONAS, ESCOLA DE ENFERMAGEM DE MANAUS, UNIVERSIDADE FEDERAL DO AMAZONAS MANAUS - AM - BRASIL.

Longitudinal studies have shown that children with B-cell acute lymphoblastic leukemia (B-ALL) are born with a deregulated immune response that, together with postnatal environmental exposures, favor the onset of the disease. In this context, Regulatory T (Treg) cells and interleukin-10 (IL-10), have a key role in immune regulation, has been shown to play a dual role in the development and progression of B-ALL. The frequency and suppressing activity of Treg cells together IL-10 high levels appear to contribute to disease maintenance. Thus, we collect peripheral blood samples from 20 B-ALL patients at 4 times of induction therapy (diagnosis baseline [D0], day 8 [D8], day 15 [D15], and at final of induction therapy [D35]), to evaluate the profile of Treg and IL-10 in peripheral blood from children with B-ALL at diagnosis and during treatment. In parallel, samples from 28 children without leukemia were collected and used as a control group (CG). Treg cells ($CD4^+CD25^+FoxP3^+$) frequency and IL-10 levels was evaluated using immunophenotyping and cytometric bead array by flow cytometry, respectively. Data demonstrated that B-ALL patients displayed at D0, a significant increase in IL-10 levels and a decreased tendency in the frequency of Treg cells, compared to the CG. In addition, kinetic analysis revealed that during remission induction therapy (D8, D15 and D35), B-ALL patients exhibited a clear decline in IL-10 levels, together with a continuous increase in the frequency of Treg cells. In conclusion, our study demonstrated that B-ALL patients have an altered profile of Treg and IL-10, suggesting that these mediators are involved in the pathophysiological process of the disease. However, additional studies are needed to assess the suppressive activity of Treg cells and their impact on the leukemic microenvironment. **Keywords:** childhood leukemia;leukemia microenvironment;immune-suppressive.

ME - 085 - **CD300a modulates the immune response during the late phase of severe experimental malaria.**

NONATO, A.; SILVA, L.P.; DE MEIRA, T.A.; JÚNIOR, C.M.Q.; MELO, N.; MACHADO, F.S.; RICCI, M.F.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Malaria caused by *Plasmodium* infection is a disease with high morbidity/mortality. Respiratory distress, severe anemia, liver dysfunction and hypoglycemia are the main symptoms of severe malaria (SM). CD300a is a receptor present in myeloid and lymphoid cells that participates in the immunoregulation, playing an important inhibitory role, influencing the host's response to infections. Herein, the role of CD300a in the development of experimental SM was investigated. BALB/c (WT) and CD300a knockout (KO) mice were infected with 10^5 *Plasmodium berghei* Anka (PbA)-parasitized red blood cells. Parasitemia, clinical score, survival, histopathology, and cytokine levels were evaluated. Deficiency of CD300 resulted in decreased parasitemia and better clinical score at the early infection time when compared to WT counterparts. Despite of these observations, 20% of the CD300 KO mice had a precocious mortality, and 80% of them presented similar susceptibility to infection than WT animals (dying at the same kinetic). At 12 days after infection, infected CD300 KO mice reduced hemoglobin, red blood cells, and the numbers of circulating leucocytes, mainly macrophages, but increased platelets, when compared with WT. Notably, in the absence of CD300 an increased basal level of IL-6, IL-1b, TNF, IFNg, and TGFb was observed in the brain, but not in lung and liver, when compared with WT. Upon infection, a drastic reduction of the cytokine levels, mainly IFN-g, was observed in the brain of CD300 KO mice. In the liver and lung, the deficiency of CD300 reduced the IL-6 and IL-1b levels, respectively. Finally, a less inflammation in the lung parenchyma, but not in the liver and brain, of KO animals was observed when compared with WT counterparts. Our results reveal an important role for CD300a during severe malaria, reinforcing the need for further investigations into the role of this inhibitory receptor in the development of pathogenesis related to this infection. **Keywords:** Plasmodium berguei ANKA;CD300a;Malaria.

ME - 086 - **Annexin A1 decreases IL-1β production without altering the ability of macrophages to kill L. braziliensis**

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Background: Cutaneous leishmaniasis (CL) is characterized by the presence of skin-ulcerated lesions. CL lesions present high levels of TNF and IL-1B, increased frequency of lymphocytes and mononuclear phagocytes and few parasites. Annexin A1 (ANXA1) is a protein belonging to the calcium-dependent phospholipid-binding annexin's superfamilies that is induced by glucocorticoids and are capable of regulating the production of inflammatory mediators. The anti-inflammatory effects of ANXA1 are mediated for the most part through binding to the formyl peptide receptor 2 (FPR2) resulting in control of the inflammatory response. Our aim was to evaluate the influence of ANXA1 in IL-1β production by L. braziliensis-infected macrophages. **Materials and Methods**: Skin biopsies, whole blood and serum were obtained from CL patients and healthy subjects (HS). Gene expression (IL1B, ANXA1, FPR2, NLRP3, CASP1 and PYCARD) were determined by RNAseq in skin biopsies and whole blood. Monocyte-derived macrophages from HS were infected with L. braziliensis (5:1) in presence or absence of rANXA1 or WRW4 (Selective FPR2 receptor inhibitor) and cultured for 8 and 48h. Levels of ANXA1 and IL-1B where determined in serum and supernatants of culture. **Results:** We observed that the genes described above were increased in CL lesions. We also observed that genes from NLRP3 inflammasome pathway were positively correlated with FPR2 and ANXA1 at lesion, while in blood only the NLRP3 gene was negatively correlated with ANXA1. In addition, we found that CL patients presented higher serum levels of ANXA1 and IL-1B when compared to HS. Leishmania-infected macrophages produced high levels of ANXA1 and IL-1β. Finally, we found that enrichment of ANXA1-infected macrophage cultures decreased IL-1β levels without altering the parasite load. **Conclusion**: Our results suggest that the increase in ANXA1 downregulates IL-1β production without affecting the ability of macrophages to kill L. braziliensis. **Keywords:** Annexin A1; IL-1β; L; braziliensis.

ME - 087 - **COMBINATION OF INTRAVITAL MICROSCOPY AND IMMUNE CELL PHENOTYPING REVEALS NEW INSIGTHS ON LIVER NKT CELL BIOLOGY DURING STERILE AND INFECTIOUS DISEASES**

SALES, S.[1]; FERREIRA, W.[1]; PEDROSA, M.L.[1]; CORRADI, P.[1]; OLIVEIRA, H.[1]; DE PAULA, C.[1]; KELLER, A.C.[2]; MENEZES, G.[1]; ANTUNES, M.M.[1]. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. UNIFESP, SAO PAULO - MG - BRASIL.

Natural killer T (NKT) cells, expressing receptors found in T cells and NK cells, are disproportionately abundant in the liver. However, the role of these cells in the development of liver diseases remains a subject of controversy. The aim of this study was investigating the putative fluctuations of NKT frequency during different models of liver diseases using *ex vivo* and *in vivo* approaches, and their relationship of other liver immune cells. Previous studies conducted by our team have shown that diet, specifically industrialized fat-rich diets including Primex-Z, can modulate the distribution of NKT cells in the liver. These diets resulted in a significant increase in NKT cell numbers and prolonged consumption increased susceptibility to E. coli infection. Similarly, drug-induced liver injury using acetaminophen caused significant variations in NKT cell numbers. However, the geographical distribution of NKT cells *in vivo* during such pathologies has not been described yet, despite its higher number in the liver. In GFP-inserted CXCR6 gene mice, intravital confocal microscopy showed numerous NKT cells in liver sinusoids, patrolling long distances at high speed. Additionally, intravenous antibody counterstaining enabled visualization of neutrophils (anti-Ly6G) and Kupffer cells (anti-F4/80), along with the NKT cells, in their native environment. Infection with *E. coli* and acetaminophen overdose caused an abrupt increase of neutrophils in the liver, along with a major reduction of Kupffer cells. Liver NKT cells maintained their intravascular location without major changes in distribution, implying their role as sentinel cells within the liver environment. This suggests that during diseases, their function may be altered rather than their distribution. Understanding real-time NKT cell function during liver injuries aims to provide new insights into NKT biology and develop potential therapies for liver diseases. **Keywords:** NKT cell;Liver disease;Innate Immunity.

ME - 088 - **CD4+ and CD8+ T cells responses in active TB patients recovered from mild COVID-19**

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The inflammation caused by COVID-19 can damage lung tissues and weaken respiratory function, creating an environment that promotes the growth of additional pathogens. This leads to more severe respiratory symptoms, impaired pulmonary function, and delayed recovery. Additionally, tuberculosis (TB), a prevalent infectious disease primarily affecting the lungs, can be worsened or progress due to comorbidities impacting the immune system. In this study, we evaluated the immune response of $CD4^+$ and $CD8^+$ T cells from patients who had mild COVID-19 and later developed TB. We collected peripheral blood mononuclear cells from three groups: control (n=9), recovered from mild COVID-19 patients (n=9), and TB patients who had mild COVID-19 (n=9) under 4 different conditions: unstimulated (medium), stimulated with SARS-CoV-2 peptides (Pool Spike CoV-2 (PS) and Pool CoV-2 (PT)) and stimulated with staphylococcal enterotoxin B. $CD4^+$ and $CD8^+$ T cells were analyzed for different markers (CD137, CD69, IL-17, TNF-α, and IFN-γ) using flow cytometry. In $CD4^+$ T cells, higher production of TNF-α, IFN-γ, and CD137 was observed in the TB group compared to the control group after peptides stimuli. When PT was used to stimulate $CD4^+$ T cells in TB group, a higher frequency of IL-17 was observed compared to the control group. In $CD8^+$ T cells, the TB group stimulated with the PS showed a higher frequency of CD137 and TNF- α than the mild COVID-19 group. Healthy patients exhibited a lower frequency of $IL\text{-}17^+CD8^+$ T cells than the mild COVID-19 and TB groups following stimulation with peptides. Furthermore, TB patients exhibited higher levels of IL-17 compared to the control group when no stimuli were applied. Thus, it can be observed that the enhanced production of IFN-γ observed in the PT or PS-treated TB group may indicate that TB patients who had mild COVID-19 could display an immune response leading to increased activation of antimicrobial mechanisms against *Mycobacterium tuberculosis*. **Keywords:** Tuberculosis;SARS-CoV-2;T lymphocytes.

ME - 089 - **Role of inflammatory mediators in the reprogramming of dendritic cells obtained from donors with acute and convalescent COVID-19**

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COVID-19 (coronavirus disease 2019) represents a public health problem worldwide. Despite being an acute respiratory infection, some of those affected continue to report symptoms after the initial infection. For that reason, understanding the mechanisms behind the acute and convalescent conditions is fundamental so that relevant pathways can be suggested, and these individuals could be treated. Thus, the aim of this work was to evaluate the cell activation profile, mainly of dendritic cells derived from monocytes of COVID-19 donors (convalescents subjects from mild and severe COVID and acute COVID), and to associate with cellular reprogramming and long-term sequelae of COVID-19. Also, we will evaluate whether HMGB-1, ATP, and lipid mediators are associated with cellular reprogramming. Using flow cytometry, we observed a reduction in the population of $CD66b^+$ cells, an activated granulocyte marker, in the PBMC of convalescent COVID-19 donors ($p<0,05$). However, in the $CD3^+CD8^+$ population, which corresponds to the lymphocytes, and the $CD14^+CD16^+$ population, a marker associated with monocytes, we have not observed any change so far. This data may be associated with the mild condition reported by the donors. In addition, we will evaluate the association and/or correlation between the cell activation profile, the biochemical parameters, and the long-term consequences of convalescent patients. These data may explain the chronic alterations reported by individuals infected with SARS-CoV-2. **Keywords:** COVID-19;inflammation;dendritic cells.

ME - 090 - **Gene Expression Profile of buffy coat in patients with severe COVID-19: Role of HMGB1 alarmin as a regulator of exhaustion response**

GONZAGA, N.A.; SILVA, J.K.A.D.O.; G.RESENDE, A.C.; BARROSO, S.P.C.; COUTINHO-SILVA, R.; VICENTINO, A.R.R.; BENJAMIM, C.F.. UFRJ, UFRJ RIO DE JANEIRO - RJ - BRASIL.

Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) infection stimulates several host immune response pathways, such as increased synthesis of type I interferons (IFNs) and increased secretion of inflammatory mediators, which are essential to limit virus spread. Although the innate and adaptive immune responses are crucial to eliminating SARS-CoV-2, the innate immune system, essential for lymphocyte activation, may in some cases cause an intense inflammatory response after the viral clearance. We recently demonstrated that the severity of Coronavirus Disease 2019 (COVID-19) is directly associated with the high release of extracellular High Mobility Group Box -1 (HMGB1). The HMGB1 acts as an alarmin in a paracrine manner by receptors such as RAGE and TLR4, activating signaling pathways and amplifying the pro-inflammatory response. Recently, the dual role of HMGB1 in regulating the immune response (activation or suppression of T lymphocyte activity) has been studied; however, its role in COVID-19 has not been yet well clarified. This project aims to understand whether the high systemic levels of HMGB1 observed in COVID-19 patients correlate with cell exhaustion response. For this, we analyzed by RT-qPCR the gene expression of HMGB1 receptors and mediators involved in inflammation, activation, and cell exhaustion in buffy coat samples from COVID-19 patients. Our preliminary data showed that the gene expression levels of TCD3 and CD14 cells were decreased in patients with severe COVID-19, which is in line with the literature, in which leukopenia is observed. In addition, we observed a downregulation of RAGE and TLR4 in such subjects, suggesting a mechanism of regulation of the innate immune response to mitigate the effect of acute hyperinflammation. This phenotype may affect the exhaustion dynamics since the levels of PD1, an immune checkpoint protein on T cells, were also decreased in our cohort. **Keywords:** COVID-19;HMGB1;cell exhaustion.

ME - 091 - **TITLE: PRODUCTION OF DENGUE VIRUS' RECOMBINANT PROTEINS FROM SEROTYPES 1 TO 4 IN EUKARYOTIC CELLS FOR THE DEVELOPMENT OF DIAGNOSTIC TESTS.**

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Dengue fever is an infectious disease that causes over 3.2 million cases annually. With the emergence of new diseases such as COVID -19 and the co-occurrence of CHIKV (chikungunya virus) and ZIKV (zika virus), diferential diagnosis of these diseases is essential, mainly because early detection and access to proper medical care are the key to reduce morbidity and mortality. There are two main types of diagnostic tests for Dengue: serological tests and molecular tests. The first one require the availability of antigens and/or specific antibodies, usually obtained as recombinant products. The production of accurate serological Dengue tests presents several limitations: recombinant proteins of this virus are difficult to express in eukaryotic cells whereas recombinant proteins produced in prokaryotic systems have shown little specificity and sensitivity. These proteins have different properties depending on the cell type in which they are produced: in eukaryotic cells, they fundergo specific post-translational modifications which can interfere with the antigen-antibody interactions. In this project, *Dengue virus* E proteins from serotypes 1 to 4 were produced using eukaryotic cells transduced with lentiviral vectors, to generate stable expression lines. The E protein sequence was designed using 80% of the gene with the transmembrane portion removed and the TPA signal peptide added for secretion. The kosak consensus sequence was also added for mRNA translation optimization. Proteins from the 4 serotypes were produced in EXPI293 cells and purified by affinity chromatography, with yields of approximately 3µg of protein per mL of culture. After production, optimizations have been carried out using cell sorting to isolate clones and labeling with specific antibodies for population enrichment. These antigens will be used in diagnostic tests and evaluated in terms of sensitivity and specificity through their ability to be recognized by specific antibodies. **Keywords:** Dengue virus;Recombinant proteins;ELISA .

ME - 092 - **The role of the DDX41 protein in the differentiation and function of regulatory T cells**

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Regulatory T lymphocytes (Tregs,) characterized as CD4+Foxp3+ T lymphocytes, are essential cells in immune homeostasis and maintenance of self-tolerance (Science 299:1057-1061). DDX41 is an RNA helicase member of the DEAD box helicase family that acts as a cytosolic DNA sensor recognizing double-stranded DNA and cyclic dinucleotides. This function occurs through interaction with the STING molecule, which culminates in the synthesis of type I interferons via activation of NFkB and IRF3 signaling pathways (Nature Immun. 12:959-965, 2011). Studies from our research group suggest that STING plays an important role in the differentiation and function of Tregs; however, the role of the DDX41 is still unknown. Therefore, the present work aims to understand the role of DDX41 in the biology of Treg cells. To achieve this, we employed a CRISPR/CAS9 approach to silence DDX41 in a murine T lymphocyte cell line (EL4). The initial characterization of the CRISPR-DDX41 plasmid transduction in murine lymphoma cells was efficiently completed and culminated in the knockout of the DDX41 protein in EL4 cells. Interestingly, during the differentiation of EL4 cells into Tregs with TGF-b, we observed a notable correspondence between the expression periods of Foxp3 and DDX41. Notably, DDX41-deficient EL4 cells showed lower expression of phenotypic and functional markers of regulatory T cells, such as CTLA-4, TIGIT, CD25, and PD-1, and lower expression of IFN-beta when activated with the cyclic dinucleotides C-di-GMP. Based on these findings, we hypothesize that DDX41 plays a crucial role in Treg cell function. As a result, the following steps of our investigation involve studying knockout mice to understand further the underlying mechanism of DDX41 in Treg cell biology. These insights may provide valuable information about the relationship between DDX41 and Tregs, making it a potential target for enhancing our comprehension of adaptive immunity and immunoregulation. **Keywords:** DDX41;Treg;inflammation.

ME - 093 - **Regulation of lipid bodies and inflammatory lipid mediators by bioactive flavonoid compounds**

LUZ, L.; WANDERLEY, J.L.. UENF, UENF CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Lipid bodies are organelles that function as a site for the synthesis of inflammatory lipid mediators, such as prostaglandins and leukotrienes, participating in the inflammatory activation of the immune system cells. Quercetin and rutin are flavonoids widely found in several plants with antioxidant, antiviral, antimicrobial and anti-inflammatory activity. We will evaluate the activity of the bioflavonoids rutin and quercetin in the formation process of lipid bodies, and inflammatory lipid mediators production in activated macrophages. With this, we intend to evaluate the role of these compounds in the regulation of the inflammatory response mediated by this pathway, suggesting a possible alternative to the use of non-steroidal anti-inflammatory drugs. The methodology consists of using cultures of macrophages from the bone marrow of C57BL/6 mice. These macrophages will be activated with TLR receptor agonists, and treated with quercetin and rutin. Stimulation with oleic acid will be used as a positive control of corpuscle production. The production of lipid bodies will be evaluated by flow cytometry after labeling with BODIPY. The location of these organelles will be done by optical or confocal microscopy after marking with Oil Red. The cellular localization of the COX2 enzyme will be submitted Western Blot essay. The production of prostaglandin E2 and leukotriene B4 will be determined by ELISA. Preliminary results showed that after 2 hours of culture there was no difference in the production of corpuscles between activated and non-activated macrophages. And after 24 hours there was a greater number of lipid bodies in the macrophages activated with oleic acid. These results allowed learning the corpuscle quantification technique that will be used to evaluate the action of bioflavonoids on activated macrophages. **Keywords:** Lipid bodies;bioflavonoids;inflammation.

ME - 094 - **Multiparameter flow cytometry for diagnosis and prognostic stratification of neuroblastic tumors – STOT tube.**

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Peripheral neuroblastic tumors (pNTs including Neuroblastoma, Ganglioneuroblastoma and Ganglioneuroma) are biologically and clinically heterogeneous extracranial tumors responsible for 15% of childhood cancer death. One of the most important prognostic values is the International Neuroblastoma Pathology Classification (INPC), which distinguishes a favorable histology (FH) and an unfavorable histology (UH). Despite high cure rates in low-risk group, patients in the high-risk group remains with survival rates less than 50% despite currently available high-intensity and multimodal therapy. As the majority of these high-risk patients are classified into the UH group according to the INPC but are molecularly heterogeneous, novel predictive markers as immunophenotypic features can be helpful to improve stratification and guide therapy. Here we describe the immunophenotypic analyzes by multiparameter flow cytometry (MFC) of 92 samples from 51 patients – 27 female and 24 males; median age 2.5y (0-9 years) with a single tube of 8-color/12-markers STOT ("Solid Tumor Orientation Tube") designed for diagnostic orientation and follow up of pediatric solid tumor. Of 92 samples 25 were tissue samples, 47 bone marrow, 18 peripheral blood and 2 lymph nodes. From 92 samples analyzed, 51 (55%) were infiltrated by neuroblastic cells and could be all correctly assigned as neuroblastic tumors at diagnosis with the combination of GD2+/ CD56+/ CD271het/ CD99-/ myogenin-/ EpCAM-/ CD45-/ smCD3-/ cyCD3-/ CD19-/ CD4-/ CD8- immunophenotype. Interestingly, inside tissue samples, local vs. metastatic tumors had a statistically significant median fluorescent intensity (MFI) difference in GD2 (MFI 3000 vs. 24000; p=0.023) and CD56 (MFI 13556 vs. 62644; p=0.05). In summary STOT strategy can be used as an auxiliary weapon at neuroblastic diagnosis and also add valuable information for prognosis and anti-GD2 treatment. **Keywords:** Multiparameter flow cytometry;Neuroblastic tumors ;Pediatric solid tumors.

ME - 095 - **Small extracellular vesicles from adipose tissue induce the production of inflammatory cytokines in human macrophages**

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Introduction: Obesity is a public health problem and a chronic condition defined as an excess of adipose tissue. In obese individuals, increased adiposity is associated with chronic inflammatory response and metabolic dysfunction, alongside dysregulated secretion of predominantly pro-inflammatory adipokines. Extracellular vesicles play an essential role in intercellular communication, modulating functions in target cells. The phenotype of macrophages is highly associated with their biochemical and immunological environment, and during obesity macrophages in the adipose tissue are majorly polarized towards M1. The objective of this study is to investigate whether metabolic dysfunction in adipose tissue from obese patients modulates macrophage polarization through small extracellular vesicles (EVs) production. Materials and Methods: Human adipose tissue was collected from bariatric surgeries or cholecystectomy, and EVs were obtained from adipose tissue explants using ultracentrifugation. Human macrophages were differentiated from peripheral blood monocytes obtained from eutrophic healthy subjects, and stimulated with 25 µg/mL of small extracellular vesicles from obese or eutrophic adipose tissue. Secretion of cytokines and NO was evaluated in culture supernatants. Results: Small EVs from obese adipose tissue induced the production of IL-1 beta, IL-6, and NO by macrophages when compared to macrophages stimulated with EVs from eutrophic adipose tissue. Conclusions: Our preliminary results show that EVs from adipose tissue of obese individuals induce an inflammatory profile in macrophages suggestive of polarization towards M1, rather than M2, phenotype, which was not induced by EVs from eutrophic subjects. The definitive induction of M1 polarization by EVs from obese adipose tissue is under investigation using other cellular markers. **Keywords:** Obesity;Small extracellular vesicles;Macrophages.

ME - 096 - **Analysis of platelet activation and interaction with epithelial cells during SARS-CoV-2 infection**

DE CAMPOS, M.M.[1]; AZEVEDO-QUINTANILHA, I.G.[2]; DIAS, S.D.S.G.[2]; SOARES, V.C.[2]; SANTOS, J.D.C.[2]; DE OLIVEIRA, D.M.[2]; GONCALVES, R.M.[3]; HOTTZ, E.D.[4]; BOZZA, F.A.[2]; BOZZA, P.T.[2]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Platelets are essential in hemostasis and thrombus architecture, but they are also capable of recognizing viral pathogens and participating in the mediation of the inflammatory response. During SARS-CoV-2 infection, platelets are activated and contribute to hypercoagulability and inflammatory amplification, which can collaborate with injury to the pulmonary vasculature and induce the process of immunothrombosis in the pulmonary epithelium. Therefore, it is essential to understand the role of platelets in the face of SARS-CoV-2 infection and their interaction with lung epithelial cells. **Objectives:** To characterize platelet activation and interaction with pulmonary epithelial cells during SARS-CoV-2 infection and understand what mechanisms and immunometabolic changes come from this interaction. **Methodology:** Epithelial lung adenocarcinoma cells-ATCC/HTB-55 (CALU-3) were infected with SARS-CoV-2 and stimulated with platelets from healthy donors. The interaction between these cells was evaluated 24 and 48 hours after infection. **Results**: Platelets are activated by SARS-CoV-2 infection but are not able to generate infectious particles. However, platelets induce increased viral replication in infected Calu-3 cells, increased production of thromboxane and tissue factor. Inhibiting pathways of platelet activation and thromboxane synthesis, viral replication was reduced. Amplification of immunometabolic changes and non-inflammatory cell death was characterized in the interaction between platelets and infected Calu-3 cells. **Conclusion:** Platelets modulate infected Calu-3 cells, which allows for increased viral replication but not progression to apoptotic cell death. The data obtained suggest that viral replication is increased in the presence of platelets due to increased release of thromboxane and platelet tissue factor that act in in platelet activation and aggregation, collaborating with thromboinflammation. **Keywords:** platelets;thromboinflammation;SARS-CoV-2.

ME - 097 - **THE EFFECTS OF CHRONIC CONSUMPTION OF SUGARY SWEET BEVERAGES ON METABOLISM, INTESTINAL MUCOSA IMMUNITY AND BEHAVIOR IN EARLY WEANING MICE**

ABREU, T.M.[1]; BARBOSA, S.C.[2]; MOREIRA, F.C.[1]; MOREIRA JUNIOR, R.E.[1]; DE OLIVEIRA, M.F.A.[1]; FARIA, A.M.C.[1]; MAIOLI, T.U.[1]. 1. UFMG, UFMG BELO HORIZONTE - MG - BRASIL; 2. USP, USP RIBERÃO PRETO - MG - BRASIL.

INTRODUCTION: The rising consumption of meals rich in fat and refined carbohydrates has been associated with the development of metabolic alterations and increased inflammation in adipose tissue and lymphoid organs. Furthermore, there is growing evidence linking this dietary pattern to the emergence of psychiatric disorders. Among the extensively consumed foods, sugar-sweetened beverages (SSBs) stand out due to their high glucose and fructose content. Thus, this study aims to investigate the effects of early-life SSB consumption. METHODS: We employed an experimental model using C57BL/6 mice subjected to early weaning. The mice were fed an AIN93G diet and received either a sugary drink containing 30% glucose and fructose in a 45:55 ratio or filtered water. After 8 weeks of treatment, we evaluated anxiety-like behavior, metabolic parameters, and immunological changes. RESULTS: The animals exposed to sugary sweet beverages for 8 weeks exhibited increased anxiety-like and compulsive behaviors. However, there were no significant differences in weight gain or fasting glycemia compared to the control group. Moreover, we observed na enhanced inflammatory profile in the ileum segment. CONCLUSION: Chronic SSB consumption was found to impact behavior and potentially lead to metabolic changes without causing weight gain. **Keywords:** sugar consumption;anxiety;imunoregulation .

ME - 098 - **Effect of the proteolytic fraction of Vasconcellea cundinamarcensis in the inflammatory response associated to murine GVHD.**

RUGGIO, G.M.F.; ROMERO, M.G.M.E.C.; LOPES, M.T.P.; LINHARES, A.F.S.; CAMPOS, Z.D.T.. UFMG, UFMG BELO HORIZONTE - MG - BRASIL.

Graft-versus-host disease occurs after hematopoietic stem cell transplantation, almost exclusively in patients who receive an allogeneic transplant. In GVHD, the donor's alloreactive T cells attack the recipient's healthy tissues such as liver and intestine caused profuse diarrhea, severe malnutrition and intestinal bleeding, these symptoms generate a poor prognosis for the patient. Currently, the use of corticosteroids is the mainly therapy available. Then, we sought to investigate P1G10, the proteolytic fraction of Vasconcellea cundinamarcensis. Studies show protective effect against gastric and colon ulcers of P1G10 in animal models, possibly by anti - inflammatory and antioxidant action. Then, the aim of the research is to investigate the protective role of P1G10 in the GVHD mice.Male, C57BL/6j and BALB/c mice were used, with C57BL/6j undergoing total bone marrow ablation followed by allogeneic transplantation (GVHD group), while the control group received syngeneic transplantation. The animals were divided into control, vehicle and treated groups (P1G10 at 0.3; 3,0 or 30,0mg/kg). In the dose-response curves the dose of 0.3mg/kg showed better results in the clinical scale, weight gain and survival of the animals. The animals used to inflammatory response analysis were euthanized 7 days after transplant and histological analysis of the intestine, colon and liver was performed, which showed mild tissue damage in the treated animals. A cytokine test was carried out in the intestine, colon and liver using the Elisa method, who is currently analysis. The data presented so far demonstrate a possible gastrointestinal protective function of P1G10 at low doses in the murine model. **Bibliographic references:** 1. **N Engl J Med.,** 43, 8:1088-1092, 2021. 2. **Br J Haematol.,** 187, 5:563-572, 2019. 3. **Nat Rev Gastroenterol Hepatol.**,12:711-726, 2017. 4. **Blood.,** 18:1657-1665, 2021. 5. **J Pharm Pharmacol.,**1:133-141, 2015. 6 **Scientific Reports.,** v. 10, p. 3074, 2020. **Keywords:** GVHD;P1G10;intestino .

ME - 099 - **AXL RECEPTOR MEDIATES THE UPTAKE OF INFECTED RED BLOOD CELLS AND REGULATES THE INFLAMMATORY RESPONSE IN PLASMODIUM CHABAUDI- INFECTED MICE**

LEITE, N.P.D.S.[1]; RAMOS, R.D.S.[2]; CORREA, A.A.D.S.[1]; RIGONI, T.D.S.[1]; SILVA, J.S.[3]; COSTA, D.L.[1]; MATTEUCCI, K.C.[1]; GAZZINELLI, R.T.[3]. 1. RIBEIRÃO PRETO MEDICAL SCHOOL - UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO MEDICAL SCHOOL - UNIVERSITY OF SÃO PAULO RIBEIRÃO PRETO - SP - BRASIL; 2. FACULTY OF PHILOSOPHY, SCIENCES AND LETTERS AT RIBEIRÃO PRETO - UNIVERSITY OF SÃO PAULO, FACULTY OF PHILOSOPHY, SCIENCES AND LETTERS AT RIBEIRÃO PRETO - UNIVERSITY OF SÃO PAULO RIBEIRÃO PRETO - SP - BRASIL; 3. BI-INSTITUTIONAL TRANSLATIONAL MEDICINE PROJECT FIOCRUZ/USP, BI-INSTITUTIONAL TRANSLATIONAL MEDICINE PROJECT FIOCRUZ/USP RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Axl receptor mediates efferocytosis and regulates the inflammatory response through its intrinsic signaling. These processes relate to the mechanisms of disease in parasitic diseases. This study aims to characterize phenotypically and functionally the biological role of Axl receptor during experimental infection with *Plasmodium chabaudi* AS (*Pc*) in mice. **Results:** Here we report that Axl expression is enhanced in monocytes, conventional dendritic cells (cDCs) and monocyte-derived dendritic cells from liver and spleen of *Pc*-infected mice. We also observed that the Axl receptor mediate the uptake of red blood cells by phagocytic cells. Importantly, the frequency of cDCs and Th1 cells was increased and parasitemia reduced in Axl-deficient mice. Moreover, myeloid cells from Axl-deficient mice produced less TNF and the plasma levels of inflammatory cytokines were reduced in Axl KO mice infected with *Pc*. **Conclusion:** Axl receptor plays and important regulatory role both in the innate and adaptive immune responses during *Pc* infection in mice. Hence, our results suggest that Axl receptor plays and important role in the immunopathogenesis of malaria. **Financial support:** The State of São Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq). **Keywords:** tam receptors;malaria;axl receptor.

ME - 100 - **Evaluation of IL-6 in the differentiation of RORγt+ Foxp3+ regulatory T lymphocytes in its participation in obesity-induced meta-inflammation**

PACHECO, T.C.F.; OLIVEIRA, J.E.; PEREIRA, Í.S.; BARBOSA, S.C.; RODRIGUES, V.F.; PEREIRA, J.A.; MACHADO, M.S.G.; SARTORI, D.C.. UNIVERSIDADE DE SÃO PAULO (USP) - FMRP, UNIVERSIDADE DE SÃO PAULO (USP) - FMRP RIBEIRÃO PRETO - SP - BRASIL.

Obesity is characterized by the accumulation of body fat and it is a chronic disease that has a multifactorial etiology and predisposes to other comorbidities. Recently, it was reported that T helper cell differentiation involves an intermediate stage FoxP3$^+$/RORγt$^+$ (Tr17 cells) in the presence of IL-6. Tr17 cells are particularly reside in the lamina propria of the intestine, where it is believed that, via IL-10 secretion, they may exert a protective role against metabolic syndrome (MS). The aim of this work was to investigate the role of IL-6 in the differentiation of Tr17 cells and in the inflammatory and metabolic alterations associated with obesity-induced MS. To evaluate IL-6 gene and protein expression in the ileum by RT-PCR and ELISA, respectively, wild-type (WT) C57BL/6 mice fed with standard diet (SD) (composed of 10% fat, 70% carbohydrate and 20% protein) or high-fat diet (HFD) (composed of 60% fat, 20% carbohydrate and 20% protein) were used. Furthermore, C57BL/6 WT and IL-6 deficient (IL-6KO) mice were divided into 3 groups: WT animals with SD; WT animals with HFD; and IL-6KO animals with HFD for assessment of body weight, glycemic levels, body fat accumulation and adiposity index. Treg cells were isolated from Foxp3-GFP mice by FACS-sorting, stimulated in vitro with recombinant IL-6 and subsequently analyzed by flow cytometry. Finally, validation of the probiotic from *Lactococcus lactis* expressing IL-6 was performed by western blot and ELISA. We observed that HFD-fed animals had a downregulation of IL-6 levels in the ileum. In addition, IL-6KO mice became more obese, had metabolic dysfunction, body fat accumulation and higher glycemic levels. Later, we also verified that IL-6 contributes to the in vitro generation of Tr17 cells and confirmed that the analyzed probiotic is expressingIL-6 and could be used in further experiments. These data suggest that IL-6 is important for Tr17 cell differentiation and in the control of obesity-induced MS. **Keywords:** Tr17;cytokines;metabolism.

ME - 101 - **Late lipid accumulation modulates dendritic cell activation, inflammatory response and bacterial burden during M. bovis BCG infection**

DA CRUZ, G.I.P.[1]; PEREIRA, J.C.[2]; DE OLIVEIRA, R.N.[2]; ROMA, E.H.[3]; SOUZA, A.L.[3]; SOARES, V.C.[4]; PEREIRA-DUTRA, F.[4]; VIOLA, P.T.B.[4]; SILVA, M.F.D.S.C.[2]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATÓRIO DE IMUNOBIOLOGIA DA INFLAMAÇÃO / UFF, NITERÓI - RJ - BRASIL; 3. LABORATÓRIO DE IMUNOLOGIA E IMUNOGENÉTICA DE DOENÇAS INFECCIOSAS - INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. LABORATÓRIO DE IMUNOFARMACOLOGIA / INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Tuberculosis represents a major challenge to public health, killing more than 1 million people each year. In the lungs, *Mycobacterium tuberculosis* (Mtb) infects mainly macrophages and dendritic cells (DCs), which are important bacterial reservoirs and initiators of the immune response. Mtb changes host metabolism as a survival mechanism. Lipid accumulation has been shown to be a key component in host-pathogen interactions, enabling bacterial persistence. Thus, herein, we evaluated the role of lipid synthesis enzymes in DC activation during *M. bovis* BCG infection. DCs obtained from C57BL/6 bone marrow were differentiated with 10 ng/ml GM-CSF for 10 days and infected with BCG (MOI 5) or Mtb H37Rv (MOI 1). Unlike macrophages, Mtb- and BCG-infected DCs presented a late increase in lipid droplets (LD) and in lipid-related (*fasn, dgat1, dgat2, acat1, plin2, plin3, atgl*) and proinflammatory (*il1b, il10, cox2, 5lo, mr1*) gene expression. Indeed, triacylglycerol (TAG) and cholesterol ester (CE) accumulate in DCs during Mtb and BCG infection. To evaluate the roles of TAG and CE synthesis, BCG-infected DCs were treated with diacylglycerol acyltransferase 1 (DGAT-1; A922500; 20μM) and the acyl-coenzyme A (CoA):cholesterol acyltransferase (ACAT; Ci976; 10μM) inhibitors. ACAT inhibition did not affect LD formation in DCs, but it increased the bacterial burden. Furthermore, ACAT inhibition reduced the production of IL-1beta, IL-6, IL-10, IL-12p40, TNF-alpha and PGE_2 by BCG-infected DCs. DGAT-1 inhibition only reduced LD accumulation and PGE_2 release by these cells. We also observed that the treatments inhibited the expression of MHCI, MHCII and CD80 by infected DCs. TAG and DGAT-1 are important for LD formation in DCs, whereas CE synthesis by ACAT is involved in the host inflammatory response. Both pathways seem to represent potential targets to modulate the DC response against tuberculosis. **Keywords:** tuberculosis ;dendritic cell;lipid metabolism.

ME - 102 - **ACTIVATION OF IMMUNE RESPONSE BY NANOPARTICULES OF OVALBUMIN WITH PDDA (POLY-DIALI-DIMETHYL-AMMONIUM CHLORIDE)**

PEREIRA, D.R.[1]; BENTACOURT, Y.P.[2]; TÁVORA, B.D.C.L.F.[1]; MISACA, R.C.[3]; OLAGUNJU, A.S.[4]; COELHO, G.R.[5]; DE CARVALHO, E.[6]; AMARANTE-MENDES, G.P.[4]; CARMONA-RIBEIRO, A.M.[2]; MAURO, E.F.D.L.[1]. 1. LABORATORY OF IMMUNOPATHOLOGY, BUTANTAN INSTITUTE, DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. DEPARTMENT OF BIOCHEMISTRY, CHEMISTRY INSTITUTE, UNIVERSITY OF SÃO PAULO, SÃO PAULO, BRAZIL., SÃO PAULO - SP - BRASIL; 3. LABORATORY OF IMMUNOPATHOLOGY, BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL; 4. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO., SÃO PAULO - SP - BRASIL; 5. LABORATORY OF BIOCHEMISTRY, BUTANTAN INSTITUTE, SÃO PAULO, BRAZIL., SÃO PAULO - SP - BRASIL; 6. LABORATORY OF BACTERIOLOGY, BUTANTAN INSTITUTE, UNIVERSITY OF SÃO PAULO., SÃO PAULO - SP - BRASIL.

Introduction: The poly-diali-dimethyl-ammonium chloride (PDDA) is a cationic polymer able to conjugate with ovalbumin (OVA) creating a turbid colloidal dispersion of OVA/PDDA-NPs. It was previously demonstrated that OVA/PDDA-NPs induce potent humoral and cellular OVA-specific immune responses. As known, Dendritic Cell (DC) exerts a relevant role in the induction of antigen-specific T cell activation and consequent cellular immune response. We aimed to evaluate the effect of OVA/PDDA-NPs on anti-OVA immune response, as well as on the functional activity of DCs. Methods and results: The structural characteristic of OVA/PDDA-NPs was analyzed by circular dichroism and no significant change was verified in the OVA/PDDA-NPs compared with soluble OVA or OVA/Alum. In addition, after 28 days of in vivo immunization, OVA/PDDA-NPs induced an increase of CD19+CD27+CD38+ cells and CD19+CD138+cell populations, as well as Th1 and Th2 cells compared with non-immunized group. OVA/PDDA-NPs were able to induce an increase of DCs in draining lymph nodes of mice immunized 3-4 days before and also induced the maturation of this cell population (expression of co-stimulatory and MHC II molecules). Conclusion: Our data indicate the immunogenic activity of OVA/PDDA-NPs and the effect on DC maturation. Financial support: CNPq and CAPES. **Keywords:** adjuvants;immunomodulation;dendritic cell.

ME - 103 - **Delivery of Anti-CD3 scFv by Saccharomyces boulardii Decreases Inflammation in DSS-Induced Colitis in Mice**

PINHATE, S.B.; OLINTO, V.G.; DO ALMO, M.M.; RÔLO, J.L.J.D.P.; MARANHÃO, A.Q.; BRIGIDO, M.D.M.. UNIVERSIDADE DE BRASILIA, BRASILIA - DF - BRASIL.

Introduction. Inflammatory Bowel Diseases, such as Ulcerative Colitis (UC), are chronic inflammatory disorders that affect the gastrointestinal tract (GIT). UC is characterized by recurrent inflammation and damage to the colon region, and treatment is based on anti-inflammatory drugs and monoclonal antibodies. *Saccharomyces boulardii* is a probiotic yeast widely used to treat intestinal disorders, and studies suggest beneficial effects in UC. Additionally, it can be genetically modified for heterologous expression and adapted for the oral delivery of molecules in the GIT. Here, we investigate the therapeutic effects of *S. boulardii* as a delivery vehicle of an immunomodulatory anti-CD3 scFv antibody fragment in a Dextran Sodium Sulfate (DSS) induced colitis mouse model. **Methods and Results**. ScFv expression in yeast was monitored by western-blot and Cytometry. All the diseased groups were given 3% DSS in water for the first five days. During the 11-day experiment, mice were orally gavaged daily with recombinant yeast, for the treated group or 0.9% saline, for the control group. Weight, fecal consistency, and rectal bleeding were monitored to evaluate the disease activity index, and on the day of euthanasia, the colon was measured and weighed. The anti-CD3 treated group showed similar inflammatory parameter values to the healthy group and significant differences from the non-treated group. **Conclusion.** Despite low antibody detection in transformed yeast in flow cytometry tests, their oral administration improved disease symptoms. Next, we are working to make the antibody more available on the yeast surface and maximize its beneficial effects to test it again in an in vivo model. **Keywords:** Ulcerative Colitis;Immunotherapy;Anti-CD3.

ME - 104 - **NEUROPROTECTIVE AND ANTI-INFLAMMATORY POTENTIAL OF EXTRACTS FROM MARINE SPONGE SPECIES**

NUNES, C.D.J.; COSTA, C.C.D.O.S.; SOUZA, L.D.J.; LANNA, E.; BATISTA, R.; DO NASCIMENTO, R.P.; COSTA, S.L.. UFBA, SALVADOR - BA - BRASIL.

INTRODUCTION: The search for new therapies investigate natural compounds with anti-inflammatory potential in the Central Nervous System for the treatment of neurodegenerative diseases. Sponges are among the most prolific marine organisms for new substances, and studies have demonstrated anti-inflammatory activities of some species. **OBJECTIVES:** To evaluate the cytotoxicity and neuroprotective potential of extracts from marine sponge species against in vitro inflammatory damage associated with the modulation of microglia response. **METHODS:** Cultures of PC12 cells treated with 20 extracts obtained with dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) solvents from marine sponges of the genera *Aplysina, Cladocroce, Condrilla, Callyspongia and Haliclona*. Cell viability was determined after 72 h of MTT treatment. PC12 cells were exposed to LPS for 12 h and treated for 24 h with *Aplysina fulva* extract (AF-MeOH), or with its purified compound (AF-H1). Cell viability was assessed by Trypan Blue. Microglia from the cerebral cortex of newborn rats were treated for 24 h with PC12 cell conditioned medium (MC-PC12) under these conditions and their phenotype was evaluated by phase contrast, Rosenfeld staining and immunocytochemistry. **RESULTS:** AF-MeOH and AF-H1 were not toxic at the tested concentrations. PC12 cells subjected to damage with LPS showed contracted cell bodies, an effect not observed in cultures treated with AF-MeOH and AF-H1 at the adopted concentrations. Microglia treated with MC-PC12 submitted to LPS showed amoeboid phenotype; in contrast, microglia subjected to MC-PC12 with LPS and AF-MeOH or LPS and AF-H1 showed a more branched phenotype, as did the control. **CONCLUSIONS:** Marine sponge extracts and AF-H1 were not toxic to neuronal cells. Treatment with these compounds contributed to a possible reversal of the inflammatory phenotype of PC12 cells and microglia. **SUPPORT:** CNPq; INNT; CAPES. **Keywords:** Neuroinflammation;Neuroprotective;Marine sponges
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ME - 105 - **Pleiotropic activity of CX3CL1 in experimental acute phase of Trypanosoma cruzi infection**

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The protozoan Trypanosoma cruzi triggers an acute, systemic and local inflammatory response causing alterations in the morphology and functionality of the heart. Understanding cardiac immunopathogenesis through the recruitment/activation of leukocytes and release of inflammatory mediators, it would be possible pharmacological intervention to mitigate this clinical condition. In this sense, the chemokine CX3CL1 and its receptor, CX3CR1, act favoring Th1 profile cells adhesion in inflammatory sites. Depending on the stimuli, the release of CX3CL1 can accelerate inflammatory resolution or promote tissue damage. The purpose of the study is to investigate the role of CX3CL1 in mice infected by T. cruzi, under allosteric blockade (AZD8797) of CX3CR1. For this, male C57BL/6 lineage mice were infected, via intraperitoneally, with 10^3 trypomastigotes forms of T. cruzi (Y strain), and grouped into: (i) T. cruzi and (ii) T. cruzi + 10mg/Kg of AZD8797, administered intraperitoneally for 10 days. In addition to the parasitemia and survival curve, heart and muscle fragments of the skeletal muscle were evaluated for relative mass, histomorphometric patterns and quantification of inflammatory mediators (IL-10 and TNF) using the method of enzyme immunoassay. Preliminary results demonstrate a delay in the detection of circulating parasites and a decrease in the parasitemia in the T. cruzi + AZD8797 animals. Likewise, this group showed a reduction in the relative mass and production of IL-10 and TNF in the cardiac tissue. On the other hand, the use of AZD8797 increased relative mass and IL-10 production in skeletal muscle tissue. Together, these data suggest a polarized action of CX3CL1 in a site-dependent way during acute T. cruzi infection, making this chemokine/receptor a promising pathway to be deepest investigated as a prognostic marker of cardiac pathogenesis in experimental models. Funding agencies: Capes, Fapemig, CNPq, UFOP, FINEP. **Keywords:** Trypanosoma cruzi;inflammation;CX3CL1/CX3CR1.

ME - 106 - **The Contribution of Neutrophil Extracellular Traps (NETs) to Amyloid Disease in a Pulmonary Context**

MARTINS, A.D.S.[1]; PIRES, T.R.C.[1]; GUIMARÃES, F.V.[2]; DE ARANTES, A.C.S.[2]; MARTINS, P.M.R.E.S.[2]; FOGUEL, D.[1]. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Amyloid fibrils (AF) are composed of misfolded proteins organized in a β-cross structure and are the hallmark of amyloid diseases, such as Alzheimer's and Parkinson's diseases. Neutrophil Extracellular Traps (NETs) are decondensed chromatin fibers decorated with antimicrobial proteins. Although NETs play an important role in immune homeostasis, they have already been associated with conditions such as tissue damage, autoimmunity, and metastasis, among others. Our group showed for the first time that human neutrophils when incubated with AF in vitro, release NETs in an NADPH oxidase (NOX-2)-dependent manner and that neutrophil-derived elastase participates in amyloid fragmentation forming toxic oligomeric species. Now, these studies were extended to mice (WT and gphox91 deficient mice - KO mice), by the instillation into the lungs of AF to evaluate the contribution of NET to the disease progression. Our data showed that after 3h of instillation of α-synuclein AF, there is a progressive recruitment of neutrophils to the lungs of the WT and KO mice causing an extensive inflammation of the tissue. AF were visualized in the lungs of both animals, but they disappear along time in the WT lungs remaining intact in the KO lungs suggesting their fragmentation and/or phagocytosis. Slices of the lung tissues of both animals revealed the presence of NETs only in the WT animals, consistent with the inactivity of NOX-2 in KO mice. Furthermore, the presence of AF altered the lung function of the WT animals in the presence of methacholine. Small oligomers derived from the digestion of AF were observed only in the lungs of WT mice, suggesting that the proteases present in NETs might be responsible for AF degradation and oligomers formation. These studies open new avenues for the participation of neutrophils and their traps in amyloid diseases contributing to the inflammation observed in these diseases but also by the generation of toxic, diffusible small oligomeric species. **Keywords:** Neutrophil;Neutrophil Extracellular Traps;Amyloid Diseases.

ME - 107 - **Role of lipid droplets in neural cell death and neuroinflammation induced by Zika virus infection**

DA SILVA, H.E.[1]; FERNANDES, T.D.C.[2]; DIAS, S.D.S.G.[2]; PEREIRA-DUTRA, F.[2]; VIOLA, P.T.B.[2]. 1. FEDERAL UNIVERSITY OF RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Zika virus is an arbovirus responsible to cause microcephaly and different neurological impairments. Neurodegeneration is the gradual loss of neurons structure and function until cell’s death.Lipid droplets are cytoplasmic organelles that are often related to neurodegeneration and widely used as a platform for viral replication.Data from our group showed the importance of LDs in the pathophysiology and viral replication in Zika disease in the brain.Some studies demonstrate the ability of the ZIKV to induce cell death, such as: apoptosis, pyroptosis and necroptosis.However, the mechanisms by which ZIKV impacts the central nervous system throughout development and whether and how alterations in LDs are involved with ZIKV-triggered cell death *in vivo* are still poorly understood.The present work seeks to investigate the role of LDs in cell death and neuroinflammation during ZIKV infection through the inhibition of DGAT-1, the last enzyme in the synthesis of triacylglycerols. Swiss Webster mice were provided by FIOCRUZ animal facility and protocols approved by the institutional ethical board (CEUA-IOC L002/2018-A2).3-day-old SW mice were infected or not with ZIKV (strain MR766) by the intraperitoneal route.To inhibit LD formation, animals were pretreated with DGAT-1 inhibitor (iDGAT; A922500; 2.5 mg/kg). At 13 days post-birth, analyzes of whole brain or regions of interest were evaluated from samples collected from euthanized animals. ZIKV infection triggered increased expression of the cleaved form of caspase-3.It was noticed the fragmentation of DNA of the neural cells from TUNEL assay. Pretreatment with iDGAT-1 was able to reduce the impact of apoptosis in the ZIKV infection.Furthermore, serum or whole brain tissue analyzes showed a significant reduction in the levels of IFN-γ, IL-10, IL-1β, MCP-1, TNF-α and IL-6 in the ZIKV group treated with iDGAT. These data presented here suggest that LD are involved in neuroinflammation and apoptosis pathway induced by ZIKV. **Keywords:** Zika virus;lipid droplet;cell death.

ME - 108 - **Comparative assessment of haematological and inflammatory profiles in ICU patients with Covid-19 during distinct SARS-CoV-2 outbreaks in Natal-RN**

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Covid-19, caused by SARS-CoV-2 infection, has emerged as the deadliest pandemic of the 21st century. The prognosis of this disease hinges on several factors, including the patient's immune response, metabolic and nutritional status, as well as the virus genetic characteristics. Therefore, this study aims to investigate the inflammatory profile, oxidative stress, and vitamin D levels among patients admitted to intensive care units (ICUs) in two different hospitals located in Natal-RN, during the outbreaks of the original and Gamma strains of SARS-CoV-2. Patients with positive RT-PCR for SARS-CoV-2 were organized into two groups: the first group of patients (HC) had their blood collected from April to May of 2020, during the outbreak of the original strain of SARS-CoV-2. The second group of patients (HGT) had their blood collected in January 2021, during the outbreak of the Gamma strain. The number of leukocytes, neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were determined. The levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), creatinine, vitamin D and malondialdehyde (MDA) were evaluated. Finally, the systemic levels of cytokines and chemokines were determined by 27-plex Luminex assay. Based on haematological characteristics, principal component analysis (PCA) revealed two distinct groups, in which HGT patients presented higher numbers of leukocytes, mainly neutrophils, with higher NLR and PLR ratios when compared to HC patients. In addition, higher levels of LDH, CXCL8 and MDA, and lower levels of vitamin D, GM-CSF and IFN-γ were found among HGT patients. The observed neutrophilia, together with higher levels of CXCL8 and LDH among HGT patients suggest the higher susceptibility to the occurrence of pyroptosis and NETosis, which can play major role in the severity of pulmonary damage and systemic impairment during Covid-19 caused by the Gamma variant of SARS-CoV-2, in contrast to HC patients infected with the original strain. **Keywords:** Inflammation;Covid-19;Neutrophilia.

ME - 109 - **Impact of the Covid-19 bivalent vaccine on the B cell repertoire dynamics and antibodies kinetics in patients immunized with different vaccine protocols.**

DA SILVA, G.M.; VALE, A.M.; MAIA, L.R.C.; MEIRA, G.D.A.C.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The etiologic agent of the COVID-19, SARS-CoV-2, has been the subject of many studies due to its high rate of transmission, which generally precedes symptoms. Viral infections trigger the clonal expansion of specific B cells that produce antibodies against structurally distinct epitopes from the viral particles. Those antibodies can prevent the binding of viruses to their cell receptors or form immune complexes, leading to the pathogen elimination. Furthermore, these specific B lymphocytes undergo class switching and somatic hypermutation favoring the selection of antibodies with increased affinity to viral antigens as the time of infection progresses. In this work, our main objective is to compare the dynamics of the repertoire of B lymphocytes potentially producing neutralizing antibodies, after the complete application of two distinct vaccine protocols (AstraZeneca x CoronaVac) and subsequent bivalent vaccine boost. For this purpose, we conducted a longitudinal analysis on a patient cohort, tracking their immune response from 2020 to the post-bivalent vaccination stage. Through Flow Cytometry, we were able to estimate the frequency of B cell populations specific to Spike protein (S). Functional studies were made by limiting dilution assay of class-switched memory B cell (swMBC; Dump-,CD19+,CD27+,CD38-,IgD-) and plasmacytes (Pc; Dump-,CD19+CD27+CD38+), both cultured over a NB21 feeder cell monolayer in the presence of polyclonal stimuli to secrete Immunoglobulin (Ig) *in vitro*. By means of enzyme-linked immunosorbent assay (ELISA), we characterized the kinetics of virus-specific Igs, as well the apparent affinity of these antibodies against the Wuhan strain and the variants of concern Omicron, Beta, Delta, and Gamma. This work emphasizes the importance of the immunological history of individuals with different responsive profiles to the same viral infection, given the complexity of different B cell populations and dynamics. **Keywords:** B Cell;SARS-CoV-2;Bivalente vaccine.

ME - 110 - **GASTROINTESTINAL INFECTIONS PROMOTE CELLULAR AND STRUCTURAL REMODELING IN THE OMENTAL ADIPOSE TISSUE IMPACTING PROTECTION AGAINST PATHOGENS**

DA SILVA, G.W.; SILVA, L.M.; GONÇALVES, L.M.; OLIVEIRA, B.D.C.; SALGADO, C.L.; GOMES, E.M.; MOREIRA, F.; RAMIREZ, J.A.Z.; AYUPE, M.C.; RODRIGUES, G.M.B.; LIMA, G.D.M.; DE ARAUJO, M.V.; DE OLIVEIRA, E.E.; DA FONSECA, D.M.. ICB-USP, SÃO PAULO - SP - BRASIL.

The intestinal mucosa is constantly exposed to the external environment and requires a network of immunological mechanisms to sustain tissue homeostasis. Our group has been studying the contribution of adipose tissue compartments to the gut firewall. In this context, the omentum is a visceral adipose tissue acting in the peritoneal cavity defense and enclosing leukocyte clusters (the milky spots). We hypothesized that the gut and the omentum may communicate supporting immunity against intestinal pathogens. Therefore, this study aimed to analyze the omental immune response to a bacterial (Yersinia pseudotuberculosis - YP) or protozoan (Toxoplasma gondii - TG) infection. We analyzed the omentum of naïve and YP or TG orally infected C57BL/6 mice 30 days post-infection and we found a significant remodeling of the omentum following both infections, characterized by an increase in milky spot size and count, along with increased leukocyte count in total tissue. For YP we detected an increase in the frequency of neutrophils, monocytes, TCD4+, TCD8+ and B cells, and a decrease in eosinophils and macrophages compared to the naive group. For TG we detected an increase of neutrophils, monocytes, TCD8+ cells and eosinophils, along with a decrease of TCD4+, macrophages and B cells. The YP omental expansion may be due to the YP-derived chylous ascites (CA) since we detected higher levels of IL-6 cytokine production by peritoneal macrophages treated in vitro with CA compared to naïve peritoneal fluid treatment. Omental cell transfer and total omental transplantation followed by YP infection were performed to analyze resistance to infection. Leukocytes derived from YP-infected mice did not interfere in the YP disease progression, while total omental transplantation aggravated the infection, indicating a possible deleterious effect of an omental stromal component. These results indicate communication between the gut and the omentum after gastrointestinal infection episodes. **Keywords:** omentum;adipose tissue;intestinal mucosa.

ME - 111 - **Role of SOCS-2 during the experimental Leishmania major infection**

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Cutaneous leishmaniasis is a public health problem causing a range of diseases from self-healing infections to chronic disfiguring disease. Currently, there is no vaccine for leishmaniasis, and drug therapy is often ineffective. The Suppressor of Cytokine Signaling (SOCS) family, consisting of eight members, are important physiological regulatory proteins of innate and adaptive immunity. The SOCS2 member plays a key role in the response regulation of immune response and development of pathogeneses during parasite infection, but its role in leishmaniosis is unclear and was investigated here. Female C57BL/6 and SOCS2 knockout (-/-) mice were infected with *L. major* ($1x10^5$). Ear thickness and lesions were measured weekly for 20 weeks. Lesions were ticker and more ulcerative in C57BL/6 compared to SOCS2-/- until 9 weeks. However, after this time point, the deficiency of SOCS2 resulted in development of severe lesion, mainly observed 10 weeks after infection, when compared with WT counterparts. Notably, SOCS2 deficient mice reinfected with L. major display better control of the lesion development in the ear when compared to WT mice. Despite being a preliminary result, our findings suggest a dual role of SOCS2 protein during *L. major i*nfection, acting favoring that the development of lesions at early time after infection, but critical at the late phase to control the lesion severity. **Keywords:** L; major;SOCS 2;LEISHMANIASIS.

ME - 112 - **Effects of protectin DX on a pain model induced by heat-killed Staphylococcus aureus**

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Pain is a sign of many bacterial infections, such as those caused by *S. aureus*. The aim of the study was to verify if protectin DX has analgesic and/or anti-inflammatory effects in a model of pain induced by heat-killed *S. aureus* (HKSA). *S. aureus* ATCC 6528 was suspended, heat-killed and HKSA was plated to prove the absence of viability. Male Swiss mice were divided into 8 groups (saline, HKSA and PDX). In the HKSA and PDX groups, 10^8 CFU of HKSA were administered intraplantarly. After 1 hour, 3 PDX groups were administered PDX (1, 10 and 100 pg) intraperitoneally and daily 1 hour before behavioral measures, and in other 3 PDX groups, PDX dosages were administered only once intrathecal route. Mechanical hyperalgesia (von Frey filaments) and thermal hyperalgesia (hot plate test) were evaluated 1 hour after the stimulus, 1, 3, 5 and 7 hours after treatment and daily until the 5th day. The spontaneous pain (the total number of paw flinches and time spent licking the paw) were counted, MPO and NAG tests of the paw and immunofluorescence staining of DRG for CGRP, NF-kB and TRPV1 were performed. Treatment with PDX reduced mechanical and thermal hyperalgesia, with the 100 pg dose showing the most analgesic effect for the IP route, while the 1 pg dose showing the most analgesic effect for the IT route. PDX treatment still reduced the spontaneous pain, but IT administration showed a better effect compared to IP. Moreover, had a reduction in HKSA-induced neutrophil and macrophage recruitment to the hind paw as observed by the reduced enzymatic activity (MPO and NAG), but there were no differences between doses and routes. PDX also reduced TRPV1 and NF-κB activation and CGRP release in mice. This research demonstrated that PDX acts on DRG and also directly inhibits inflammation resulting from *S. aureus* infection. In conclusion, treatment with PDX is able to reduce neuropeptides related to pain and inflammation, which demonstrates its analgesic and anti-inflammatory capacity. **Keywords:** Pain;Protectin DX;Staphylococcus aureus.

ME - 113 - **Uncovering the Neuroprotective Effect of Vitamin B12 in Pneumococcal Meningitis: Insights into Its Pleiotropic Mode of Action at the Transcriptional Level**

OLIVEIRA, M.D.S.[1]; CASSIANO, L.M.G.[1]; DE QUEIROZ, K.B.[1]; AMANCIO, A.M.[1]; SALIM, A.C.D.M.[1]; FERNANDES, G.D.R.[1]; CARNEIRO, C.M.[2]; COIMBRA, R.S.[1]. 1. INSTITUTO RENÉ RACHOU/FIOCRUZ, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO - MG - BRASIL.

During pneumococcal meningitis (PM), the interplay between bacterial virulence factors and host innate immune response could result in uncontrolled neuroinflammation, which may induce apoptotic death of progenitor cells and post-mitotic neurons in the hippocampal dentate gyrus, resulting in cognitive impairment. In PM, vitamin B12 is known to attenuate hippocampal damage and reduce the expression of some key inflammatory genes, promoting DNA methylation through increased S-adenosyl-methionine production. In this regard, this study aims to demonstrate the effect of adjuvant therapy with vitamin B12 on the hippocampal histopathology and transcriptome in infant rats with PM. Eleven-days-old Wistar rats were infected with *Streptococcus pneumoniae* (serotype 3) by intracisternal injection, and administered vitamin B12 or placebo three and 18h post-infection (p.i.). At 24h p.i., animals were euthanized and brain samples were used to perform histopathological, immunohistochemical, RNA-Seq and RT-qPCR analysis. Adjuvant B12 therapy was found to modulate hippocampal transcriptional signature induced by PM, mitigating the effects of the disease in canonical pathways related to signaling via NF-kB, production of proinflammatory cytokines, migration of peripheral leukocytes into the central nervous system (CNS) and production of reactive species. Phenotypic analysis revealed that B12 effectively inhibited microglia activation in the hippocampus and reduced inflammatory infiltrate in the CNS of infected animals. No adverse effects of B12 were predicted or observed reinforcing the safety profile of this epidrug. In conclusion, B12 effectively mitigates the impact of PM on pivotal neuroinflammatory pathways, reducing microglia activation and inflammatory infiltrate within the CNS, and attenuating hippocampal damage. These pleiotropic neuroprotective and anti-inflammatory effects of B12 are partly regulated by alterations in histone methylation markings in hippocampal neural cells. **Keywords:** Pneumococcal meningitis;Vitamin B12;Neuroinflammation.

ME - 114 - **EFFECT OF TH2 IMMUNIZATION AND PROBIOTIC TREATMENT IN THE INTESTINAL BARRIER POST-INFECTION**

RODRIGUES, G.M.B.; GONÇALVES, L.M.; MOREIRA, F.; LIMA, G.D.M.; DA SILVA, G.W.; OLIVEIRA, B.D.C.; DE OLIVEIRA, E.E.; DE ARAUJO, M.V.; SALGADO, C.L.; CAÇADOR, M.A.; DA FONSECA, D.M.. INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRASIL.

The intestinal mucosa is constantly exposed to environmental antigens, such as pathogen- dietary- and commensal microbiota-derived antigens, influencing local immune homeostasis. In the Laboratory of Mucosal Immunology, we study how environmental factors such as diet, probiotics or infection can shape the mucosal immune system. Our group observed that after the resolution of the intestinal infection by *Yersinia pseudotuberculosis* (YP), there is a permanent remodeling of the intestinal immune and lymphatic systems, which leads to microbiota translocation to the mesentery, resulting in chronic local inflammation that blocks tissue-specific responses. This process, named by us as “immunological scar”, is directly related to the development of inflammatory diseases. Therefore, restoring the immunological scar and the mesenteric immune homeostasis post-infection could prevent this process. This project aims to address strategies to reverse the immunological scar following YP infection by using 2 strategies: (1) the induction of a repairing type 2 immune tone in the mesentery, or (2) by using probiotic treatment. For the first strategy, YP-infected mice were immunized with ovalbumin (OVA)+ alum followed by oral challenge with OVA to recruit the Th2 cells to the gut and mesentery. Although naïve animals were capable to promote type immunity in the gut, as shown by the recruitment of Th2, ILC2, M2 and eosinophils to the small intestine and mesentery, YP-infected mice failed to promote the Th2 cell recruitment. Even after the type 2-driven sensitization, YP-infected mice were uncapable to promote type 2 homeostatic immune tone and the reversion of the immunological scar. In our next steps, we will test different probiotic strains to in order to promote regulatory and healing responses in the mesentery. **Keywords:** Mucosal Immunology;Probiotics;Infection.

ME - 115 - **Melatonin exhibits an antiproliferative effect and induces lytic death in pancreatic cancer cells**

BEZERRA, S.P.; LAGO, N.C.S.; SANTOS, C.M.A.P.; MACHADO, S.A.; DE MELO, H.A.B.; LAGO, F.G.; MAGALHÃES, K.G.. UNIVERSITY OF BRASILIA - UNB, BRASÍLIA - DF - BRASIL.

Introduction: Pancreatic cancer is one of the most lethal types of cancer, with no effective treatment options for advanced disease stags. Pancreatic malignancies have an immunosuppressive tumor microenvironment and are resistant to conventional therapy. Previous studies have pictured melatonin, a natural indoleamine synthesized from tryptophan, as an emerging tool with the potential for adjuvant treatment in cancer therapy. Melatonin can alleviate the side effects of chemotherapy and radiotherapy can present anti-proliferative effects and modulate cell death pathways in different types of cancer. In this context, this project aimed to characterize the effects of melatonin on the modulation of carcinogenic parameters in the human pancreatic adenocarcinoma cell line (PANC-1). **Methods:** PANC-1 cells were cultured and stimulated with distinct melatonin concentrations for different time points. Mitochondrial cell viability was performed using MTT and analyzed by spectrophotometry. Cell death, cell proliferation, cell cycle, nuclear fragmentation, and lipid droplet biogenesis were assessed by flow cytometry. Membrane pore formation, lactate dehydrogenase (LDH) enzyme release, and reactive species (RS) generation were investigated via spectrophotometry. **Results:** Our data showed that melatonin induced cytotoxicity in PANC-1 cells in a dose- and time-dependent manner, by decreasing the plasma membrane integrity, which correlates with lytic cell death occurrence and increased LDH release. Melatonin treatment led to augmented RS production and nuclear fragmentation in PANC-1 cells. In addition, melatonin treatment correlated with G0/G1 cell cycle phase arrest, and a reduction of cell proliferation and lipids droplet biogenesis. **Conclusion:** This study demonstrates the potential antitumor effect of melatonin in vitro, unveiling new possibilities to be applied as a tool for pancreatic cancer therapy. **Keywords:** Melatonin;Pancreatic cancer;Lytic death.

ME - 116 - **Evaluation of the use of gold nanoparticles and collagen matrix associated with the indirect effects of oral tolerance on wound repair**

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Currently there are several strategies to improve cutaneous wound repair and the use of gold nanoparticles (AuNP) is one of them. AuNP improves wound healing with neovascularization and anti-inflammatory effects, which have been seen also, after oral intake of a tolerated protein and its topical application (Immunology, 151:314-323, 2017). Thus, this study aimed to investigate the effects of a previously tolerated protein (ovalbumin) associated with AuNP and heterologous hydrolyzed collagen biogel (HCol) to improve wound repair. In this study, an excisional wound model (7mm-diameter) and an incisional wound model (1cm-length) were used in male Swiss mice (8 weeks old) approved by the Ethics Committee on Animal Use (No. 024/2020). Mice were divided into 5 topical treatment groups: HCol, HCol+OVA, HCol+AuNP, HCol+AuNP-OVA and saline, which received the dressing containing the respective treatment delimited to the group. Before surgery, animals in the experimental groups with topical treatment containing OVA, received orally a 1:5 egg white solution ad libitum for 5 consecutive days. The mice of the other experimental groups ingested water. After 7 days, the animals were anesthetized and after trichotomy the incisional and excisional wounds were performed. Then, topical treatments were applied to the wounds according to the experimental groups. After the surgical procedure, the lesions were covered with micropore tape, which remained for 5 days. The scar area was collected 7 days after injury and after histological processing was stained with Hematoxylin and Eosin (HE) and Gomori trichrome for semiquantitative and quantitative histopathological analyses. Our findings demonstrate that animals of the ColH+AuNP-Ova showed a reduction in inflammatory cells and fibroblasts, with better formation of granulation tissue 7 days after injury. Our results demonstrate improvement in the healing process and re-epithelialization, indicating a therapeutic potential for wound healing. **Keywords:** Wound repair;Gold nanoparticles;Oral tolerance.

ME - 117 - **THE PROLIFERATIVE CAPACITY AND THE VIABILITY OF L. (L.) AMAZONENSIS WERE SIGNIFICANTLY REDUCED BY LIDOCAINE TREATMENT**

ROCHA, J.P.; DA SILVA, P.D.C.; ALVES, É.A.R.. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Tegumentary leishmaniasis (TL) consists of a complex of vector-borne diseases caused by protozoa of the genus *Leishmania*. During TL treatment, severe signs of toxicity and therapeutic failures may occur. Due to these problems, the development of new therapies against TL has been encouraged. Studies with different local anesthetics have shown that these drugs inhibited or delayed the development of cutaneous lesions in hamsters, but the mechanisms responsible for the clinical improvement of the animals have not been elucidated. Therefore, the present project aims to investigate whether lidocaine exerts a direct anti-*Leishmania* activity and/or modulates the functions of infected macrophages, leading to a reduction in the intracellular parasitism. To achieve the proposed objective, axenic promastigotes of *L. (L.) amazonensis* were incubated with lidocaine in concentrations ranging from 0 to 5 mg/mL, at 26°C, for 24h, 48h, and 72h e the number of viable parasites were analyzed by trypan blue exclusion test. The results showed that 1.25 mg/mL, 2.5 mg/mL, and 5.0 mg/mL of lidocaine significantly reduced the proliferation and viability of promastigotes at all incubation periods tested. The concentration of 0.625 mg/mL also reduced parasite viability at 24h. For the proliferation and viability assays, the calculated inhibitory concentration 50% (IC_{50}) were, respectively, 0.52 mg/mL and 1.02 mg/mL at 24h, 0.51 mg/mL and 0.23 mg/mL at 48h, and 0.06 mg/mL and 0.23 mg/mL at 72h. Taken together, our data showed that lidocaine exhibits parasitostatic and parasiticidal activity against *L. (L.) amazonensis* at concentrations considered clinically relevant in the context of TL. It is intended to continue the study by evaluating the direct effect of lidocaine against axenic amastigotes. Additionally, it will be assessed the infection index, as well as ROS/RNS and cytokines production by *L. (L.) amazonensis*-infected macrophages derived from human blood after incubation with lidocaine. **Keywords:** Leishmania;Lidocaine;Macrophages.

ME - 118 - **ROLE OF RECEPTORS FcRs DURING MONOCYTIC RESPONSE AGAINST SARS-COV-2**

CONSTANCIO, C.D.S.; RODRIGUES, D.A.S.; FERNANDES, H.D.D.P.; GAMA, A.M.D.S.; SANTOS, C.M.R.; VIDAL, V.M.; RENAULT, L.Z.; BASTOS, V.C.; OTA, V.A.; DE LIRA, G.S.; JÚNIOR, O.D.C.F.; TANURI, A.; CASTIÑEIRAS, T.M.P.P.; VALE, A.; BOZZA, M.T.; COBOS, E.M.; DE LIMA, J.E.N.. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Classical, intermediated, and non-classical monocyte subpopulations are altered in coronavirus disease (COVID-19) patients by exacerbated inflammatory responses mediated by cytokines/chemokines (The Journal of infection 80(6):607–613, 2020). The monocytes express FcγR (CD16, CD32, CD64) and FcαR (CD89), which roles involve activation and inhibition of cellular response (Nat Rev Immunol 20:633–643, 2020). Thus, the aim of this work was to study the FcR levels and their function in monocyte activation from COVID-19 patients. **Methods and results:** Using a biotinylated Spike (S) protein from SARS-CoV2 we observed its binding in monocytes surface from COVID-19 patients. This interaction occurred via the S protein and immunoglobulin (Ig) light chains. Furthermore, we analyzed the levels of FcγR and the frequency of monocytes expressing CD64, CD32 e CD89 from uninfected individuals, COVID-19 patients, or convalescent donors. Classic monocytes exhibited a significant reduction in CD64 levels in 15-25 days since symptom onset (DSSO). The frequency of CD89 positive classical and intermediated monocytes was reduced after 100 DSSO. To evaluate if FcRs engagement modulates monocyte activation, PBMCs from healthy donors were stimulated with R848 (TLR7/8 ligand) and Pam3CSK4 (TLR2 ligand) in the presence or absence of heat-inactivated plasmas from COVID-19 patients or uninfected donors. The presence of COVID-19 plasma did not modulate TNFα or IL-6 production induced by Pam3CSK4. However, TNFα secretion induced by R848 was reduced by plasma from COVID-19 patients. **Conclusion:** Together, the results suggest that FcγR and FcαR levels are modulated in monocytes during the acute and convalescent phase of COVID-19. Moreover, viral binding is mediated by immunoglobulins via FcRs, and these interactions can alter monocyte activation. **Keywords:** COVID-19;Monocytes;FcγR.

ME - 119 - **Mygalin: anti-inflammatory effect via TLR2/1, IFN-γ and NFκB expression.**

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Introduction: Toll-like receptors (TLR) are present in several immune cells, including macrophages. Recognition via TLR begins the activation of signal transduction pathway, fundamental to the inflammatory response. Several molecules are studied to modulate the inflammatory response. Mygalin is a synthetic analog of spermidine, isolated from spider hemocytes *Acanthoscurria Gomesiana*. This molecule has microbicidal activity against *E. coli* and reduces LPS inflammatory response in macrophages. **Objective**: Evaluate the effect of Mygalin on J774 macrophage cell line, activated or not with IFN-ɤ and stimulated with the TLR2/1 agonist (Pam3CSK4), followed by the analysis of pro-inflammatory mediators: nitric oxide (NO) and IL-6, and the expression of the transcription factor NF-κB. **Methods**: J774A.1 line macrophages were pre-treated with Mygalin (90 and 360 µm) and later stimulated with TLR 2/1 agonist, PAM3CSK4 (300 ng/ml), stimulated or not with IFN-γ (10 ng/ml) for 20 hours, for nitric oxide dosage (NO) by Griess method and IL-6 by Elisa. For NF-κB/P65 tests, the cells were collected after 6 hours of activation. For Western Blotting assay, the proteins were extracted with Ripa Buffer, and used specific antibodies. **Results**: The pretreatment of cells with Mygalin during activation with Pam3CSK4 reduced NO production, regardless of the dose used. There was a reduction of IL-6 levels only with 360 µM of Mygalin, suggesting a dependent dose effect. Similar results were obtained with the cells activated with IFN-γ, but less significant. Stimulation of cells with Pam3CSK4 positively regulated the expression of NFKB and the presence of Mygalin in the highest dose inhibited the expression of this factor. **Conclusion**: Mygalin reduces inflammatory response via TLR2/1, inhibiting NFKB activation, a transcription factor that regulates inflammatory cytokines synthesis. **Support**: FAPESP No. 2013/11212-9, 2020/08182-4, Butantan Foundation and Capes Scholarship No. 8887.497904/2020-00. **Keywords:** Acylpoliamine;Mygalin, inflammatory response;Cytokines.

ME - 120 - **EVALUATION OF SARS-COV-2-SPECIFIC T LYMPHOCYTES IN CHILDREN INFECTED WITH DENGUE**

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The dengue virus is a flavivirus that causes a disease that ranges from mild presentation to severe illness that can cause death. A fine balance between the pro- and the anti-inflammatory responses are thought to be important for the clinical presentation of the disease. Previous infections can promote protection or worsening of the disease through cross-reactivity or trained immunity, as observed in patients infected with Covid-19 who had already had contact with other coronaviruses or BCG vaccination. Since children usually present mild Covid-19 infection, one hypothesis is the presence of previous immunity steamed by seasonal coronavirus cross-reactivity or trained immunity induced by unrelated infection. Therefore, T lymphocytes may play an essential role in eliminating infection and controlling inflammation through cross-reactivity or by a mechanism of bystander modulation. This work aimed to verify the activation of T cells against SARS-COV-2 in pre-pandemic samples of children infected with dengue. PBMC's from children (1-13 years) collected in 2013-2014 were used, which were thawed and cultured in the presence of the peptide library DENV1 Envelope (ENV) or SARS-COV-2 Nucleocapsid (NC) for 14 hours. Then the cells were labeled for flow cytometry. Interestingly, the antigenic stimulation with ENV decreased the frequency of T cells, CD4 and CD8, producing TNF and CD8 producing IL10 in febrile phase patients. On the other hand, there was an increase in the frequency of CD4 cells producing IL4/IL13 when PBMCs were stimulated with ENV in defervescence. During the convalescence period, we observed a rise in IL10-producing CD8 cells specific to ENV. SARS-COV-2 NC did not induce T cells producing IFNg, TNF, IL10, IL17a, IL4 and IL13. We conclude that infection or exposure to dengue virus does not generate T lymphocytes capable of cross-reactivity to SARS-COV-2, neither we find evidence related to dengue-mediated immunity training against Covid-19. **Keywords:** Dengue;SARS-COV-2;Cross-reactivity.

ME - 121 - **Effects of Zileuton treatment during Plasmodium berghei ANKA infection.**

SANTOS, L.L.D.O.; MELO, N.; SANTANA, L.F.D.; SILVA, L.P.; RICCI, M.F.; NONATO, A.; PEREIRA, R.D.D.; TEIXEIRA, S.L.; RABELO, R.A.N.; ALVARENGA, V.B.S.; MACHADO, F.S.. UFMG, BELO HORIZONTE - MG - BRASIL.

Plasmodium infection is a global concern, as it can lead to the development of severe malaria, resulting in significant morbidity and mortality rates. Despite this, malaria is still considered by World Health Organization a neglected disease. Plasmodium berghei ANKA (PbA) recapitulates in many aspects the human severe malaria, including respiratory distress and cerebral malaria (CM). Zileuton (Z) is a 5-lipoxygenase inhibitor used for prophylaxis and treatment of chronic and aspirin-induced asthma. However, the efficacy of Z in treating malaria infection remains uncertain. C57BL/6 (WT) mice were infected with 10 5 red blood cells parasitized with PbA and subjected to different treatment regimens. Zileuton administration was initiated at 3º (Z3) or 5º (Z5) day post infection (dpi) and continued for 10 days. Parasitemia, body weight, survival and clinical score were evaluated. The Z5 treated mice presented lower parasitemia at 6° dpi when compared to the Z3 and untreated groups. Furthermore, the untreated group presented precocious death when compared to the treated groups, specially Z5, that increased survival of all animals until 24° dpi. In addition, Z5 animals presented a better clinical score in all the analyzed days and the body weight was similar to the control group. Collectively, our preliminary results demonstrate a potential protection of zileuton during experimental severe malaria. **Keywords:** Malaria;Zileuton;Plasmodium berghei ANKA.

ME - 122 - **Transfection of human monocyte-derived dendritic cells (moDC) with total mRNA from neoplastic cells: evaluation of the strategy in a model with glioblastoma tumor lineage cells and moDC from donors healthy**

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Glioblastoma is the most aggressive and frequent type of central nervous system tumor and standard treatment for this disease still leaves much to be desired. In our laboratory, we use therapeutic vaccination using dendritic cells derived from monocytes (moDC) from healthy donors, fused with cells patients' autologous tumors. However, some limitations are present in the production process of this vaccine. A strategy to replace tumor cells as a source of antigens for presentation by moDC would be the use of mRNA from tumor cells, amplified in vitro and transfected into moDC. To evaluate such a strategy, we tried to introduce into cells of the glioblastoma, U87MG, the GFP marker (through transfection of plasmid carrying the GFP gene), which was used, along with the GFAP protein, (a typical protein from glial cells) as an indicator of moDC transfection by mRNA extracted of tumor cells.Once the conditions for tumor RNA extraction and transfection, the moDC were transfected with the tumor mRNA amplified in vitro and its membrane phenotype and its expression of GFP and GFAP were evaluated. We observed that after 24 hours of mRNA transfection, the phenotype of the mature and immature moDC change with an intense reduction in the expression of PDL1 compared to moDCs that did not receive the mRNA, suggesting the possible ability of mRNA to stimulate moDC to an activating phenotype. We observed that electroporation is not as effective, for viability and delivery, as the other transfection methods, and that despite the low expression of GFAP and GFP in transfected moDC, it was possible to detect their expression through flow cytometry. Thus, having expression of genes in neoplastic cells was obtained by moDC transfected with mRNA from them obtained and amplified in vitro, the proposed strategy seems to be viable and applicable to a more advanced test. **Keywords:** Vaccine;Tumor;mRNA.

ME - 123 - **The role of HIF-1α in PD-L1 expression in the NETs production during sepsis**

LEANDRO, M.D.O.; CEBINELLI, G.C.M.; RAMOS, A.D.S.; COSTA, V.F.; RODRIGUES, F.C.; CUNHA, T.M.; ALVES FILHO, J.C.F.; CUNHA, F.Q.. UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Sepsis is a condition caused by a dysregulated host response to an infection, resulting in life-threatening organ dysfunction. In preliminary results obtained from analyses conducted by our group, we observed an increase in the Cd274 and HIF-1α expression in neutrophils from non-survive septic mice. Additionally, it has been demonstrated that HIF-1α could modulate PD-L1 expression in some diseases and is also associated with a greater production of neutrophil extracellular traps (NETs). In this way, the objective of this study is to evaluate the effects of the inhibition of HIF-1α expression in neutrophils during sepsis, analyzing its involvement in the NETs production and PD-L1 expression. To gain further insights, we developed a conditional HIF-1α knockout mouse strain in neutrophils. We isolate bone marrow neutrophils from HIF-1α $^{flox/flox}$ Ly6G $^{WT/CRE}$ heterozygous mice and HIF-1α $^{flox/flox}$ Ly6G $^{WT/WT}$ control mice and subsequently, the neutrophils were stimulated with LPS and INF-γ for 4 hours to analyze the expression of PD-L1 by flow cytometry and the production of NETs (MPO/DNA assay). Although no difference was found in the production of NETs, the levels of $Ly6G^{+}$ $CD11b^{+}$ neutrophils that were positive for PD-L1 increased significantly in neutrophils from heterozygous mice with HIF-1α deletion, indicating a possible negative regulatory effect for the expression of PD-L1. Our next steps involve the induction of sepsis by CLP (cecal ligation and puncture) in conditioned knockout mice to evaluate its effect during the illness. **Keywords:** Sepsis;NETs;HIF1alfa.

ME - 124 - **The C/T genotype of the rs2234246 variant of the TREM1 gene favors an increase in the concentration of soluble mediators in COVID-19 in a cohort from the Brazilian Amazon**

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The relationship between the immune response and COVID-19 has been widely investigated, aiming at a better understanding of the dynamics involving the immune response and SARS-CoV-2 infection. In this regard, we present the Trigger Receptor Expressed in Myeloid Cells 1 (TREM-1), which is upregulated in inflammation and is part of an extensive family of immunoglobulin (Ig) receptors discovered in the 2000s. The TREM-1 receptor is widely expressed in neutrophils, monocytes and macrophages, and their activation potentiates the inflammatory response. In this context, some genetic variants in the TREM1 gene have been studied and associated with a worse prognosis in some diseases. We evaluated the influence of the rs2234246 variant on the TREM-1 receptor gene and its association with the profile of soluble mediators, in 50 blood donors, 36 patients with COVID-19 and 52 convalescing individuals from SARS-CoV-2 infection, at times 30, 60 and 90 days after the clinical cure of the disease. The variant was genotyped by qPCR, and the soluble mediators CXCL8, CCL3, CCL2, IL-1β, IL-6, TNF-α and IFN-γ were measured using the Luminex assay. Our data show that individuals with the C/T genotype of the rs2234246 variant of the TREM1 gene have increased levels of CXCL8, CCL3, IL-1β, TNF-α and IFN-γ. New studies including other variants of the TREM1 gene are needed for a better understanding of the role of this receptor in the immune system and in the modulation of the release of immune mediators in response to SARS-CoV-2 infection. **Keywords:** TREM-1;COVID-19;INFLAMMATION.

ME - 125 - **Ex vivo Characterization of Natural Killer Cells Phenotypic-functional Profile from Patients with COVID-19**

DOS SANTOS, T.M.A.F.[1]; CARVALHO, N.O.[1]; DE OLIVEIRA, G.[1]; MIRANDA, V.H.S.[1]; PEREIRA, A.A.S.[1]; PRADO, R.O.[1]; COELHO-DOS-REIS, J.G.[2]; MARTINS-FILHO, O.A.[1]; SPEZIALI, E.[1]; CARVALHO, A.T.[1]; PERUHYPE-MAGALHÃES, V.[1]. 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

COVID-19 is a systemic disease which triggered a pandemic and was responsible for the greatest cause of morbidity and mortality in Brazil in recent years. It is known that the individual's immune response plays a critical role in the evolution of the disease, in which the Natural Killer (NK) cell population seems to play an important role against the virus. In this sense, our goal was to evaluate the phenotypic-functional profile of NK cells in patients with COVID-19 in relation to admission and disease outcome. Whole peripheral blood samples were collected of 49 patients with a positive diagnosis for COVID-19, and 12 volunteers with a negative diagnosis. The samples were processed and the phenotypic-functional profile of NK cells was evaluated by *ex vivo* context employing a mix of monoclonal antibodies (CD3, CD56, CD161, CD38, NKG2D and CD107A), and analyzed by Flow Cytometry. Data showed decreased percentage of $CD56^+CD161^+$ NK cells expressing the early activation marker CD69 in COVID-19 group, as compared to control group, although an increase in activation markers $CD38^+$ and $NKG2D^+$, associated with NK cells response, was seen. $CD107A^+$, marker of cytotoxic activity, was also increased. Regarding the hospitalization, patients admitted to the Intensive Care Unit (ICU) showed a decrease in NK cells compared to patients in ward. However, an increase in $CD56^+CD161^+$ expressing CD69 was observed. Patients who died showed decreased NK cells $CD56^+CD161^+CD38^+$ and $CD56^+CD161^+NKG2D^+$, as compared to patients discharged from hospital. Furthermore, when categorizing patients by age, decreased frequency of NK cells was found in patients over 69 years. Decrease in the expression of $CD38^+$ and $NKG2D^+$ activation receptors in patients over 69 years of age was also observed. It is suggested that the percentage of circulating cells and activation and cytotoxicity phenotypes of NK cells would be associated with a worse COVID-19 prognosis, especially in patients >60 years in ICU. **Keywords:** NK cells;COVID-19;Flow Cytometry.

ME - 126 - **Mechanisms of pulmonary inflammation involving platelets and platelet-leukocyte interactions in experimental obesity and asthma**

DE SOUZA, V.P.[1]; MENEGATI, L.M.[2]; DE OLIVEIRA, E.E.[3]; DIB, P.R.B.[1]; MACEDO, G.C.[1]; HOTTZ, E.D.[1]; SILVA, F.M.D.C.E.[2]. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

INTRODUCTION: Allergic asthma is an inflammatory disease initiated in the airways through exposure to allergens. Classically, asthma presents a Th2 eosinophilic immune response profile, but in conjunction with obesity there is an increase in Th17 profile and neutrophilia. Additionally, platelets are known to play a crucial role in leukocyte infiltration into the lungs. However, the importance of platelets in neutrophilic or eosinophil response during asthma with or without obesity has not been investigated. Therefore, this study aims to evaluate the role of platelets to leukocyte recruitment in the lungs during experimental obesity and asthma. **METHODS:** Female Balb/c mice were fed a high-fat diet (HFD) or standard diet and were subjected to the induction allergic asthma by 2 sensitizations and 3 ovalbumin (OVA) challenges. The animals were weighed throughout the protocol, euthanized at 24 h and 48 h to collect biological materials of interest for subsequent analyses**. RESULTS:** Elevated levels of leptin in the lungs were observed in obese animals, which was consistent with the higher accumulation of perigonadal fat. Interestingly, both perigonadal fat and leptin levels in the lung were reduced in the presence of allergy. We also investigated the differential blood cell count and found that allergic animals showed an increase in eosinophils and platelets regardless of obesity, but not neutrophils, which were increased due to HFD. Increased eosinophil counts were consistent with the levels of the chemokine CCL11 in the lung tissue of allergic animals. **CONCLUSION**: Our data demonstrate that allergic asthma reduces perigonadal fat and leptin levels in obese mice. Asthma also increases platelet, and eosinophil counts in the blood, regardless of diet-induced obesity. Further assays will be necessary to confirm the involvement of platelets and platelet-leukocyte interaction in the pathogenesis of obesity and asthma. **Keywords:** Asthma;Obesity;Platelets.

ME - 127 - **Cytokine profile in murine splenocytes after immunization with polyepitope T-cell antigen and challenge by Leishmania infantum**

SANTOS, A.D.C.F.[1]; MOREIRA, G.J.L.[1]; BARCELOS, A.C.A.[1]; DA SILVA, F.O.[1]; GONÇALVES, L.C.[1]; DE ALMEIDA, R.E.[1]; PEREIRA, H.D.R.[1]; REIS, T.P.[1]; NETTO, M.E.G.[1]; ROATT, B.M.[1]; DE BRITO, R.C.F.[2]; SOARES, R.D.D.O.A.[1]; OSTOLIN, T.L.V.D.P.[1]; REIS, A.B.[1]. 1. UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO - MG - BRASIL; 2. THE PIRBRIGHT INSTITUTE, PIRBRIGHT - INGLATERRA.

Many Leishmania (***L.***) spp. antigens candidates have been purified and assessed to develop a specific immune response against experimental Leishmania infection. Nevertheless, different ***Leishmania*** species, as well as different proteins, can trigger distinct protective immune mechanisms. Therefore, immunoinformatic-based vaccines are a viable tool considering their potential to induce a desired immune response due to extremely accurate specific B and T cells prediction. Furthermore, allows the development of a vaccine that can be deployed on a broad scale for prophylaxis and disease control. This study evaluated the immunogenicity of a polyepitope T-cell antigen against visceral leishmaniasis (VL). Female BALB/c mice were divided into groups Saline, Poly-ICLC adjuvant, Chimera A isolated (Chi-A), or combined with the adjuvant (Chi-A/Poly-ICLC). The vaccination protocol consisted of three subcutaneous (SC) or intradermal (ID) immunizations, with a fifteen-day interval between doses. Fifteen days after the third immunization, the animals were challenged with $1x10^6$ ***L. infantum*** promastigotes and euthanized after 45 days. The parameters evaluated were the intracellular cytokines (IL-2, IL-10, TNF, and IFN-γ) produced by CD4+ and CD8+ T cells using multiparametric flow cytometry. Immunized mice with Chi-A/Poly-ICLC showed an increase in the frequency of activated CD4+ and CD8+ T cells producers of IL-2, TNF, and IFN-γ. Moreover, SC immunization with Chi-A/Poly-ICLC increased the production of these cytokines when compared to ID immunization, except for CD8+IL-2+. IL-10-producing CD4+ T cells were reduced after immunization with Chi-A/Poly-ICLC, regardless of the route. In addition, IL-10-producing CD8+ T cells were reduced after ID immunization. Our data reveal that our vaccine candidate is capable of inducing a specific immune response and has potential against VL. Acknowledgments: CAPES, CNPq, FAPEMIG, FINEP, UFOP, PROPPI, LIMP, CCA-UFOP, and INCT-DT. **Keywords:** visceral leishmaniasis;polyepitope vaccine;immunogenicity.

ME - 128 - **Early transcribed membrane proteins applied on chemiluminescent and enzyme-linked immunoassay as potential diagnostic tools for Plasmodium infection.**

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Malaria remains the most lethal parasitic disease in the world, having a long history of combat and prevention measures over the years in the world. Among the etiological agents of the disease, Plasmodium vivax has significant importance in Asia and the Americas, where it is the most prevalent species in Brazil. Endemic areas routinely employ methods that include light microscopy and rapid tests, which use the detection of specific antigens for the diagnosis of malaria. However, new strategies for diagnosis are still crucial for controlling the disease. Therefore, the present project aims to validate two recombinant antigens, here named ETRAMP A and ETRAMP B, as potential diagnostic antigens for malaria using enzyme immunoassay and chemiluminescent platforms. The antigens were produced through heterologous expression in Escherichia coli and purified by affinity chromatography with a nickel-sepharose matrix. After purification, the antigens were quantified and used to standardize both immunoenzymatic and chemiluminescence assays. The conditions employed for the indirect ELISA remained the optimal choice for chemiluminescence platform testing, being 12,5 ng/well, 2% of serum, and secondary antibody diluted to 1:20K. Subsequently, individual samples were tested to assess the sensitivity and specificity of the diagnostic tests for P. vivax and P. falciparum. Among the tested antigens, ETRAMP A exhibited the most favorable outcomes in both tests, displaying a sensitivity greater than 84% and specificity higher than 95%, aligning with previous findings in the literature. While ETRAMP B exhibited sensitivity greater than 60% for both platforms, with specificity higher than 95%. When evaluated considering accuracy, the area under the curve (AUC), ETRAMP A presented AUC>0.9 for both platforms and ETRAMP B presented AUC>0.8. Although both antigens have shown good results, ETRAMP A seems to be the best candidate for a diagnostic test on both platforms. **Keywords:** malaria;CLIA;ETRAMP.

ME - 129 - **Protective efficacy and modulation of macrophage functionality induced by intranasal and intramuscular vaccination with total Leishmania amazonensis antigens (LaAg) against Leishmania amazonensis infection in the hamster model**

PRATES, M.C.N.; COUTO, L.D.S.; SANTOS, G.M.P.; SAAVEDRA, A.F.; RIBEIRO-ROMÃO, R.P.; DA SILVA, A.G.; DA-CRUZ, A.M.; PINTO, E.F.. INSTITUTO OSWALDO CRUZ - FIOCRUZ/RJ, RIO DE JANEIRO - RJ - BRASIL.

Previous studies in the murine model demonstrated that protective immunity induced by the total antigens of *Leishmania amazonensis* (LaAg) is critically dependent on the immunization route. While the vaccination with LaAg by the intramuscular (IM) routes induced an exacerbation in the lesion development, intranasal (IN) vaccination induced protection against infection by *L. amazonensis.* In recent years, we demonstrated the efficacy of LaAg vaccine administered intranasally against infection by *L. braziliensis* in the hamster model. In this work, we investigated, in the hamster model, the protective efficacy and modulation of macrophage functionality induced by IN and IM vaccination with LaAg against *L. amazonensis* infection. Hamsters were immunized with two doses of 20 µg of LaAg by the IN or IM route and challenged in the paw with 1 x 10^5 promastigotes of *L. amazonensis.* The control group was infected but not vaccinated. After 100 days of infection the quantification of the parasite load and the evaluation of the mRNA expression of enzymes and cytokines by RT qPCR were performed and peritoneal macrophages were collected and infected in vitro with *L. amazonensis*. LaAg IN group showed a significant reduction in lesion growth and parasite load of *L. amazonensis* infected hamsters when compared to the control group (unvaccinated), whereas the LaAg group IM presented an exacerbation of the lesion. LaAg IM group showed a significant decrease in the iNOS expression compared to the LaAg IN group and an increase in expression of Arginase, IL-10 and IL-4 on infected paws. In preliminary studies in vitro with infected-macrophages, LaAg IM group presented an increase of the infection rate (% infected macrophage). These results demonstrated that IN vaccination with LaAg induces protection against *L. amazonensis* in the hamster model, indicating that IN vaccination using LaAg is highly promising in the development of an effective vaccine against tegumentar leishmaniasis. **Keywords:** Leishmania amazonensis;intranasal vaccination;hamster model.

ME - 130 - **Recombinant human antibodies fragment (Fab) selection by Phage Display against TIM-3 as a promising tool for immunotherapy**

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Immune checkpoints are negative regulatory receptors expressed on immune cells and its function is to act as a brake for the immune system, maintaining self-tolerance. However, these molecules also participate in immune escape mechanisms, and TIM-3 stand out for their involvement causing dysfunctions in T-cell populations in a variety of diseases such as cancer and COVID-19. The TIM-3 interaction inhibition with its ligands by therapeutic antibodies showed promising results as an antitumor agent in clinical studies. Antibodies are an excellent molecule for the design of high-affinity, protein-based binding reagents used for different purposes, including immunotherapy. Using recombinant DNA technology and methods such as Phage Display, it is also possible to obtain fragments of recombinant antibodies (rAbs), such as Fab, with in vitro immunological repertoires, without the need for direct immunization of live hosts. In the present work, we used a human synthetic Fab fragment library to select high-affinity binders against TIM-3 by phage display to characterize promising tools for immunotherapy. Selection of antibodies was performed using a naive human Fab fragment phage library panned against immobilized commercial TIM-3-ECD (His-tagged, Elabscience, USA). The selected phages were analyzed for affinity by single-point competitive phage ELISA assay and sequencing, the Fab sequence was amplified, cloned, and expressed in a bacterial system, followed by purification by Immobilized-metal affinity chromatography. Three Fab antibody fragments were obtained with high affinity against TIM-3. Interaction between antibodies and glycosylated and glycosylated TIM-3 ectodomains is ongoing to confirm the functional state of the purified Fabs and determine the involvement of glycosylated epitopes in target recognition. Once confirmed these Fab fragments could raise as promising tools as therapeutic molecules for immunotherapy. **Keywords:** TIM-3;recombinant antibody;immune checkpoints.

ME - 131 - **The impact of malnutrition-diet, gut microbiota, and host's sexual dimorphism in defense against antimicrobial-resistant bacterial infection using the fruit flies as an in vivo model**

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Dietary changes associated with the Western-lifestyle affect the gut microbiota, and are associated with increased incidence of inflammatory diseases and susceptibility to infections. Furthermore, the enterobacteria *Klebsiella pneumonia (Kp)* commonly found and prevalent in gut microbiota are a major reservoir for multiple resistance genes and thus, a bacterial of great clinical relevance for the ability to disseminate by escape the host immune response. In this way, we aimed to investigate the direct connection between diet and microbiota to host resistance against infection caused by a multiresistant strain of *Kp*. To this aim, we first developed the gnotobiotic (germ-free) *Drosophila melanogaster* infected with systemically with AMR *Kp*. We observed that results in female *versus* male suggest that the hosts may represent very different environments to microbiota colonization that affects the outcome *Kp* infection. To evaluate the impact of different diets on *Kp* infection we treated fruit flies orally with different malnutrition diets (high sucrose, high fat and ketogenic), followed by oral *Kp* infection. We observed that both fat and ketogenic diets increased host susceptibility to *Kp* antimicrobial-resistant infection with stronger phenotype in female-infected flies compared to male-infected flies. Our results, even preliminary indicated that malnutrition favors AMR bacterial dissemination by compromising host defense and might be involved in the exacerbation and spread of the antimicrobial resistance alarming rate worldwide. **Keywords:** gut microbiota;diet;Drosophila melanogaster.

ME - 132 - **NOVEL BENZOTHIAZOLE ANALOGS AS POTENTIAL ANTIVIRAL AGENTS AGAINST CHIKUNGUNYA VIRUS**

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Introduction: Chikungunya fever is an arthropod-borne viral disease characterized by intense inflammatory polyarthralgia. Currently, there is no licensed vaccine or antivirals for this disease. In this way, the aim of this study was to evaluate the antiviral activity of novel benzothiazoles compounds against CHIKV *in vitro*. **Methods and Results:** Vero E6 cells were seeded in 96 well plates, and after 24h, monolayers were treated with different concentrations of 19 benzothiazoles (50−200 µM). After 48h of incubation at 37 ºC with a 5% CO_2 atmosphere, cell viability was assessed by the MTT colorimetric assay. The 50% cytotoxic concentration (CC50), the concentration that promotes 50% viral inhibition (EC50), and the selectivity index (SI = CC50/EC50) for each compound were determined using non-linear regression curve fit. Out of 19 benzothiazoles tested, 8 demonstrated cytotoxicity at 50 µM. Therefore, 11 compounds were tested in antiviral assays (concentrations from 6,25 to 50 µM) against CHIKV *in vitro* (MOI 0.01). Three of these compounds, namely EdCHIK10, EdCHIK39, and EdCHIK82, exhibited significant antiviral activity in the MTT assay after 48h, with viral inhibition percentages above 83%. Moreover, the EC50 values were 9.73 ± 2.92 µM, 5.88 ± 0.93 µM and 9.58 ± 1.16 µM and SI > 20.55, > 30.58, > 20.87 for EdCHIK10, EdCHIK39, and EdCHIK82, respectively. The intracellular flow cytometry of CHIKV-infected cells showed a reduction of infection from 40.96 % ± 9.84 (untreated) to 8.40 % ± 1.81 (EdCHIK10), 11.13 % ± 0.35 (EdCHIK39) and 21.86 % ± 12.04 (EdCHIK82). **Conclusion:** This study revealed the promising antiviral activity of novel benzothiazoles against CHIKV *in vitro*, thus contributing for the design of drugs targeting this arbovirus. **Keywords:** Chikungunya virus;Antiviral activity;Cyclopropane analogs.

ME - 133 - **Interferon-primed resident tissue macrophage is key in immunosenescence burden in Triple-Negative Breast Cancer microenvironment**

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The triple-negative breast cancer (TNBC) subtype is a very aggressive and heterogeneous tumor, affecting women worldwide. Despite cytotoxic chemotherapy, TNBC patients exhibit limited response to therapy, with a median overall survival of only 12-18 months, making it the subtype with the poorest prognosis among BC types. Understanding the tumor microenvironment (TME) is crucial for assessing the impact of different cell compositions on clinical outcomes and identifying new specific targets, enhancing precision medicine strategies. Macrophages (Mø) are highly plastic cells with different phenotypes and functions, and their balance determines whether the response will favor tumor growth or inhibition. Immunosenescence is the aging process that occurs in immune cells, which leads to the development of chronic low-grade inflammation in tissues. Single-cell (scRNA-seq) analyses allow the understanding of rare subpopulations in complex tissues. In this study, we integrated a total of 416,075 cells. By clustering cells and analyzing the expression of canonical genes, we identified 43 cell subpopulations/states in different molecular BRCA subtypes. We characterize immune and non-immune cells, totaling 43 cell subpopulations/states. It was possible to identify six Mø subpopulations, highlighting the RTM_IFN that expresses both resident and interferon associated genes, as well as genes related to senescence. Furthermore, ligand-receptor analysis identified a possible communication between these cells and malignant cells, which may facilitate tumor progression. We hope to dissect the crosstalking and their role in the TME of TNBC, mainly with regard to Mø's immunosenescence. Our findings provide insight into the cellular phenotypes and their roles in the TME of TNBC, particularly regarding Mø's immunosenescence, which may contribute to the development of new treatment strategies, and provide a more comprehensive understanding of the TME of TNBC. **Keywords:** Macrophages;Senescence ;Tumor Microenvironment.

ME - 134 - **Characterization of the vaccine immune response of mice immunized with the recombinant influenza virus carrying the murine IL-7 gene**

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Influenza is a disease caused by influenza, a virus that has a major impact on the world's health system and can cause serious illness. It is known that vaccination is essential to reduce these impacts. The elucidation of a rapid and effective immune response modulation against the virus is still a challenge. IL-7 is important in the adaptive immune response, in the development and maintenance of T lymphocytes and expansion of B cells. However, the role of IL-7 in vaccination has not yet been clarified. Using the reverse genetics technique, a recombinant influenza virus that carries the murine IL-7 gene (FluIL-7) was generated. This work aimed to evaluate the role of IL-7 in modulating the effector immune response in immunization in a murine model. C57BL/6 mice were immunized via IN and euthanized on days 1, 5 and 10. Lung mechanics were evaluated and BALF, blood and lung were collected for analysis. Our results demonstrated that FluIL-7 is safe and the promoted immune response is related to different mechanisms, such as the preservation of lung mechanics, early formation of iBALT, which results in a faster and more effective induction of the local immune response, in addition to preservation of lung tissue. Assessment of CBA in the lung demonstrated early expansion of populations important in the antiviral and regulatory response. Measurement of antibodies (total IgG, IgG1, IgG2c, IgA) in serum and BALF demonstrated the induction of higher IgA titers in the FluIL-7 group. Our results suggest that vaccination with FluIL7 induces an early and robust specific cellular and humoral immunity dependent on IL-7, with formation of iBALT, induction of T lymphocytes, production of anti-H1N1 influenza IgA in the respiratory mucosa of mice. From this project, it is expected to establish the proof of concept for the development of safe and effective vaccines capable of modulating the immune response in a comprehensive and effective way against influenza infection. **Keywords:** influenza;reverse genetics;interleukin 7.

ME - 135 - **Integrative transcriptomic analysis of human monocyte revealed common signatures in cancer patients**

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Introduction: Our previous study demonstrated that monocytes from breast cancer patients display alterations in their transcriptomic profile that include the down regulation of genes involved in their differentiation and in their ability to stimulate the immune system, in addition to producing reduced levels of inflammatory cytokines when compared to healthy donors' monocytes. We aim here to reveal common gene signatures present in monocytes from patients with distinct hematologic and solid cancer. **Methods:** An integrative bioinformatics analysis was performed to investigate monocyte signatures in ten public RNA-seq datasets from hematologic tumor (HT) (Chronic Myeloid Leukemia, acute myeloid leukemia and Diffuse large B-cell lymphoma) and solid tumors (ST) (esophageal, lung, head and neck, brain, colon). We generated a list of differentially expressed genes (DEGs) between monocytes from healthy donors versus cancer patients or between healthy and tumoral tissues using the DESeq2 package in R-studio and performed the functional enrichment analysis using Gene Ontology (GO) and protein-protein interactions considering the common up-regulated genes. **Results**: In blood monocytes from the HT datasets, we found 13 common genes, including *MSR1*, *CAMP*, *ELANE* and *CCL3*. These genes are mainly involved in the signaling pathways for Phagocytosis and Calcium Ion Homeostasis. For monocytes in ST tissues, we found 161 commonly expressed genes in all datasets, including genes belonging to the immunoglobulin family (*IGHG3, IGHM, IGHG4*), genes involved in the organization of the extracellular matrix (*MMP3, MMP8, MMP9, MMP12*) and genes from the CXCL chemokine family (*CXCL10, CXCL9, CXCL13*). **Conclusion:** Our data suggest that monocytes are involved in extracellular matrix organization, cell migration and antigen recognition in HT and TS. We will next evaluate the expression of those molecules in monocytes from multiple myeloma patients and correlate with clinical data. **Keywords:** Monocyte ;Transcriptomic ;tumor.

ME - 136 - **CROSSTALK OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM: INFLAMMATION MODULATION AND TISSUE REPAIR IN COVID-19 PATIENTS**

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Studies suggest that SARS-CoV-2, the etiological agent of COVID-19, may affect the renin-angiotensin-aldosterone system (RAAS) which is critical for blood pressure regulation, blood volume control, and electrolyte balance. The objective of the study was to analyze the differential gene expression of the RAAS receptors: angiotensin converting enzyme (ACE) and angiotensin II converting enzyme (ACE2), quantify the soluble proteins ACE2 and Angiotensinogen (Ang), and determine their correlation with cytokines and growth factors in individuals with COVID-19. We included ninety individuals hospitalized at the COVID-19 Center (INI-FIOCRUZ/RJ), with samples collected on the first and 300 days after hospitalization. For gene expression determination we performed RT-qPCR, whereas soluble ACE2 and Ang proteins were quantified by ELISA. Luminex assay was used to quantify cytokines, chemokines, and growth factors. For statistical analysis, we used the ANOVA test and Spearman's correlation. During hospitalization, we observed a downregulation of both receptors, with ACE2 remaining suppressed at 300 days. Soluble protein analysis revealed a decrease in ACE2 (*P*=0.007) and an increase in Ang (*P*=0.005) during the COVID-19 acute phase compared to controls. We found a negative correlation between Ang and TNF-α (*P*=0.005) and ACE2 with hepatocyte growth factor (HGF), stem cell factor (SCF), and fibroblast growth factor (FGF), with P<0.05. We also found a positive correlation between Ang and HGF (*P*=0.04), IL-9 (*P*=0.03), and Interferon γ-induced protein 10 (IP-10,*P*=0.01). These findings suggest that the SARS-CoV-2 infection induces long-lasting alterations in RAAS, influencing inflammation and tissue regeneration processes, indicating a crosstalk between RAAS regulation and COVID-19 immunopathology. Therefore, our findings offer valuable insights for comprehending the disease and provide an essential tool for investigating prognostic biomarkers in COVID-19 and post-COVID-19 scenarios. **Keywords:** SARS-COV-2;RAAS;ACE/ACE2.

ME - 137 - **HIGH NEUTRALIZING TITERS AFTER 2 DOSES OF VACCINATION IN PACIENTS WITH PERSISTENT SYMPTOMS AFTER COVID-19**

CEZAR, P.A.[1]; FERNANDES, T.F.B.[1]; SCHWARZ, M.G.A.[1]; DE SÁ, N.B.R.[1]; CAZOTE, A.D.S.[1]; GERALDO, K.M.[2]; RIBEIRO, M.P.D.[2]; GRINSZTEJN, B.[2]; VELOSO, V.G.[2]; CARDOSO, S.W.[2]; GIACOIA-GRIPP, C.B.W.[1]; CORTES, F.H.[1]; GUIMARÃES, M.L.[1]; MORGADO, M.G.[1]; DE ALMEIDA, D.V.[1]; PILOTTO, J.H.[1]. 1. OSWALDO CRUZ INSTITUTE - IOC/ FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. EVANDRO CHAGAS NATIONAL INSTITUTE OF INFECTIOUS DISEASES - INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

The long-term effects observed in patients who have recovered from COVID-19 have emerged as a new public health concern. Post-acute COVID-19 syndrome (PASC) is the persistence of symptoms beyond four weeks from the onset of the illness. This study aimed to investigate the relationship between the presence of PASC-associated symptoms and the level of neutralizing antibodies (Nab). Samples from 240 individuals with PASC under follow-up at the COVID-Long center of the Evandro Chagas National Institute of Infectious Diseases (INI-FIOCRUZ) were collected. Additionally, 35 samples were selected from patients who did not exhibit any symptoms as a control group. We performed an anti-SARS-CoV-2 neutralization assay using pseudovirus to determine the Nab titers. The statistical analysis was done using the Krustal-Wallis test. Among the 240 participants, only those who received two identical doses of the COVID-19 vaccine were selected for further analysis. Seven participants received the CoronaVac (PASC C), 5 received the AstraZeneca (PASC A), and 4 received the Pfizer vaccine (PASC P). In the control group, 14 participants were unvaccinated (No Vac control), 11 received the CoronaVac (Vac C control), and 10 received the AstraZeneca (Vac A control). The No Vac control group exhibited a geometric mean titer (GMT) of 136.1 [95%CI:61-302]. There was no significant difference among control group participants according to the vaccine received, GMT Vac C= 171 [95%CI: 69-419] and Vac A 191 [95% CI: 82-441]. However, a significant difference was observed between the GMT of the groups with and without symptoms (*P*<0.05). Those with symptoms: PASC C had a GMT of 2,590 [95%CI:61-302], PASC A had a GMT of 6,467 [95%CI:5,498-7,606], and PASC P had a GMT of 4,060 [95%CI:1,453-11,344]. The results indicate a post-vaccine humoral response with high levels of neutralizing antibodies in individuals with PASC, suggesting a robust immune response following vaccination. **Keywords:** PASC;Neutralizing antibodies;Vaccine.

ME - 138 - **Disparity between morbidity and mortality rates for Multiple Sclerosis in Brazil: An ecological and time series study**

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Introduction: Multiple Sclerosis (MS) is an autoimmune, demyelinating disease that initially affects the nervous system and causes severe damage to health. The disease has a chronic and progressive inflammatory nature that affects mainly adults. Global data indicate that the prevalence of MS has increased since 2013 and that epidemiological data are needed to close gaps in knowledge about MS and its relationships. Objectives: Thus, this study aimed to evaluate the epidemiology of MS in Brazil and its federative units, guaranteeing morbidity and mortality indicators for the disease. **Methods:** In order to achieve the objectives, MS morbidity and mortality data were obtained from the Ministry of Health database. Hospitalization data were evaluated from 2007 to 2020 and mortality data was evaluated from 2000 to 2019. Rates were obtained after normalization by the population number according to IBGE. The confidence level used will be 5%. **Results/discussion:** The data showed a positive and significant correlation ($r^2 = 0.86$) for the rate of hospitalization due to MS in Brazil, for the evaluated period. On the other hand, a negative and significant correlation rate for mortality ($r^2 = 0.41$) was observed for the study period. There was also heterogeneity for the distribution of morbidity and mortality rates among the Brazilian federative units ($p<0.05$). **Conclusions:** However, it was possible to conclude that there is a clear increase in hospital morbidity where mortality rates are controlled, and there is inequality in the control of morbidity and mortality rates to MS in the country. The study allows us to indicate weaknesses in the fight against the disease. **Keywords:** Multiple Sclerosis;Morbidity, Mortality;Health Expenditures.

ME - 139 - **Identification of cancer/testis antigens in individuals affected by gastric cancer in Pará**

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Gastric cancer (GC) stands out as one of the neoplasms with the highest incidence and mortality. Precise identification of biomarkers is crucial to improve the clinical management of patients. In this context, cancer/testis antigens (CTAs) stand out, which are self-antigens expressed in the embryonic phase, in testes, and tumors. Due to this expression pattern, CTAs may be potential tumor biomarkers or therapeutic targets. Thus, the aim of this study was to investigate the expression of CTAs in patients diagnosed with GC in Pará. Paired samples of tumor and non-tumor tissue adjacent to the tumor (JDA) were collected from 42 patients. Then, total RNA sequencing was performed. Based on the readings obtained, a differential gene expression analysis was performed using the DESeq2 package, in which the CTAs that showed a difference in expression |Log2(Fold-Change)| greater than 2 and a p-value less than 0.05 were identified. To elucidate the biological roles of DE genes, genetic ontology was performed. Additionally, the impact of CTAs expression on overall survival (OS) was evaluated. In total, 1894 ED genes were identified among tumor and ADJ samples, of which 57 belong to the CTA family. To explore the impact of CTAs on tumor biology, 29 biological process terms were enriched, including cellular processes involved in reproduction, germ cell development, and the cell cycle. Among the evaluated CTAs, 16 seem to impact patients' OS. Notably, it was observed that high expression levels of VENTXP1 (p:0.00086), CCDC110 (p: 0.014), TDRD6 (p:0.025), as well as low expression of OTOA (p:0.0082), ADAM29 (p:0.0046), and IHO1 (p:0.019) are associated with poor OS outcomes. Our results suggest potential candidates that can guide future investigations for the development of accurate and effective tumor biomarkers. **Keywords:** gastriccancer;cancer/testis antigens(CTAs);tumor biomarkers.

ME - 140 - **Novel Immune Therapy to Promote Functional Recovery After Intracerebral Hemorrhage**

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Intracerebral hemorrhage (ICH) is the most devastating type of stroke, with a disproportionately high mortality approaching 40%. Current investigations in ICH treatment have focused on limiting hematoma expansion which unfortunately, have not led to an effective treatment. Neuroinflammation play a critical role in secondary injury post-ICH. Exposure of brain parenchyma to blood products initiates a complex inflammatory cascade start with activation of resident microglia. Resulting cytokine release recruits circulating monocytes and lymphocytes, further enhancing inflammation, and contributing to secondary injury. The cellular and molecular mechanisms leading to neurological deficits following ICH are poorly understood, and there is no effective therapy to modulate the mitigate CNS injury and promote recovery. The mucosal immune system is a unique tolerogenic organ that provides a physiological approach for the induction of regulatory T cells (Tregs). We found that nasal anti-CD3 monoclonal antibody induces IL-10-secreting Tregs that migrate to the brain and suppresses microglial inflammation in an EAE model. The therapeutic potential of the Tregs/IL-10 axis in ICH is largely unexplored.We employed the collagenases model of ICH in mice and administered nasal anti-CD3 treatment within 24 hours of ICH which was continued up to 1-month post-bleed. We performed Flow cytometry, histopathology and behavioral analyses to characterize the cells and assess the effects of treatment on the behavioral outcomes at 1-month post-ICH. Nasal anti-CD3 increased FoxP3+ Tregs and IL10 producing FoxP3+ Tregs in the brain. It also reduced microglial and astrocytes activation and increased hematoma resolution post-ICH. In addition, the treatment has improved behavioral outcomes, including motor, spatial learning, and hippocampal-dependent working memory functions at 1-month post-ICH. Our findings suggest that nasal anti-CD3 may represent a novel therapeutic approach for treating ICH.Nue. **Keywords:** Intracerebral Hemorrhage;Mucosal Immunity;Neuroinflammation.

ME - 141 - **New vaccine preparation with reactivity for Wuhan and Omicron variants: evaluation of IgG and IgM antibodies for more than one year in an experimental model**

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SARS-CoV-2 is a virus that causes COVID-19, which caused the pandemic from 2019 to 2023. We studied the immunization with the receptor binding domain (RBD) associated with the adjuvant mixtures Dimethyldioctadecylammonium and Saponin (DDA/Sap) or Outer membrane vesicles of Neisseria meningitidis C:2a:P1.5 strain and Aluminum Hydroxide (OMV/AH). Swiss adult female mice were immunized with two intramuscular doses. Blood collections were taken on days 0 (pre-immune), 21, 47, 176 and 368. Maternal-fetal transference of IgG was studied in the RBD+DDA/Sap group. IgM, IgG, IgG1, IgG2a and IgG3 were evaluated by ELISA. Functionality was analyzed by avidity and a surrogate neutralization assay for Wuhan and Omicron variants. Cellular response was evaluated by IL-4 and IFN-Y ELISpot. Groups RBD+DDA/Sap and RBD+OMV/AH showed higher IgM and IgG levels. RBD+OMV/AH mainly induced IgG1, IgG2a and IgG2b, whereas RBD+DDA/Sap induced all IgG isotypes. The adjuvants contributed to achieve high avidity in both groups. When avidity of isotypes were checked, IgG1 and IgG2a presented the higher avidity and IgG3, the lowest. Neutralization was higher on day 47, the same point where we observed higher IgG and IgM levels. Thus, this time point presented neutralization against the Omicron strain as well, mainly for the RBD+OMV/AH group. Cytokines corroborated isotype analysis and RBD+DDA/Sap induced a Th1/Th2 profile, with IFN-Y and IL-4 secretion. Considering the offspring, IgG levels were maintained until 45 days after birth and the main IgG isotypes were IgG2a and IgG2b. The results suggest that DDA/Sap is a promising adjuvant mixture to enhance the humoral and cellular immune response against SARS-CoV-2, even when the Omicron variant is considered, and to support maternal-fetal transference of antibodies. Interestingly, antibody levels were maintained for one year, suggesting persistence of the immune response. **Keywords:** SARS-CoV-2; RBD; OMV; Saponin;Dioctadecyldimethylammonium Bromide;;Maternal-Fetal transfer.

PD - 016 - **Use of Celecoxib showed direct antifungal activity and improved neutrophil microbicidal activity against Paracoccidioides brasiliensis**

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Paracoccidioidomycosis (PCM) is a mycosis caused by the fungus *Paracoccidioides brasiliensis*. The use of Celecoxib can be a new alternative to complement antifungal-therapy. We evaluated the effect of Celecoxib (6mg/Kg) either introduced directly into the air-pouch or administered systemically, by employing two experimental models: the effect of Celecoxib treatment in the initial aspects of the immune response, mice were inoculated via subcutaneous (sc) air-pouch with the virulent Pb18 *P. brasiliensis* strain and on the 5th day of infection, treatment was initiated and maintained daily until 3 days before cell collection. After 8 days of infection and treatment, the cells present at the air-pouch were collected to evaluate their antifungal activity and the supernatants were obtained to determine the concentration of catalase and peroxidase. Mice were infected intraperitoneally with Pb18 and after three days of infection, Celecoxib treatment by gavage was initiated and maintained daily for 15/120 days. Antibody determination was performed using ELISA. At 15 days of the infection, treated mice showed higher levels of catalase and peroxidase production than controls. At his time, Celecoxib also increased the antifungal activity of PMNs, mainly in relation to their phagocytic capacity, at 90 minutes of incubation than control. Regarding antibody titers, lower concentrations of IgM, IgG and IgG1a were observed at both 15 and 60 days of infection and treatment in the sera of infected and treated mice as compared to only infected controls. The treatment of experimental PCM with the anti-inflammatory drug Celecoxib not only reduced the intense inflammatory response but also had direct antifungal activity. Grants: CNPq-309917/2020-4, FAPEMIG-PPM-00497-18 and FAPEMIG-BPD-00341-22. L.A.Santos holds a scholarship from CNPq within the Young Doctors Fixation in Brazil Support Program. T.D.Andrade and J.C.Dutra are recipient CAPES and E.M.Picoli of CNPq-PIBIC scholarships. **Keywords:** Paracoccidioides brasiliensis;Celecoxib;Paracoccidioidomycosis .

PD - 017 - **Recruitment of monocytes/macrophages into alveolar septa of lungs of SARS-CoV-2 infected animals**

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Upon a viral infection, one of the first cells to interact with pathogens are macrophages and monocytes. These cells can also get infected and become instrumental to virus spreading by direct cell-to-cell contact and interaction with other cell types. During their active migration to the sites of injury, monocytes and macrophages produce chemokines and cytokines that are crucial for immune and inflammatory processes. Due to the significant morbidity and mortality caused by severe clinical symptoms of SARSCoV-2 infection, including cytokine storm and multiple organ failure, we still need better understanding of immune responses in the lungs. In this study, we divided Syrian golden hamsters into 6 groups. The uninfected group served as control. Three groups of SARS-CoV-2 only infected (unvaccinated) animals were studied at 2-, 5-, and 14-days post infection (p.i.). Two infected+vaccinated groups were studied at 5- and 14-days p.i. (n=4 per group). We quantified both the percentage of lung parenchyma occupied by fluorescent stained cells and the normalized fluorescent intensity ratio (FIR) of each immune marker (myeloperoxidase for neutrophils, CCR2 and CX3CR1 for classical and non-classical monocytes/macrophages, IBA-1 for macrophages and CD3 for T cells). The levels of the immune cell types were elevated in unvaccinated animals, 2 days p.i, as measured by FIR. A higher presence ofmacrophages, predominantly septal, was detected on days 2 (72.7%, $p<0.01$), 5 (63.6%, $p<0.05$) and 14 (65.2%, $p<0.05$) p.i. for unvaccinated animals when compared to noninfected control (34.4%), indicating the importance of these cells in the development of the disease. The FIR of IBA-1+ and CX3CR1+ cells, was higher 2 days p.i. for unvaccinated animals, when compared to vaccinated ones and to 5- and 14- days p.i ($p<0.05$). Our research suggests recruitment of septal macrophages into the lungs of SARS-CoV-2 infected animals and a reduced recruitment of these cells in vaccinated-infected animals. **Keywords:** pulmonary intravascular macrophages;SARS-CoV-2;monocytes.

PD - 018 - **MECHANISMS OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs) INDUCED BY SARS-CoV-2**

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COVID-19 is a disease caused by the SARS-CoV-2 and can cause mild flu-like symptoms. However, in some individuals, this disease takes a severe form and progress to multiple organ failure. The mechanisms involved during the severe form of this disease are not well understood. However, neutrophils have been described to participate during COVID-19. Neutrophil Extracellular Traps are a defense mechanism that can contribute to the resolution of a pathology, or act as a mediator in the inflammatory response, promoting tissue damage and organic damage. The molecular mechanisms involved in COVID-19 are constantly discussed in the literature. However, in the context of SARS-CoV-2 infection, the proteins involved in this response are still poorly elucidated. Therefore, this work seeks to understand which target proteins participate in this extracellular DNA release process and which signaling pathways are being activated during SARS-CoV-2 infection. We isolated neutrophils from patients affected by COVID-19 (CONEP – CAAE: 30248420.9.0000.5440) using percoll gradient. We demonstrate that the release of NETs in neutrophils isolated from healthy donors is elastase and PI3K. Furthermore, we observed that in neutrophils isolated from patients with COVID-19, a release of NETs occurs involving activation of mitochondrial ROS pathways. We observed that pharmacological inhibition of mitochondrial and NOX2-dependent ROS pathways leads to reduced NET release by neutrophils from healthy donors challenged with the SARS-CoV-2 virus. We suggests that the mechanism of NETs release in response to the SARS-CoV-2 involves elastase and PI3K, PKC delta and ROS activation. **Keywords:** Neutrophils;Neutrophil Extracellular Traps;COVID-19.

PD - 019 - **PRELIMINARY STUDY OF THE EARLY IMMUNOMODULATORY ACTIVITY OF SECRETOMA OF Leishmania braziliensis in vivo.**

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American Tegumentary Leishmaniasis(ATL) caused by *Leishmania braziliensis* is associated with clinical forms ranging from localized cutaneous lesions(LCL) to multiple disseminated lesions(DL) and mucocutaneous forms involving metastatic dissemination of parasites.However, the mechanisms underlying the metastatic capability remain understood. When we compared the immunomodulatory capability *in vitro* of the secretome from strains of *L. braziliensis* associated with two polar forms of ATL:auto-resolving LCL and DL, we observed significant differences in protein abundance between LCL and DL secretomes. The treatment of peritoneal macrophages with LCL secretome and/or infection with the LCL strain induced the production of pro-inflammatory molecules involved in cell activation and recruitment, but LD secretome/parasites did not. These results suggested that the secretomes could differentially recruit cells to the site of infection *in vivo*. To test this hypothesis,BALB/c mice were inoculated in the ears with LCL or DL strain secretome and euthanized 24h after stimulation. Ears were analyzed by flow cytometry for the frequency of tissue macrophages(TM), inflammatory monocytes(IM), and neutrophils(N). The arginase+(Arg+) cell frequency was analyzed. The Committee for Animal Care and Use approved the protocol(L-008/2020). We observed a significant increase in N(CD11b+Ly6G+Ly6Cint) and IM(CD11b+Ly6G-Ly6Chi) in animals stimulated with secretomes($p<0.01$). Interestingly, we found less TM(CD11b+Ly6G-Ly6C-CD206+) in animals that received the DL secretome compared to those that received the LCL secretome($p=0.0039$) and control animals($p=0.0023$). The frequency of TM-Arg+ reduced concerning the control in LCL($p=0.0022$) and DL($p=0.0268$). Only animals stimulated with LCL secretome showed a reduced frequency of IM-Arg+ compared to the control($p=0.0126$). These results suggest that both secretomes could promote N and IM recruitment but seem to activate IM at the stimulus site differentially. **Keywords:** LEISHMANIA BRAZILIENSIS;SECRETOME;IMMUNOMODULATION.

PD - 020 - **ACE-2, TMPRSS2, AND TLR7 GENES POLYMORPHISMS AND TIME TO COVID-19 PROGRESSION RELATED TO VENTILATORY SUPPORT USE AND/OR DEATH IN A CLINICAL COHORT FROM A REFERENCE HOSPITAL IN RIO DE JANEIRO, BRAZIL**

DE SÁ, N.B.R.[1]; DE LUCA, D.M.[2]; GOULART, M.N.G.[2]; ALVES, M.R.[3]; GERALDO, K.M.[3]; RIBEIRO, M.P.D.[3]; CARDOSO, S.W.[3]; GRINSZTEJN, B.[3]; VELOSO, V.G.[3]; GOMES, L.R.[4]; CAZOTE, A.D.S.[2]; ALMEIDA, D.V.[2]; GIACOIA-GRIPP, C.B.W.[2]; CORTES, F.H.[2]; MORGADO, M.G.[2]. 1. INSTITUTO OSWALDO CRUZ (IOC)/FIOCRUZ, INSTITUTO OSWALDO CRUZ (IOC)/FIOCRUZ RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ/FIOCRUZ, INSTITUTO OSWALDO CRUZ/FIOCRUZ RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS (INI)/FIOCRUZ, INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS (INI)/FIOCRUZ RIO DE JANEIRO - RJ - BRASIL; 4. CENTRO DE DESENVOLVIMENTO TECNOLÓGICO EM SAÚDE (CDTS)/FIOCRUZ, CENTRO DE DESENVOLVIMENTO TECNOLÓGICO EM SAÚDE (CDTS)/FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

Background: COVID-19 presents a broad spectrum of clinical manifestations, from asymptomatic to severe forms requiring hospitalization. Molecules involved in SARS-CoV-2 cell entry and downstream signaling cascade of mediators of innate immunity may influence the progression to severe forms of COVID-19. We investigated the role of 10 single base polymorphisms (SNPs) in ACE2, TMPRSS2, MX1, and TLR7 genes in SARS-CoV-2 infected patients. **Methods:** The study included 384 inpatients of the Hospital Center for COVID-19 (INI/FIOCRUZ). SNP genotyping was performed by RT-PCR and analyzed as risk factors for time to the use of mechanical ventilatory support (MVS) (n=164[42.7%]) or death (n=154[40.1%]) or MVS+death (n=128[33,3%]), as result of COVID-19, by Cox proportional hazard models. **Results:** For demographic and genetic analyses, COVID-19 inpatients were divided according to the outcomes of hospital discharge or death considering or not using MVS. Considering both genders, T/T genotype (aHR=3.686; P=0.02) in the TMPRSS2 rs12329760 was associated with faster progression to MVS. Differences were observed between the chromosome X-located ACE2 and TLR7 genes between men and women. Considering only women, carrier-T (aHR=11.142; P=0.008) in the ACE2 rs4240157 was associated with a risk for a faster progression to death, whereas those bearing the C/T genotype (aHR=10.065; P=0.013), T/T genotype (aHR=17.842; P=0.003), or carrier-T (aHR=12.117; P=0.005) in the same gene were associated with faster progression to MVS+death. Considering only the male gender, only Carrier-G (aHR=0.42; P=0.018) in the TMPRSS2 rs2070788 was associated with slower progression to MVS. Other SNPs in the ACE2, TMPRSS2, and TLR7 genes were associated with slower progression in both genders. **Conclusions:** Our results demonstrated for the first-time significant differences in SNPs frequencies of ACE2, TMPRSS2, MX1, and TLR7 genes related to the time to progression to distinct events in severe forms of COVID-19. **Keywords:** COVID-19;Single nucleotide polymorphisms (SNPs);risk factors.

PD - 021 - **Chronic infection with atypical strain of Toxoplasma gondii induces neuroinflammation and contributes with behavioral changes in murine host**

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The protozoan *Toxoplasma gondii* exhibit a great diverse population structure, with high distribution of atypical strains in South America, mainly in Brazil. The establishment of the immune response, with the production of IFN-γ, is crucial to control *T. gondii* infection and to maintain the chronic stage, however, chronic inflammation may be detrimental to the host homeostasis. In this context, the work presented here sought to investigate the impact of *T. gondii* atypical strain infection on neuro-inflammation and its consequences to possible behavioral alterations in experimental murine model. BALB/c male mice were orally infected with ten tissue cysts of clonal ME49 or atypical TgCkBrRN2 (CK2) *T. gondii* strains. 8 weeks post-infection, mice were submitted to behavior analysis, to determine possible behavioral changes induced by infection. Afterwards, blood was harvested, and serum was used to quantify IgG. Brain tissue was harvested for cyst count, quantification of neurotrophic factors and cytokines by ELISA and cell phenotyping by multiparametric flow cytometry. During chronic infection, mice infected with both strains displayed similar levels of IgG, however, animals infected with CK2 atypical strain displayed higher cyst count and higher inflammatory cell infiltrate in the brain parenchyma with a dramatic decrease in microglia population and elevated numbers of T cells and Ly6C^{hi} monocytes. CK2 infected animals showed higher expression of IFNɣ and TNF in brain, as well as lower levels of NGF (nerve growth factor) in pre-frontal cortex and striatum modified levels of CXCL1 (fractalkine) in prefrontal cortex and hippocampus. We showed that the inflammatory levels contribute to elevated anxiety and depression levels found in CK2 infected animals. Our study shed light on how infection with different strains of *T. gondii* may activate the immune response differently in the brain and trigger behavioral changes in the host. **Keywords:** neuroinflammation;depression;Toxoplasma gondii.

PD - 022 - **Lipid droplet biogenesis and function during the Zika virus infection**

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The infection by the arbovirus Zika virus (ZIKV) is a global public health problem due to its association with adult neurological disorders and congenital diseases in newborns. Several RNA viruses have the ability to manipulate lipid metabolism and exploit lipid droplets (LD) to facilitate viral replication and promote pathogenesis. LDs are organelles with crucial functions in lipid metabolism, energy regulation, intracellular transport, and their involvement in infections and inflammation is well recognized. However, the mechanisms of LD formation and their roles in ZIKV infection in neural cells are still unclear. Thus, the objective of this study is to investigate the molecular mechanisms governing LD formation and function during ZIKV infection in neural cells. Our findings reveal that LDs play a significant role in ZIKV infection by promoting viral replication. ZIKV infection modulates the expression of lipid metabolism pathways, including the upregulation of lipogenesis proteins and decreased the lipolysis-associated proteins, resulting in LD accumulation in human neural cells. Inhibition of DGAT-1, an enzyme involved in LD synthesis, reduces both LD accumulation and ZIKV replication in vitro. Similarly, pharmacological and genetic inhibition of SREBPs, transcription factors that regulate lipid metabolism, also reduces ZIKV replication in vitro. Importantly, inhibition of DGAT-1 not only decreases inflammatory cytokine production in the brain but also mitigates weight loss and mortality associated with ZIKV infection in a mouse model (CEUA- IOC/ license number L-001/2018). Our findings highlight the crucial role of lipid metabolic reprogramming and LD formation in ZIKV replication and pathogenesis, opening new perspectives for ZIKV therapies development. **Keywords:** Zika virus;Lipid droplet;Lipid metabolism.

PD - 023 - **Temporal patterns of cytokine and injury biomarkers in hospitalized COVID-19 patients treated with methylprednisolone**

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Introduction: The COVID-19 presents with complex pathophysiological effects in various organ systems. Understanding the profiles of biomarkers and cytokines in infection, as well as the changes that occur after administration of systemic corticosteroid treatment, is critical to assessing the benefits and/or risks of this treatment. **Objective**: To determine the effect of methylprednisolone (MP) on issue injury biomarkers and cytokine profiles compared to placebo in patients with COVID-19. **Methods**: The analysis was performed on 50 negative controls, 49 MP-treated and 52 placebo-treated serum samples. Samples collected on D1 (pre-treatment), D7 and D14 were analyzed. Luminex assay quantified the HMGB1, FABP3, myoglobin, troponin I and NTproBNP. Immune mediators (CXCL8, CCL2, CXCL9, CXCL10, TNF, IFN-γ, IL-17A, IL-12p70, IL-10, IL-6, IL-4, IL-2, and IL-1β) were quantified using cytometric bead array. **Results**: At baseline, injury biomarkers (HMGB1, TnI, myoglobin, FABP3) were elevated. At D7, HMGB1 was higher in the MP group (p=0.0448) compared to the placebo group, while HMGB1 in the placebo group diminished by D14 (p=0.0115). Compared to healthy control, immune mediators (IL-17A, IL-6, IL-10, MIG, MCP-1, and IP-10) were elevated at baseline (all p≤0.05). At D7, MIG and IP-10 of the MP-group were lower than in the placebo-group (p=0.0431, p=0.0069, respectively). IL-2 (MP-group) and IL-17A (placebo-group) had increased by D14. In placebo group, IL-2 and IL-17A increased, as IL-12p70, IL-10 and IP-10 steadily decreased during follow-up. The MP treated group had IL-2, IFN-γ, IL-17A and IL-12p70 increase, while IL-1β and IL-10 decreased towards D14. **Conclusions:** Overall, MP use in COVID-19 management has implications on the immunological response and injury biomarker profile following the infection. More future studies are needed to fortify methylprednisolone's use in the management of inflammatory reactions associated with corona virus infections. **Keywords:** COVID-19;methylprednisolone;immune mediators.

PD - 024 - **Synthesis and use of chimeric zika virus proteins for serological diagnosis of low cross-reactivity**

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Arboviruses constitute a group of viruses transmitted primarily by mosquitoes. They represent a major public health problem worldwide. The emergence of Zika in Brazil (2015) drew attention because despite being an acute and self-limiting disease, part of the individuals had severe neurological manifestations, congenital complications, and Guillain-Barré syndrome. The great antigenic similarity between flaviviruses makes diagnosis difficult. Thus, there is a need to develop new diagnostic tools. This work aimed to generate chimeric proteins capable of serologically differentiating ZIKV or DENV infections. The recombinant proteins (ZIKV-1, ZIKV-2, ZIKV-3) were designed and their respective genes were subcloned into a pET21a expression vector. The recombinant proteins were expressed and purified and antigenicity was validated. Purified proteins were tested as solid-phase antigens in standard ELISA protocols for the detection of anti-ZIKV IgG antibodies. The results obtained after standardization are promising, and the tests elaborated with the recombinant antigens showing high sensitivity and specificity, in addition to low cross-reactivity with the interferent with other viruses. The ZIKV-1 protein showed 91% sensitivity and 97% specificity, the ZIKV-2 protein showed 95% sensitivity and 96% specificity, and the ZIKV-3 protein showed 66% sensitivity and 84% specificity. Regarding the DENV interferent, there was 10% cross-reactivity for ZIKV-1 and 19% for ZIKV-3, ZIKV-2 did not show cross-reactivity. The assays were also carried out in another laboratory and presented similar results, demonstrating reproducibility and robustness. The results found for ZIKV-1 and ZIKV-2 show great potential for developing a specific diagnostic test for detecting IgG antibodies to ZIKV using the produced chimeric proteins. **Keywords:** Zika virus;Dengue virus;Serological Diagnosis.

PD - 025 - **The immunomodulation effect of a chemical chaperone in the intestinal mucosa of patients with Crohn's disease.**

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Background: Endoplasmic reticulum stress (ER stress) has been linked to inflammatory bowel disease (IBD)[(1)]. The overproduction of pro-inflammatory cytokines activates ER stress markers in order to restore homeostasis. Although the use of a chemical chaperone seemed to decrease the production of pro-inflammatory cytokines by inhibiting ER stress[(2)], there is no study that evaluates its use in human intestinal mucosa from Crohn's disease (CD) patients. **Objective:** To evaluate the expression of inflammatory cytokines in the intestinal mucosa from CD patients after the treatment of a chemical chaperone. **Methods:** After the approval of Ethics Committee, the biopsies were collected, by colonoscopy, from CD patients with CD and from controls. The samples were treated with the chemical chaperone, PBA, during an *ex vivo* culture, for transcriptional analysis, whereas inflammatory cytokines levels were evaluated in the medium, via multiplex assay. **Results:** CD group was composed of 10 patients with activated disease and the control group of 6 individuals. The expression of inflammatory cytokines, as well as ER stress-related genes, were significantly increased in the CD group when compared to the control group. After PBA treatment, all these markers decreased significantly. Pro-inflammatory cytokines levels in the medium were higher in the CD group, and decreased after PBA, compared to the controls. **Conclusions:** Our results strongly suggest that the chemical chaperone promoted a modulationof inflammatory markers in CD patients, attenuating inflammation and bringing up a potential new therapeutical target for treating inflammatory diseases. **References:** 1 *PLoS One*, *14*(9), e0223105, 2019. 2 Toxicol Lett. 271:26-37, 2017. **Financial support:** This study was supported by the São Paulo State Research Support Foundation (FAPESP) (2020/04407-1), and B.L.R. received a post-doctoral scholarship from Funding for Education, Research and Extension Support (FAEPEX). **Keywords:** Crohn's disease;ER stress;chaperone.

PD - 026 - **Continuous use of anticholesterolemics and diuretics are indicative of poor prognosis in patients with COVID-19**

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There is satisfactory information, which reports how comorbidities that affect the population, impact the outcome of patients with COVID-19. However, little is known about the effects that the continuous use drugs used to treat such comorbidities may impact the prognosis of patients with COVID-19. The purpose of this study is to evaluate the interference of the continuous use of drug classes in the expression of biomarkers during the first week of hospitalization. This is observational retrospective study was conducted from July 21, 2020 through March 20, 2021 and included 176 hospitalized patients diagnosed with COVID-19 in Belo Horizonte, Brazil. The patients diagnosed with COVID-19 were underwent collection of fasting whole blood samples for further analysis. Other data extracted for this study included age, sex, clinical symptoms, related comorbidities, and classes of continuous use. Routine serum biochemical parameters, including alanine aminotransferase, aspartate aminotransferase, Lactate dehydrogenase, C-reactive protein, N-terminal fragment of B-type natriuretic peptide and cardiac troponin were measured. Among the drug classes evaluated, we verified that the continuous use of diuretic and antihypercholesterolemic drug classes presented a significant relative risk of death as outcome, when compared to the group of patients who was discharged. In this study, we verified for the first time that hospitalized COVID-19 patients on continuous use of anticholesterolemic and diuretic classes are likely to have a worst outcome. Moreover, we conclude that patients using anticholesterolemic and diuretic drug classes show some correlations that are indicative of unbalancing between the injuries caused by SARS-CoV-2 infection and the immune response. **Keywords:** Coronavirus disease 2019;Biomarkers;Antihypercholesterolemic, Diuretics.

PD - 027 - **Continuous use of anticholesterolemics and diuretics are indicative of poor prognosis in patients with COVID-19**

OLIVEIRA, F.M.S.[1]; CAETANO, M.M.M.[1]; DE GODOY, A.R.V.[1]; DE OLIVEIRA1, L.L.[1]; MAMBRINI, J.V.D.M.[1]; REZENDE4, M.S.[2]; FANTINI, M.P.R.[3]; MENDES, T.A.[4]; DE MEDEIROS, N.I.[1]; GUIMARÃES, H.C.[2]; FIUZA, J.A.[1]; GAZE, S.T.[1]. 1. RENE RACHOU INSTITUTE, OSWALDO CRUZ FOUNDATION, RENE RACHOU INSTITUTE, OSWALDO CRUZ FOUNDATION BELO HORIZONTE - MG - BRASIL; 2. RISOLETA TOLENTINO NEVES HOSPITAL, RISOLETA TOLENTINO NEVES HOSPITAL BELO HORIZONTE - MG - BRASIL; 3. MATERDEI HOSPITAL, MATERDEI HOSPITAL BELO HORIZONTE - MG - BRASIL; 4. FEDERAL UNIVERSITY OF VIÇOSA, FEDERAL UNIVERSITY OF VIÇOSA BELO HORIZONTE - MG - BRASIL.

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PD - 028 - **Continuous use of anticholesterolemics and diuretics are indicative of poor prognosis in patients with COVID-19**

OLIVEIRA, F.M.S.[1]; CAETANO, M.M.M.[1]; DE GODOY, A.R.V.[1]; DE OLIVEIRA1, L.L.[1]; MAMBRINI, J.V.D.M.[1]; REZENDE4, M.S.[2]; FANTINI, M.P.R.[3]; MENDES, T.A.[4]; DE MEDEIROS, N.I.[1]; GUIMARÃES, H.C.[2]; FIUZA, J.A.[1]; GAZE, S.T.[1]. 1. RENE RACHOU INSTITUTE, OSWALDO CRUZ FOUNDATION, RENE RACHOU INSTITUTE, OSWALDO CRUZ FOUNDATION BELO HORIZONTE - MG - BRASIL; 2. RISOLETA TOLENTINO NEVES HOSPITAL, RISOLETA TOLENTINO NEVES HOSPITAL BELO HORIZONTE - MG - BRASIL; 3. MATERDEI HOSPITAL, MATERDEI HOSPITAL BELO HORIZONTE - MG - BRASIL; 4. FEDERAL UNIVERSITY OF VIÇOSA, FEDERAL UNIVERSITY OF VIÇOSA BELO HORIZONTE - MG - BRASIL.

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PD - 029 - **In vivo AND In vitro Trypanosoma cruzi INFECTION: STUDY OF A REGULATOR EFFECT OF ANNEXIN A1**

RICCI, M.F.; OLIVEIRA, F.B.R.; RABELO, R.A.N.; MARQUES, M.D.; BARBOSA, C.L.N.; DE ALMEIDA, V.M.; REZENDE, I.C.; MELO, N.; SANTOS, L.L.O.; SANTANA, L.F.D.; VIEIRA, A.T.; MACHADO, F.S.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, UNIVERSIDADE FEDERAL DE MINAS GERAIS BELO HORIZONTE - MG - BRASIL.

Chagas disease (CD) is a neglected disease caused by the protozoan *Trypanosoma cruzi (Tc)*, whose complications include cardiac, digestive and neurological dysfunction. The imbalanced inflammatory response is associated with the clinical forms development, thus Annexin A1 (ANXA1) emerges as an important protein with a central role in the inflammatory process resolution. Herein, the influence of ANXA1 on gut microbiota composition and in the development of Tc-induced pathogenesis were investigated. For *in vivo* analysis, female BALB/c (WT) and ANXA1 knockout (KO) mice, 8 to 9 weeks old, were infected ip with 10^3 trypomastigotes forms (Y strain). Parasitemia, body weight and survival were evaluated. We collected fresh stool samples at 10 and 20 day post-infection (dpi) for cultivable fecal microbiota analysis. For *in vitro* analysis, we used the primary myenteric neurons culture assays. The supernatant was collected at 24, 48 and 72 hpi with *Tc* Y strain (10:1) for lactate dehydrogenase (LDH), nitric oxide (NO) and ELISA for the cytokines IL-6, TNF-α, IFN-γ and IL-10. The results showed that the deficiency of ANXA1 resulted in higher parasitemia, weight loss, and mortality rate compared with WT. The cultivable fecal microbiota quantification showed that ANXA1 KO mice had less *Bacteroides*, *Staphylococcus* and *Enterococcus* at 10 and 20 dpi. *Staphylococcus* in WT mice decreased with *Tc* infection, whereas it increased in ANXA1 KO mice. *In vitro*, at 24 hpi, myenteric neurons from infected-ANXA1 KO mice showed higher levels of NO, IL-6, TNF-α and IFN-γ and lower levels of IL -10 production compared with WT. Collectively, these results suggest that ANXA1 is a vital regulator of the development of CD pathogenesis, which may be influenced by the intestinal microbiota affecting communication between gut/immune/central system. This helps portraying new insights into disease pathogenesis and may provide new potential therapeutic approaches. **Keywords:** Trypanosoma cruzi;Annexin A1;Microbiota.

PD - 030 - **PGC1a at the interface between mitochondrial activity and regulatory T cell biology**

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Regulatory T (T_{reg}) cells play important role in the maintenance of immunologic tolerance and control of immune responses. The transcription factor Foxp3 is essential for the development, stability and suppressive function of T_{reg} cells. In the last few years, it has been evidenced different metabolic pathways driving the phenotype of T_{reg} cells, which require mitochondrial integrity and metabolism for exerting their functions. Mitochondria are sites of biochemical processes including lipid oxidation, tricarboxylic acid cycle and oxidative phosphorylation, that culminate in generation of energy. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a) acts as an important regulator of oxidative metabolism, biogenesis and mitochondrial dynamics through the activation of different transcription factors, being widely described in tissues with high energy demand. Although a low expression of PGC1a has been related to the exacerbation of inflammatory responses, little is known about its intrinsic role in immune cells, especially in immunoregulatory functions. This project aims to investigate how PGC1a connects the regulation of cellular metabolism to the generation and function of T_{reg} cells. Our preliminary results demonstrated that higher mitochondrial content is closely related with the differentiation of T_{reg} cells. Moreover, these cells showed a substantial increase in the expression of PGC1a. In line, increased PGC1a activity promoted higher levels of Foxp3 expression, which effect was reduced by the inhibition of PGC1a, suggesting a contribution of PGC1a in the development of T_{reg} cells. Also, the activation of PGC1a accompanied increased mitochondrial mass in T_{reg} cell cultures. These preliminary findings shed light on the PGC1a potential as a pharmacological target when manipulating T_{reg} cells as a therapeutic strategy. **Keywords:** Mitochondria;PGC1a;Treg cells.

PD - 031 - **CSP vaccine for Plasmodium vivax malaria**

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Malaria is caused by *Plasmodium* parasites, which are transmitted by the bite of the Anopheles mosquito in tropical regions. The development of alternative tools to control malaria is increasingly important due to the emergence of drug-resistant parasites and insecticide-resistant mosquitoes. According to the WHO, in 2019, there were an estimated 229 million cases of malaria and 409,000 deaths caused by malaria worldwide. The most advanced malaria vaccine today is Mosquirix, also known as RTS,S, which consists of a recombinant vaccine based on the circumsporozoite protein (CSP) of *P. falciparum*, and does not offer any protection against *P. vivax*. The present work consists of the generation of recombinant proteins based on the primary sequences of the three allelic forms of the CSP protein of *P. vivax* (VK210, VK247 and *P. vivax*-like). A recombinant hybrid polypeptide containing the repeating central region of the three allelic variants, called PvCSP-*All epitopes*, was expressed in the yeast system *Pichia pastoris*. A preliminary stability study of both the IFA and the vaccine formulation was performed to evaluate physicochemical and antigenicity parameters for quality control at long-term (5°C) and accelerated (30°C) temperatures at 0,7,15,30,60,90 and 180 days. In order to evaluate the vaccine potential of PvCSP, C57BL/6 mice were immunized with three doses of the protein formulated with different nanoemulsion-based adjuvants and a double-stranded RNA analogue that interacts t8jwith Toll3-type receptors (TLR3). The humoral and cellular response was evaluated and a significant production of Total IgG, IgG1 and IgG2c antibodies specific for both the PvCSP chimera and all subunits was observed in the immunized groups. In addition, we observed a significant production of IFN-γ by splenocytes stimulated with the chimera protein. In conclusion, the PvCSP chimera was stable and capable of inducing a robust and specific immune response in the malaria vaccine formulation. **Keywords:** Malaria;proteina recombinante;resposta imune.

PD - 032 - **Chronic Chagas disease cardiomyopathy patients display a higher frequency of circulating Th1 cells than indeterminate phase patients**

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Chagas disease (CD) is caused by Trypanosoma cruzi, with about 1.2 million people infected in Brazil. Approximately 30% of the infected develop chronic chagasic heart disease (CCC), which is characterized by myocarditis and it is one of the main causes of hospitalization from heart failure in Latin America. Cardiac biopsy of CCC patients show higher frequency of Th1 lymphocytes. In addition, IFNg-producing cells are more frequent in peripheral blood of CCC when compared to indeterminate phase individuals (IF). Conversely, previous research shows that Th17 cells and IL-17 are less pronounced in CCC than IF. Currently, Th1 and Th17 populations in CD are only characterized by markers such as CD3, CD4, IFNg and IL-17. However, these cell subtypes have never been studied together in the same group of patients and with more specific markers for CD4+ Th1 (CD4+CD45RO+CD25-CD161-CXCR3+CCR4-CCR6-IFNg+) and Th17 (CD4+CD45RO+CD25-CD161+CXCR3-CCR4+CCR6+IL-17+) T cell. Our goal was to simultaneously evaluate Th1 and Th17 cells in CCC and FI patients. PBMC from CCC (n=4) and IF (n=7) were incubated with PMA (50 ng/ml), ionomycin (500 ug/ml) and Brefeldin A for 5h. After stimulation, cells were stained with anti-CD3, CD4, CD45RO, CD25, CD161, CXCR3, CCR4 and CCR6; fixed/permeabilized and incubated with anti-IFNg and IL-17. All stains were performed for 30 min at 10°C. The samples were acquired on FACS Canto II and analyzed with FlowJo and GraphPad Prism 9.0. Our results showed that CCC had higher Th1 frequency than FI patients (p=0.042). There were no significant differences in cytokine production by the Th1 and Th17 subpopulations of CCC and IF patients. Although the frequency of Th17 did not differ significantly between the two groups of patients, there is a tendency for Th17 cells from IF to produce more IL-17 than CCC. These results highlight that Th1 cells may play a significant role in Chagas' heart disease and suggest expanding the sample size for Th17 assessment. **Keywords:** Chagas Disease;Th1 Cell;chronic chagasic heart disease.

PD - 033 - **Allergy to cassava: identification of new allergens and cross-reactivity beyond latex**

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Cassava (*Manihot esculenta*) is a widely consumed food in many parts of the world. Belonging to the Euphorbiaceae family, the same as latex, it can cause mild to severe allergic reactions. Man e 5, the only known cassava allergen, cross-reacts with the latex allergen Hev b 5. Some patients allergic to cassava do not cross react with latex indicating there are specific allergens. Thus, the aim of this study was to identify and characterize new cassava allergens. For that, protein extract of this root was submitted to a 2D-SDS-PAGE followed by an immunoblotting with sera from 10 individuals with confirmed cassava allergy. IgE-reactive proteins were identified by mass spectrometry. Six proteins were recognized by IgE: alpha-1,4 glycan phosphorylase, peptidase_S9, and cytosol_AP described here as allergens for the first time. The three remaining proteins are homologues already described for cassava and identified in other sources, including latex: ATP synthase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and fructose biphosphate aldolase (FBA). Out of these proteins, FBA was recombinantly produced in Expi293 cells and tested with allergic patients' serum. Protein stability to pepsin digestion was evaluated *in vitro*. Our recombinant FBA molecule was IgE-recognized by 80% of our cohort, proved to be stable to pepsin digestion and, interestingly, was recognized by individuals with no history of latex allergy or sensitization. This is the first report of an IgE-reactive protein present in cassava that do not cross-react with latex, thus our molecule may be important for *in vivo* diagnostic tests, overcoming extract standardization issues. As Brazil is very rich in biodiversity, allergens not yet identified can be important triggers of allergies. Furthermore, in the future, the molecule here identified can be used in desensitization protocols, in which the use of an isolated molecule would be more effective in comparison to extracts, avoiding secondary sensitization. **Keywords:** Food Allergy;Manihot esculenta ;Fructose Biphosphate Aldolase .

PD - 034 - **Signature of memory CD8+ T cell in recovered COVID-19 individuals**

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COVID-19 is caused by SARS-CoV-2 and exhibits a range of clinical conditions, in which the immune response plays a crucial role in the disease. Long-lived SARS-CoV-2-specific CD8+ memory T cells provide long-term immune protection by limiting viral replication upon reinfection. This study investigated immunomodulatory mechanisms mediated by CD8+ T lymphocyte subtypes in individuals who had recovered from mild and severe COVID-19 prior to vaccination. Peripheral Blood Mononuclear Cells (PBMCs) were collected from control (n = 9), mild (n=9), and severe (n=6) groups, and incubated under 3 different conditions: unstimulated (medium), and stimulated with SARS-CoV-2 peptides (Pool Spike CoV-2 and Pool CoV-2). In this study, naïve, effector memory that reexpresses CD45RA (TEMRA), effector memory (TEM), and central memory (TCM) CD8+ T cells, as well as IL-10, IFN-y, TNF-α, and IL-17 cytokines, and activation markers (CD69, CD137, and Ki67) were analyzed by flow cytometry. Pool Spike CoV-2 and Pool CoV-2 stimulus elicited a higher frequency of CD8+ TCM cells in the recovered mild group. CD8+ TCM and TEM cells showed heterogeneity in CD137 and CD69 activation marker expressions between mild and severe recovered groups. Also, we observed a predominance in CD137 expression by naive CD8+ cells, TCM, and TEM from the mild recovered group when stimulated with antigenic pools. Additionality, a higher CD69 expression from the severe recovered group by CD8+ TEMRA cells was observed under SARS-CoV-2 Epitope Pools. CD8+ naïve, TCM, and TEM cell subsets from recovered mild volunteers had higher expression of TNF-α while the expression partner of IFN-γ, IL-10, and IL-17 point to an antiviral signature by TEMRA CD8+ cells. Our findings contribute to the elucidation of the functional capabilities of each subpopulation of memory T cells during SARS CoV-2 antigenic reexposure, as well as their role in disease outcomes in individuals who recovered from COVID-19. **Keywords:** Immunological Memory;Immune response;SARS-CoV-2 infection.

PD - 035 - **A multi-omics investigation of the Fingerprint of Small Extracellular Vesicles in Zika virus**

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Mass spectrometry diagnostics is already a reality for a number of diseases, such as several types of cancers and bacterial infections. Because of this, the inclusion of ZIKV in the portfolio of diseases diagnosed by mass spectrometry targeting a laboratory that already has this equipment would imply the reduction of the unit cost of the test to only US$ 0.65. This study, was collected from 30 children with confirmed CZS (CZS+) and 15 control children (CZS-). The study included a prospective cohort of children living in the state of Maranhão, in northeastern Brazil (CAAE: 86696618.7.0000.5467). Data from the literature indicate that localizations may determine functions such as: 1) Extracellular - Cell shrinkage causes the cytosol to push EVs to the perinuclear region with "address labels" for rapid transport; 2) Plasma Membrane - specificity for membrane ligands and recognize Molecular Patterns Associated with Pathogens; 3) Intracellular - driving the formation of multivesicular bodies and actively act in intracellular signaling pathways; and 5) Nucleus - intracellular signaling and modulation of immune response. Indeed, EVs infected with ZIKV showed specific molecular pathways dysregulated leading to cell death and abnormal differentiation. A strain-specific protein modulation in EVs was detected with EVs inducing profound changes in EVs neurospheres compared to ZIKV. These data highlight the importance of viral adaptation and its correlation with the disease. Moreover, the analysis of neurons differentiated after infection was applied as in vitro model of embryonic neurodevelopment upon ZIKV exposure. Infected mature neurons showed not only extensive downregulation of synapse-related proteins but also decreased synapse density. This study broadens the understanding of protein expression changes in EVs-infected ZIKV and neurons, revealing a strain-specific viral adaptation and functional impairment of surviving neurons. **Keywords:** biomarker;exosomes;multi-omics.

PD - 036 - **CHARACTERIZATION OF IMMUNE CELL INFILTRATED IN BREAST CANCER MODEL AND THE INFLUENCE OF MODERATE AEROBIC TRAINING**

SILVEIRA, L.S.; TEIXEIRA, A.A.D.S.; BIONDO, L.A.; DE LIMA JR, E.A.; SANTIAGO-CARVALHO, I.; LIMA, M.R.D.; NETO, J.C.R.. UNIVERSITY OF SAO PAULO, UNIVERSITY OF SAO PAULO SÃO PAULO - SP - BRASIL.

BACKGROUND: Lower levels of physical activity is considered one of the main external factors in cancer development. Based on the protective role of exercise many works have emerged trying to explain key mechanisms of interaction with tumour immune cells. In some types of cancers, including breast cancer, it has been hypothesized that PPARγ has anti-tumorigenic functions. Thus, the purpose of this study was to evaluate the effect of both, PPARγ deletion in macrophages and a moderate exercise protocol in the immune cell profile of triple-negative breast cancer in mice. METHODS: Female mice with deletion of PPARγ in myeloid cells and their control littermates were orthotopically injected with a triple negative breast cancer cell line (E0771) or saline (control). The group that received the tumour cells was subdivided in: a) moderate aerobic training protocol (60% of maximum speed, 5 days/week for 4 weeks) or b) sedentary. After exercise protocol, tumour growth, inflammatory parameters and subpopulations of tumour associated macrophages (TAMs) and infiltrated lymphocytes (TIL) were analysed. RESULTS: Exercise was efficient in reducing tumour volume regardless of the genotype, however no significant change in the proliferation marker KI-67 was found. Percentages of TAMs subpopulation ($CD45^+CD11b^+F4/80^+$) M0 ($CD86^-CD206^-$) M1 ($CD206^-CD86^+$) or M2 ($CD86^-CD206^+$) were not affected by genotype nor exercise. The relative number of $CD4^+$ T cells and cytokines such as IL-4 and IL-6 were increased in tumour of wild type exercised mice, while MCP-1 was increased. IKBKE (inhibitor of nuclear factor kappa b kinase subunit epsilon), a breast cancer known oncogene was slightly lower in tumour infiltrated cells from exercised WT mice compared to KO. CONCLUSION: Exercise reduced tumour volume and this positive effect seems to be related to increased tumour infiltrated $CD4^+$ T lymphocytes. **Keywords:** exercise;breast cancer;immune system.

PD - 037 - **Pro-resolution Pathway in Experimental Colitis – A New Possible Therapeutic Approach for Inflammatory Bowel Diseases**

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Background: Inflammatory bowel diseases (IBD) are idiopathic disorders characterized by chronic inflammation of the gastrointestinal tract. Recent studies have highlighted the role of specialized pro-resolution lipid mediators (SPMs) in the resolution of chronic inflammation. Therefore, this study aimed to investigate the SPM Resolvin D2 (RvD2) and its precursor, omega-3 polyunsaturated fatty acid. **Methods:** An experimental model was carried out. Mice were separated into a control group, and a DSS (dextran sulfate sodium)-induced colitis group (3% dilution in drinking water); both groups were subdivided and received either a standard diet or an experimental diet enriched with 20% of oil rich in omega-3. Another group of mice, after acquiring DSS-induced colitis, was subdivided and treated with RvD2 or anti-TNFα. Throughout the experimental protocols, the body weight, food intake, Disease Activity Index (DAI), metabolic phenotype, and inflammatory profile of these animals were assessed by real-time PCR, ELISA, histology, Western blotting, and lipid analysis. The study was approved by the research ethics committee. **Results:** The animals with experimental colitis showed an increase in TNFα and IL22 transcriptional expression besides a reduction in the enzymes involved in the endogenous biosynthesis of RvD2, such as PLA2, 15-LOX, 5-LOX, and its receptor GPR18. Dietary supplementation with omega-3-rich oil increased RvD2 and its precursor besides reducing the DAI, weight loss, colonic shortening, and the histologic colonic inflammation score. Treatment with RvD2 effectively attenuated experimental colitis, reducing TNFα transcriptional levels and p-JNK protein expression. Besides, RvD2 increased the immediate precursor of RvD2 (7,8-epoxy-17-HDHA). **Conclusion:** The beneficial effect of RvD2 and its precursor omega-3 may bring about a new therapeutic approach for IBD. **Reference:** Clin Rev Allergy Immunol. 58:82-91, 2020. **Keywords:** Pro-resolving lipid mediators;Resolvins;Inflammatory bowel disease.

PD - 038 - **Developmental origin of transitional dendritic cells: a novel DC population**

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Dendritic cells (DCs) are a group of antigen-presenting cells divided into subsets based on their origin and function. Plasmacytoid DCs (pDCs) are able to secrete high levels of type I interferon (IFN-I) during viral infections, whereas conventional type 1 DCs (cDC1s) and conventional type 2 DCs (cDC2s) excel at the activation of antigen-specific $CD8^+$ and $CD4^+$ T cells, respectively. Recently, high-dimensional single-cell technologies revealed emerging DC populations. One of these are transitional DCs (tDCs), which have features of both pDCs and cDCs. Yet, we lack an understanding of the developmental origin of tDCs and their relationship with pDCs and cDCs. Here, we used high-dimensional analyzes, adoptive cell transfer experiments and lineage tracing mouse model to evaluate tDC origins from bone marrow progenitor cells. Despite that tDCs and pDCs share several developmental features, we found that tDCs do not derive from pDCs at homeostasis after adoptive cell transfer. Instead, tDCs originate from a bone marrow progenitor shared with pDCs (pro-pDCs). We report that tDCs can convert into a subpopulation of cDC2s expressing ESAM. However, despite tDC capacity to convert into cDC2, these are different from previously described cDC2 precursors (pre-cDC2s), indicating they are a novel population. Our data clarify tDC ontogeny, and suggest that the DC network should be expanded to encompassed tDCs, a population of DCs developmentally related to pDCs that contributes to the heterogeneity of cDC2. REFERENCE: Nat. Rev. Immunol. 14, 571–578 (2014). Immunity 50, 37–50 (2019). Cell Rep. 29, 3736–3750.e8 (2019). Nature Immunology, 41590-023-01545-7, (2023). **Keywords:** Dendritic cells;Plasmacytoid dendritic cells;Transitional dendritic cells.

PD - 039 - **Differential Expression of Clock Genes in M1 and M2 Macrophages, Revealing Significant Upregulation of RORb Gene in M1 Phenotype**

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Monocytes and macrophages exhibit robust circadian rhythms with high amplitude of clock gene expression. Consequently, various functions of macrophages and monocytes display circadian rhythms, such as pattern recognition receptor (PRR) response, cytokine expression in response to endotoxin challenge, tissue recruitment, and phagocytosis. It is intriguing to note that during the differentiation process from monocytes to macrophages with classical or alternative phenotypes, significant alterations in the energetic metabolism of these cells are observed. Clock genes are known to assist in regulating the transcription of various genes that govern metabolic pathways in different cell types. Considering that changes in energetic metabolism are essential prerequisites for macrophage differentiation, we hypothesize that there must be a modification in the expression pattern of clock genes during the process of macrophage differentiation. Preliminary results from our research group have demonstrated that this phenomenon indeed occurs. During the differentiation process from M0 to M1 or M0 to M2 macrophages, there is a clear modification in the regulated clock genes, with time-dependent differences. Hence, we emphasize the intimate relationship between the differentiation of macrophage subtypes and clock genes, with a particular emphasis on the RORb gene, which exhibits a significant increase in the M1 phenotype. **Keywords:** Clock genes ;macrophages ;innate immunity.

PD - 040 - **Gut dysbiosis induced by amoxicillin influences the estrous cycle and the expression of IL-1β and IL-10 in the ovary and caecum of mice.**

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Gut dysbiosis refers to an imbalance of the gut microbiota and it is characterized by an exacerbated or decreased proliferation of bacterial population. Since intestinal dysbiosis is associated with a wide range of immunopathological and reproductive conditions, the main goal of this study was to evaluate amoxicillin-induced gut dysbiosis and its influence on the estrous cycle in mice. Therefore, mice were treated with amoxicillin or phosphate buffer saline (PBS), and the fecal microbiota was evaluated by 16S rDNA metagenomic sequencing. The estrous cycle was evaluated by vaginal cytology, vaginal opening, and vaginal wash by flow cytometry. After the induction of gut dysbiosis, the ovaries and the caecum sample were analyzed for differential expression of *IL-1β* and *IL-10* genes and histological analysis. Amoxicillin-treated mice presented differing bacterial groups in the fecal microbiota when compared to the PBS-treated group indicating that amoxicillin treatment induced gut dysbiosis and they gained weight. The vaginal cytology analysis showed that amoxicillin-induced gut dysbiosis decreased the number of cells but increased the relative number of leucocytes and altered the estrous cycle. *IL-1β* was shown to be upregulated in the caecum and the ovary of the dysbiotic mice. On the other hand, *IL-10* expression was shown to be diminished in both organs of the dysbiotic mice. The oocyte area from the dysbiotic group presented lower than non-dysbiotic mice with increasing thickness of the pellucid zone. The follicular teak from dysbiotic mice showed lower thickness than non-dysbiotic mice. In conclusion, the results indicate that amoxicillin induces gut dysbiosis and influences the estrous cycle and the inflammatory status of the ovary and the caecum of the mices. **Keywords:** Dysbiosis;Reproduction;Cytokine expression.

PD - 041 - **Characterization of diversity and patterns of humoral immunogenicity for HIV-1 antisense protein (ASP)**

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HIV-1 has an antisense gene overlapping *env* that encodes the ASP protein. ASP functions are still unknown, but it has been associated with gp120 in the viral envelope and membrane of infected cells, making it a potential target for immune response. Despite this, immune response patterns against ASP are poorly described and can be influenced by the high genetic variability of the env gene.To explore this, we analyzed 100k HIV-1 ASP sequences from Los Alamos HIV sequence database using phylogenetic, Shannon entropy (H_s), and logo tools to study ASP variability in worldwide and Brazilian sequences from the most prevalent HIV-1 subtypes in Brazil (B, C, and F1). Data obtained in silico guided the design and synthesis of 15-mer overlapping peptides through Spot Synthesis on a cellulose membrane. Peptide arrays were screened to assess IGG/IGM reactivity (IR) in pooled plasma samples from HIV controllers and individuals with acute or recent HIV infection. Discarding regions with low alignment accuracy, several sites with higher variability (H_s >1.5) were identified among the datasets (25 worldwide, 20 for Brazilian). Among sites with H_s<1.5, sequence logos allowed the identification of 23 other sites with subtype-specific signatures. Altogether, aa variations with frequencies >20% in the 48 variable sites identified were included in 92 peptides, divided into 15 sets, representing near full-length ASP. At immune screening, IGM and IGG IR did not significantly differ for most peptide sets. The strongest IRs were observed in 4 sets from the middle and one at the C-terminus of the protein. 3/5 of those sets presented lower IR variance intraset, indicating cross-reactivity between B/C/F1 epitopes. Our data provides a map of ASP regions preferentially targeted by IGG and IGM humoral response. Despite B/C/F1 subtype signatures in ASP, protein diversity did not negatively impact the response against regions with higher immunogenicity. **Keywords:** HIV-1 ASP;Genetic diversity;Humoral response.

PD - 042 - **Purinergic signaling in Cryptococcus neoformans infection: molecular and pharmacological studies.**

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Cryptococcosis is a systemic fungal disease usually caused by *Cryptococcus neoformans*. The infection initially occurs in the lung and can spread and infect other organs. Currently, there is no efficient treatment for late-discovered cases, requiring the search for new therapeutic targets. Purinergic signaling is a cell signaling pathway activated by extracellular nucleotides and nucleosides, which are relevant in initiating and maintaining inflammatory response against pathogens. Nevertheless, the role of purinergic signaling in cryptococcosis is unclear. Thus, this work aims to elucidate the implications of purinergic signaling in cryptococcosis. For this, female Balb/c mice (6-8 weeks) were randomly divided into three groups: control (CTRL – not submitted to any procedure), Sham and infected group (H99). Sham and H99 groups were later subdivided into two subgroups: animals treated with PBS (SHAM and H99), and those treated with Brilliant Blue G (BBG) (SHAM-BBG and H99-BBG), a P2X7 antagonist. On day 0, H99 groups were intratracheally instilled with $1x10^5$ *C. neoformans var. grubii* H99. On days -1, 2 and 5, BBG (50 mg/kg) was injected intraperitoneally. On day 7, mice were submitted to lung mechanics analysis, followed by euthanasia, for lung collection to further molecular evaluation. Compared to Sham, H99 group showed increased: lung elastance (16%), pulmonary resistance (12%), mRNA levels of the purinergic receptors P2X7 (85%), $P2Y_2$ (131%) and $P2Y_{12}$ (66%), mRNA levels of pro-inflammatory cytokines (TNF-α-200%, IL-1β-187%, and IL-6-161%), IL-10 (334%) and IL-1 β protein levels (214%). BBG treatment reduced gene expression of P2X7 receptor (-46%) and pro-inflammatory cytokines (TNF-α-54%, IL-1β-57%, and IL-6-70%) in H99 infected animals, suggesting a role of purinergic signaling in the inflammatory response during Cryptococcosis. Ongoing experiments with P2 receptor inhibitors will clarify the role of purinergic signaling in *C.neoformans* infection. **Keywords:** C;neoformans;purinergic signalization;BBG.

PD - 043 - **Citrullination impacts on the peptide coupling with the high-risk rheumatoid arthritis HLA-DRB1*04:01 molecule**

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Citrullination is a post-translational process that contributes to rheumatoid arthritis (RA) pathogenesis, modifying joint tissue proteins, eliciting the production of anti-citrullinated protein antibodies (ACPA), which have been associated with the presence of the *HLA-DRB1**04:01, *04:04, *04:05, *14:02, and *10:01 alleles. These alleles encode a shared conserved amino acid sequence (QKRAA) inside the peptide-binding groove, known as the RA shared epitope. Taken advantage of computational approaches, we evaluated the molecular interactions between the HLA-DRB1*04:01 molecule (encoded by the high-risk *04:01 allele) with citrullinated and non-citrullinated peptide-ligands derived from the synovial structure proteins (vimentin). Crystal structure of the HLA-DRB1*04:01 binding to a citrullinated peptide (SAVRL**C**SSVPGVR) was recovered from the Protein Data Bank (PDB ID: 4MCY; resolution: 2.3 Å). From citrullinated peptide we constructed the native peptide, mutating the citrulline residue to arginine using the Mutagenesis tool on PyMol v.2.4. The pHLA complexes were used to input four independent 50 ns molecular dynamic simulations (MD), using Gromacs v.2019. No significant conformational differences were observed along time for the both pHLA constructs. Root Mean Square (RMS) Deviation and RMS Fluctuations showed similar average results for both systems, with less than 1Å of difference between them. Notwithstanding, the pHLA citrullinated contruct showed higher standard deviation as compared to the native pHLA. The citrullinated pHLA exhibited more residues involved in intermolecular hydrogen bonds during MD (e.g. present in more than 50% of the simulation), which might in turn confer higher stability as compared to the native pHLA. In conclusion, the citrullination process affects pHLA complex stability and dynamics, which may impact on the immune response associated with RA development. **Keywords:** rheumatoid arthritis;HLA-DRB1*04:01;molecular dynamic simulation.

PD - 044 - **CIRCULATING MICROVESICLES OF ENDOTHELIAL CELL AND NEUTROPHIL-DERIVED AS A SCREENING TOOL OF BREAST CANCER**

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According to the World Health Organization, in 2020, 2.2 million new breast cancer (BC) cases were diagnosed in women. The search for tools that expand access and early diagnosis is important for public health policies, increasing the chances of cure. The microvesicles (MVs), vesicles released by cells and identified for carrying specific markers that allow identifying your origin, are responsible for cell communication, can transport different types of molecules, and are in large quantities in the blood. Endothelial cell and neutrophil-derived MVs (EdMVs and NeuMVs, respectively) play a role in cancer development and progression through neoangiogenesis and immune response evasion. Therefore, this study investigated the potential of EdMVs and NeuMVs as biomarkers of breast cancer and their possible use in the clinic. Blood was collected from 100 patients with breast cancer before treatment at the Mário Penna Institute, and 50 women without breast cancer were included as healthy controls. MVs were identified by immunophenotyping using flow cytometry. The profile of the MVs was evaluated according to prognostic and predictive factors, such as histological grade of the tumor and metastasis, and the performance analysis of these MVs was carried out. BC patients have more EdMVs and NeuMVs than the control group. Moreover, patients with moderately differentiated tumors and presenting metastasis before treatment had also an increased number of EdMVs and NeuMVs than the control group. These findings suggest that these MVs are involved in tumors with a more aggressive profile. Performance tests in Leave-one-out cross-validation demonstrated that EdMVs and NeuMVs can identify patients with BC with 88.5% accuracy. Due to their sensitivity and importance in tumorigenesis, these MVs may be considered BC biomarkers and an interesting tool in screening and early diagnosis campaigns. **Keywords:** Breast cancer;Microvesicles;Biomarker.

PD - 045 - **Performance of Chagas-Flow ATE serology in the clinical monitoring of Chagas disease and optimization of this methodology for the genotype-specific diagnosis of T. cruzi infection**

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Trypanosoma cruzi was subdivided in six distinct genetic groups, Discrete Typing Units (DTUs), TcI-TcVI. These DTUs can influence in the parasite biologics proprieties as well as in the clinic and treatment of the Chagas disease (ChD). In this context, the serological method by flow cytometry (Chagas-Flow ATE) was standardized for detecting anti-amastigote (AMA-A), trypomastigote (TRYPO-T) and epimastigote (EPI-E) antibodies of *T. cruzi* for the genotype-specific diagnosis of the ChD. The present study evaluated the performance of Chagas-Flow ATE for clinic monitoring of the ChD and optimizes this technique for genotype-specific diagnosis of *T. cruzi* infection. A total of 20 serum samples from CH patients with the indeterminate (n=8) and cardiac (n=12) clinical forms were tested for IgG1 reactivity to A, T and E antigens along the titration curve (1:250-1:32,000) in parallel genotype-specific platforms (TcI/TcVI/TcII). For the optimization of Chagas-Flow ATE the *T. cruzi* antigens were labeled with different fluorochromes on a single platform: ATE TcI with Violet Proliferation Dye (VPD), ATE TcII with fluorescein isothiocyanate (FITC) and ATE TcVI with Alexa Fluor 647. The results demonstrated greater reactivity of the serum samples from CH patients with the cardiac form compared to those with the indeterminate form. Moreover, the TRYPO TcII antigen, at 1:250 dilution, with 40% cut-off showed the best performance indices for clinical monitoring of the ChD. Concentrations of the fluorochromes were standardized for *T. cruzi* antigens labeling to be used in single platform in the genotype-specific diagnosis of ChD by Chagas-Flow ATE. Differential reactivity of serum samples from CH patients infected with *T. cruzi* TcI, TcVI or TcII was observed. This technique will enable in a single platform to infer which *T. cruzi* DTU would be infecting the patient and be used for clinical monitoring of ChD. **Keywords:** Chagas disease;genotype-specific diagnosis;clinical monitoring.

PD - 046 - **COMPARISON OF THE ANTIBODY REPERTOIRE OF HORSES BEFORE AND AFTER IMMUNIZATION WITH LOXOSCELES SPIDER VENOM**

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Horse polyclonal antibodies are used for disease treatment and prevention for more than a century. In Brazil, serotherapy is the primary treatment for *Loxosceles* spider bites. Although effective, serum therapy has limitations and potential risks for patients. Despite the extensive use of serum therapy, there remains a substantial knowledge gap regarding the antibody composition of immunized horses, as well as the influence of this immunization on their antibody repertoire. In this way, this work aims to gain insight into horse antibody repertoire after *Loxosceles* venom immunization. For this, we compared the heavy chain (IGH) repertoire of horse antibodies before and after immunization with *Loxosceles* spider venom using HTS technology. Unimmunized horses (UH) yielded an average of 66,141 unique IgG clones from four domestic horses, with 75% coverage. Immunized horses (IH) showed an average of 114,933 IgG clones with 80% coverage. Using clones from pre- and post-immunization repertoires, we analyzed expanded clones (EC) and compared repertoire characteristics. IGHV4-37N, IGHV4-65, and IGHV4-37 were the most prevalent IGHV segment used in the expanded clones. Size distribution analysis of the CDR-H3 region in UH, IH, and EC horses revealed similar average CDR-H3 sizes. The hydrophobicity index of CDR-H3 indicated that the EC had antibodies with a statistically lower mean (-0.4464) compared to the IH (-0.3864) ($p < 0.05$). We also compared the IgG subclasses between the three groups studied, in the case of IGHG4/IGHG7 the frequency of the EC is higher (19.8%) when compared to the UH (8.9%). In the case of IGHG3, it is the opposite, the UH group has a higher relative frequency (23.7%) when compared to the IH (11.0%) and EC (3.3 %) groups. Our analysis provides new insights into horse antibody composition, potentially aiding the rational design of horse synthetic antibodies for therapeutic use. **Keywords:** Horse;Immunoglobulin;Repertoire.

PD - 047 - **Intranasal Vaccination with LaAg against Leishmania infantum infection in Golden Hamsters (Mesocricetus auratus)**

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Leishmaniasis is a group of diseases caused by protozoa belonging to the genus *Leishmania*, which exhibit diverse clinical manifestations and have a global distribution. The disease can present itself in various forms and is classified as cutaneous (localized cutaneous, diffuse, and mucosal forms) or visceral, the latter being fatal if left untreated. The available drugs for leishmaniasis treatment are highly toxic and have several contraindications, and there is currently no approved vaccine for human use. Therefore, the development of a vaccine for the prevention of leishmaniasis is of utmost importance. Our research group investigated the potential of administering *Leishmania amazonensis* whole antigen (LaAg) intranasally as a means of conferring protection against the disease. LaAg intranasal demonstrated protection in experiments using mice C57BL/6 and BALB/c. Furthermore, oral immunization with LaAg was found to confer protection against *L. braziliensis* in golden hamsters. In this study, we aimed to assess the effectiveness of LaAg in golden hamsters against *L. infantum* infection. Golden hamsters were immunized intranasally with LaAg and received a booster dose 15 days after the initial immunization. Two weeks later, the animals were challenged with a high dose of *L. infantum* promastigotes (2×10^7) via intraperitoneal injection. The hamsters were monitored for a period of 6 months to observe any weight loss, and after that, they were euthanized for analysis of parasitic loads in the spleen, liver, and bone marrow. The results obtained indicate that intranasal immunization with LaAg, under the challenge of a high dose of *L. infantum* infection, was not able to reduce the parasite load in the hamsters. As a result, we plan to explore new experimental conditions in future studies. These may involve modifying the vaccination schedules, as well as reducing the dose of *L. infantum* infection used during the challenge phase. **Keywords:** Leishmaniasis;Vaccine;LaAg.

PD - 048 - **DEVELOPMENT OF RNA-LOADED LIPID NANOPARTICLES TO BE USED AS NEW VACCINE PLATFORMS: EVALUATION OF BIODISTRIBUTION AND IMMUNE RESPONSE IN MICE**

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Lipid nanoparticles (LNP) are effective delivery vehicles for messenger RNA (mRNA) that have shown excelent results regarding both safety and efficacy for vaccine applications. It has recently gained much attention because of their remarkable success as a delivery platform for COVID-19 vaccines. To face one of the main challenges in the development of RNA vaccines, we tested a new and versatile LNP formulation as an alternative to those already on the market and that is capable of encapsulating and deliver to cells different mRNAs. Using Firefly luciferase (FLuc) as a reporter mRNA model we evaluated *in vivo* biodistribution of our formulation. Using the dengue serotype 2 NS1 protein and the *Leishmania infantum* DTL8 antigen we evaluated the immune response in mice after intramuscular injection with the corresponding mRNA-encapsulated LNP. LNP were synthesized using a microfluidic organic-aqueous precipitation method. Dynamic light scattering was performed to determine the hydrodynamic size, polydispersity index, and zeta potential. Quant-iT™ RiboGreen™ RNA Assay kit was employed to calculate mRNA encapsulation efficiency. For all tested mRNAs, mRNA-LNPs showed a diameter around 100 nm and encapsulation efficiency higher than 85%. BALB/c mice inoculated with 10µg of FLuc mRNA-LNP via intramuscular followed by intraperiton injection of luciferase substrate showed fluorescence signals, collected by IVIS Spectrum instrument, in the injection site, as well as in the liver and spleen, which were detected up to 24 hours after administration. C57Bl/6 mice immunized with 10µg of NS1 mRNA-LNP or DTL8 mRNA-LNP per dose via intramuscular showed a high titers of total IgG antibodies against NS1 and DTL8 antigens. Altogether the results indicated that the developed mRNA-LNPs can be used as new vaccine platform, which is able stimulate the immune response against pathogens that are causative agents of various diseases. **Keywords:** vaccines;lipid nanoparticles ;mRNA.

PD - 049 - **Distinct cellular immune responses are associated with pathogenesis, disease progression, and late-relapsing hepatitis in yellow fever patients**

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Yellow fever (YF) is a hemorrhagic, infectious, febrile viral disease of great importance to public health, due to its clinical severity and high potential for dissemination in urban areas. From July/2016 to June/2018, Minas Gerais faced the largest outbreak of YF, with more than 1,000 cases and 340 deaths confirmed by the disease. There are few studies t addressing humoral and cellular immunity during human infection with the yellow fever virus. Thus, this work aimed to evaluate the cellular immunity of individuals infected by the yellow fever virus. Cellular immunity was assessed by quantification of serum chemokines, cytokines, and systemic growth factors by multiplex assay. The study population included patients with YF in the acute (Days 1 to 15 after symptoms onset: D1-15; n = 92) and convalescent (D16-315; n = 262) phases. The analysis of the soluble factors showed a massive storm of soluble mediators in acute YF. Augmented levels of soluble mediators were observed in YF patients with higher morbidity scores, patients under intensive care, and those progressing to death. On the other hand, lower levels were observed in YF patients who progressed to late-relapsing hepatitis (L-Hep) compared to those without L-Hep. A unimodal peak of biomarkers around D4-6 with a progressive decrease towards D181-315 was observed in patients without L-Hep, in contrast, a bimodal pattern, with a second peak around D61-90, was associated with L-Hep. Patients progressing to discharge presented a continuous decrease of biomarkers and a compact/integrated soluble mediator network, while those evolving to death showed a stable/increased profile and a segregated network. This study provided a comprehensive overview of cellular immunity during YF infection, highlighting that distinct cellular immune responses are associated with pathogenesis and disease progression in wild yellow fever infection. **Keywords:** yellow fever;wild-type virus;cellular immunity.

PD - 050 - **microRNAs-mRNA molecular networks potentially involved in SARS-CoV-2 infection of cardiomyocytes**

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Beyond the respiratory syndrome, patients with COVID-19 may experience cardiac complications, including viral myocarditis and arrhythmias, due to mechanisms poorly characterized. We histologically analyzed the heart tissue of patients who died from SARS-CoV-2 infection, presenting myocarditis, inflammatory infiltrate of lymphoid and myeloid cells. Hence, we conducted an integrative analysis of publicly available RNAseq datasets of SARS-CoV-2-infected cardiomyocytes. We analyzed 42 transcriptomes of cardiomyocytes (including primary human cardiomyocytes, iPSC-CMs, or hESC-CMs) infected with SARS-CoV-2 compared to 36 uninfected control samples. We characterized the expression profile of messenger RNA (mRNA) and microRNAs, a class of small noncoding RNAs that post-transcriptionally regulate gene expression with therapeutic potential in several pathologies. We identified a network of 331 potential microRNAs associated with 601 common DEGs among the datasets. Besides microRNAs related to genes encoding proteins of the extracellular matrix and coagulation, we found microRNAs linked to immune response genes, such as the miR-155-5p, a well-known modulator of the inflammatory response and interferon type I signaling pathway. To better understand the alteration caused by genes associated with extracellular matrix remodeling, we performed a fractal dimension analysis that showed the disorganization of matrix nuclear regions in the cardiac tissues of patients who died from COVID-19. We identified microRNA modulators of genes involved in cardiac muscle contraction, such as miR-29a-3p, miR-29b-3p, and miR-146b-3p. Our study profiled the miRs involved with cardiac tissue inflammation and matrix remodeling-associated miRs after tissue injury, which compromises cardiac function in patients with COVID-19. Hence, our study suggests new potential microRNA-mRNA molecular networks and pathways involved in the inflammatory state of SARS-CoV-2 infection of cardiomyocytes. **Keywords:** COVID-19;microRNAs;Myocarditis.

PD - 051 - **DEVELOPMENT OF RNA VACCINES FOR LEISHMANIASIS AND COMPARISON WITH VACCINES BASED ON RECOMBINANT ANTIGENS**

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Despite intensive efforts and studies in animal models indicating variable levels of protection achieved by immunization with defined subunit vaccines, to date, no vaccine for human leishmaniasis is available. Because, as demonstrated during COVID-19 pandemic, RNA vaccines have proven to be a technological breakthrough, we decided to develop an RNA vaccine and compare its protection level with the immune response and protection obtained with a recombinant vaccine based on the same antigen. The protein associated with kinetoplast (PAK), identified after two-dimensional gel analysis and mass spectrometry of L. amazonensis proteins using serum from mice immunized with total extract of the parasite, was selected as a target antigen. Immunization of mice with recombinant PAK, named DTL8, was able to generate a Th1 response that partially reduced the parasite load of animals after challenge with L. infantum. In vitro transcribed DTL8 RNA containing the appropriated 5' and 3' UTRs and a poly-A tail encapsulated in a lipidic nanoparticle (LNP) formulation was used to immunize C57BL/6 and BALB/c mice. In contrast to a weak antibody response in BALB/c mice, immunization of C57BL/6 mice with DTL8 RNA resulted in the induction of higher levels of antibodies compared to immunization with recombinant DTL8. To investigate whether the composition of the LNP influences antibody production, we immunized mice with the DTL8 RNA encapsulated in our LNP formulation or in a LNP formulation present in the Moderna Covid-19 RNA vaccine, but no differences in the antibody levels were observed. After challenging immunized BALB/c mice with L. infantum, only animals immunized with recombinant DTL8 were partially protected against the infection, a result that is probably due to the low antibody levels observed in this animal model. To *evaluate the protection against infection* with L. amazonensis, we are currently immunizing C57BL/6 mice with DLT8 RNA and with the recombinant antigen. **Keywords:** Leishmaniasis;RNA vaccine;LNP formulation.

PD - 052 - **Interleukin-27 Promotes Divergent Effects on HIV-1 Infection in Peripheral Blood Mononuclear Cells through BST-2/Tetherin**

TEMEROZO, J.R.; FERREIRA, P.L.C.; LACERDA, L.L.; VIEIRA, R.C.; ALVES, B.C.; GOBBO, L.; ALVES, M.R.; MENNA-BARRETO, R.F.S.; BOU-HABIB, D.C.. FIOCRUZ, FIOCRUZ RIO DE JANEIRO - RJ - BRASIL.

IL-27 has been considered a potent inhibitor of HIV-1 replication, as several reports showing that this cytokine controls HIV-1 infection in peripheral blood mononuclear cells (PBMCs), monocyte-derived macrophages, and dendritic cells. However, our present results are contrary to the current knowledge that IL-27 acts only as a downregulator of HIV-1 replication. We observed that IL-27 can either prevent or enhance viral growth in PBMCs, depending on when is added to the infected cells. We detected that the increase of HIV-1 dissemination is due to enhanced cell-to-cell transmission with the involvement of the interferon-induced HIV-1 restriction factor BST-2/Tetherin and CD11a (LFA-1), an integrin that participates in formation of virological synapse. IL-27 inhibited HIV-1 replication when added to cells 2 h after infection, promoting the prototypical BST-2/Tetherin-induced virion accumulation at cell membrane of HIV-1-infected PBMCs. BST-2/Tetherin gene expression was significantly upregulated in the IL-27-treated PBMCs, with a simultaneous increase in the number of BST-2/Tetherin+ cells, and BST-2/Tetherin silencing diminished the anti-HIV-1 effect of IL-27. In contrast, IL-27 increased HIV-1 production when added to cells 4 days after infection, which was prevented by BST-2/Tetherin gene knockdown, returning IL-27 to function again as an HIV-1 inhibitory factor. These contrasting roles of IL-27 were associated with the dynamic of viral production, since the IL-27-mediated enhancement of viral replication was prevented by antiretrovirals, as well as by keeping cells under agitation to avoid cell-to-cell contact. Likewise, inhibition of CD11a, an integrin associated with HIV-1 cell-to-cell transmission, abrogated the IL-27 enhancement of HIV-1 production. Our findings illustrate the complexity of the HIV-1-host interactions and may impact the potential therapeutic use of IL-27 and other soluble mediators that induce BST-2/Tetherin expression for HIV-1 infection. **Keywords:** IL-27;HIV-1;BST-2.

PD - 053 - **Neutrophil dialogues: vital NETosis activation as a source of danger signal molecules during L. infantum infection.**

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Neutrophils are the first immune-cell to arrive at the site of infection. In order to contain pathogen multiplication, they release neutrophil extracellular traps (NETs), nuclear or mitochondrial DNA webs decorated with antimicrobial peptides. *Leishmania infantum* have been shown as a NET activator however, there is a lack in our comprehension on NETosis activation pathways and immune mechanisms triggered by NETs in *L. infantum* infection. Ten healthy human blood donors were recruited to this study. Neutrophils were separated by Ficoll and incubated with PMA, PHA, LPS or *L. infantum* for 1h, 4h and 24h at 37°. Culture supernatant was collected for cell-free DNA and elastase activity evaluation, as well as, determination of nuclear or mitochondrial source of DNA by qPCR. Neutrophils viability was evaluated by flow cytometry. Means or median were compared by ANOVA or Mann-Whitney test, $p<0.05$ was considered as significant. After 1h of human neutrophils co-culture with chemicals or *L. infantum*, NETs were released. Although NETosis mechanisms are activated by *L. infantum* infection, infected neutrophils shows survival rates higher than neutrophils chemically stimulated, but not different from control. Furthermore, *L. infantum*-infected neutrophils show similar survival rate 4 h and 24 h post-infection accompanied by increase in cell-free DNA and elastase activity. *L. infantum*-infected neutrophils significantly released more mitochondrial DNA, while the levels of nuclear DNA were unchanged when compared to control. Additionally, mitochondrial DNA continues to be released by *L. infantum* infected neutrophils 4 h and even 24 h compared to control. Chemically activated neutrophils released similar amounts of nuclear and mitochondrial DNA. Taken together, those results indicate that neutrophils uses vital NETosis pathway to produce and secret danger signal molecules as mitochondrial DNA to communicate other cells that an infectious event is going on. **Keywords:** Neutrophils;Mitochondrial DNA;Leishmaniasis.

PD - 054 - **STUDY OF THE INHIBITORY ACTIVITY OF ORGANOSELENIUMS IN SARS-CoV-2 VIRUS REPLICATION**

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The SARS-CoV-2 is the etiological agent for COVID-19 with global dissemination. Studies have been directed towards for the development of an effective treatment against COVID-19, mainly in critically ill hospitalized patients, helping to control the emergence of new coronavirus mutations. Ebselen (EbSe) is an organoselenium compound safe for humans and has antioxidant, anti-inflammatory and antimicrobial properties. Diphenyl diselenide ($(PhSe)_2$) has similar chemical and pharmacological properties to EbSe. Herein, we compare EbSe and $(PhSe)_2$ *in vitro* anti-SARS-CoV-2 activity, as well as its predictive mechanism of interaction with M^{pro} (virus main protease) *in silico*. Calu-3 (human type II pneumocytes) cells were infected with SARS-CoV-2 (GenBank MT710714, SisGen AC58AE2) at a multiplicity of infection (MOI) of 0.01 and 0.1 for 1 hour at 37°C. After that, the culture medium was removed, and treatment (0.78-12.5 µM) was performed for 24 and 48 hours. The replicative ability of SARS-CoV-2 in supernatant of infected cultures with or without treatment was evaluated by counting the plaque-forming units (PFU/mL), and the cytotoxicity was assessed by MTT assay. The EC_{50} values for EbSe and $(PhSe)_2$ after 24 hours post infection (hpi) was 3.8 µM and 3.9 µM, respectively, and after 48 hpi were 2.6 µM and 3.4 µM. These concentrations are safe to non-infected cells, since CC_{50} found was greater than 200 µM, for both molecules. *In silico* data suggested that the antiviral mechanism of $(PhSe)_2$ against SARS-CoV-2 occurs through covalent binding to C145 residue of M^{pro}. Our results indicate that EbSe and $(PhSe)_2$ have a relevant inhibitory action against the replication of SARS-CoV-2 *in vitro*, demonstrating the functional importance of these compounds, and their scaffolds, in the development of possible therapeutic drugs for the COVID-19 treatment. At this moment, our studies are moving forward with water-soluble $(PhSe)_2$ analogues and its antiviral and anti-inflammatory properties. **Keywords:** organoselenium compounds;antiviral activity;SARS-CoV-2.

PD - 055 - **Vitamin D3 is a potential adjuvant for the LaAg mucosal vaccine against leishmaniasis.**

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Introduction: Leishmaniasis is a complex of neglected diseases that represents a significant global health problem caused by parasites of the genus Leishmania. Many people live in endemic areas worldwide; however, the available treatments are toxic to the host, and there are no vaccines for human use. *Leishmania amazonensis* is the etiological agent of diffuse cutaneous leishmaniasis in Brazil, and individuals infected with this species can develop multiple non-ulcerative lesions throughout the body. Therefore, our objective was to enhance the efficacy of a first-generation vaccine composed of total antigens from *L. amazonensis* (LaAg). **Methods:** We immunized C57BL/6 mice twice (at days 0 and 7) with LaAg or LaAg + vitamin D3 or PBS via intranasal administration. For oral administration, we used olive oil as a vehicle due to the liposolubility of vitamin D3. On day 14, the mice were challenged with 5x10^5 promastigotes of L. amazonensis on the right hind paw (intradermal), and hypersensitivity was measured weekly until the animals were euthanized at the peak or chronic phase. **Results:** Both at the peak of infection and in the chronic phase, immunization with LaAg + vit D3 demonstrated a protective effect compared to immunization with LaAg alone. When we used olive oil as the vehicle for oral administration, the LaAg vaccines lost their protective capacity in immunized and infected mice. Additionally, immunization with LaAg + vitamin D3 increased the expression of activation molecules in dendritic cells. **Conclusion:** Therefore, the use of vitamin D3 as a potential vaccine adjuvant reduces hypersensitivity (inflammation) at the site of infection and enhances the expression of activation molecules in dendritic cells. These results suggest that a vaccine formulation containing LaAg and vitamin D3, as an adjuvant, may be a promising strategy for the development of an effective vaccine against diffuse cutaneous leishmaniasis caused by *L. amazonensis*. **Keywords:** Vaccine;Leishmania amazonensis;Vitamin D3.

PD - 056 - **Presence of endogenous' mediator on muscle exudate trigged by Snake Metalloproteinase (SVMPs) and its local damage relationship**

GIMENES, S.N.C.[1]; TIOYAMA, E.[2]; COLOMBINI, M.[1]; FREITAS, L.A.S.[1]; JEONG-JIN, P.[3]; SHERMAN, N.E.[3]; FOX, J.W.[3]; MAURO, E.F.D.L.[1]; DA SILVA, A.M.M.[1]. 1. INSTITUTO BUTANTAN, INSTITUTO BUTANTAN SAO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, INSTITUTO BUTANTAN SÃO PAULO - SP - BRASIL; 3. UNIVERSITY OF VIRGINIA, UNIVERSITY OF VIRGINIA CHARLOTTESVILLE - ESTADOS UNIDOS DA AMERICA.

Introduction: It has been extensively showed that the catalytic effect of SVMPs to cleave extracellular matrix (ECM) proteins is responsible for the dramatic local damage on snakebite. Many works have investigated the pathophysiology of local damage and correlate with the reasons for lower efficiency of immunotherapy. In human snakebite, the pathophysiology of local damage can be related to release of proinflammatory molecules right after snakebite, mainly DAMPs, immunomodulators and cytokines. **Objective:** We sought to understand the pro-inflammatory effect of a P-III-SVMPs (*Batroxrhagin*), from *Bothrops atrox* venom on local site. **Methods:** We analyzed by proteomic and CBA kit, the proinflammatory protein profile and cytokine presence on exudate released after 15 minutes, 3 and 24 hours of PIII-SVMP injection in muscle mice and their capacity to stimulate migration of macrophage cells. **Results:** The proteomic analysis elucidated that the toxin has capacity to release inflammatory molecules, DAMPs and Immunomodulators, since the first time evaluated. *Batroxrhagin*, induced an early and fast pro-inflammatory effect right after the first time of toxin's injection. Going further, we identified the presence of cytokines in the exudates content, such as IL-10, TNF-α, IL-2 and a high concentration of IL-6. The identification of cytokines on mice exudate is one more evidence of SVMPs' capacity on trigger inflammatory response on the local site. Considering the presence of all inflammatory molecules in muscle's exudate, we investigated their chemotaxis' ability using macrophage suspension by Transwell assay. SVMP-PIII exudate was able to induce a highest cell migration when compared to negative (medium) and positive (LPS) control. According to the relevance of current snakebite scenario, our results broaden the understanding of the processes involved in the local effects of human envenoming by *Bothrops spp.,* and brought a better understanding of local lesion reactions. **Keywords:** Toxinology;Local damage;Inflammation.

PD - 057 - **EFFECTS OF DIETARY INTERVETIONS OVER THE LIVER IMMUNE SYSTEM**

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Introduction: Caloric restriction is profoundly linked to physiological modulation of the immune system. Recently, dietary interventions have been appreciated as beneficial for health, as support therapy for many diseases. Whereas mammals evolved to adapt to periodic food shortage, the impact of nutritional scarcity over immunity is poorly understood. The aim of this project is to assess the effects of time restricted feeding (TRF) over the immune system. **Methods and results**: To characterize the overall distribution of leukocytes in homeostasis versus TRF, C57BL/6J mice were kept under *ad libitum* and 12h TRF (namely day fasting or night fasting) regimen for 5 weeks. Leukocytes were isolated from several tissues for immunophenotyping by multiparametric spectral flow cytometry. Our results indicate that most leukocytes display circadian fluctuations over the 24h of the day. However, the liver, displays a unique fluctuation of cells that coincides with the expected feeding time. Night-fasted mice display abnormal fluctuation of total $CD45^+$ cells in the liver. Strikingly, fasting acts as a signal that triggers activation and accumulation of invariant natural killer T (iNKT) cells in the liver. iNKT cells are critical components of immune surveillance and regulation in the liver. Moreover, these cells are important for the maintenance of metabolic homeostasis. Further results suggest that fast-dependent hepatic iNKT accumulation is microbiota-independent. Collectively, our results suggest that the liver is exceptionally affected by TRF, which leads accumulation of iNKT cells. For a more comprehensive understanding of the mechanisms involved in this phenomenon, experiments are currently underway. Understanding how TRF modulates immunity has the potential to form the basis of new approaches and treatments to manage human pathologies. **Support:** Presidential Postdoctoral Award - Fred Hutchinson Cancer Center. **Keywords:** Invariant Natural Killer T cells;Time restricted feeding;Circadian rhythm.

PD - 058 - **Participation of NETs as a biomarker of autoimmune rheumatic diseases**

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Neutrophil extracellular traps (NETs) in rheumatoid arthritis (RA), is characterized by damage to joint cartilage and bones, with increased infiltration of neutrophils into the synovial cartilage and these are more likely to develop NETs when compared to patients without RA. Increased occurrence of neutrophils expressing the Programmed Cell Death Ligant 1 (PD-L1) were associated with RA progression. In this way, we investigate the NETs and disease progression in a sample of healthy patients without RA, with untreated RA; we also investigate, in an experimental model, the role of PD-L1^{+} neutrophils and their possible relationship with increase in NETs. Initially, we observed the occurrence of NETs in plasma samples from RA patients (RA) (n=30) and from healthy people without a positive diagnosis for RA (Control) (n=15) by measuring DNA free cell conjugated with myeloperoxidase by spectrophotometry and it was possible to observe the increase in the occurrence of NETs in the RA group. In vitro, murine neutrophils were maintained in culture for 24 hours, in the presence of IFN-γ to increase PD-L1 expression, subsequently; these neutrophils were stimulated or not with LPS to induce NETs and the supernatant was collected after 4 and 8 hours of stimulus, for measurement of NETs and cytokines. We observed the increase of NETs in neutrophils cultured with IFN-γ after 8 hours of stimulation with LPS. IL-1ß concentration was higher in the supernatant of neutrophils maintained in culture with INF-γ to 8 hours. TNF-a concentration was higher in neutrophils supernatant maintained in culture with INF-γ and stimulated with LPS for both 4 and 8 hours. These results show that PD-L1 expression by IFN-γ induction in neutrophils, can result in increase of NETs and regulation of cytokine secretion. This experiment will provide knowledge about possible susceptibility markers for RA, facilitating the diagnosis and treatment of the disease.**Keywords:** Inflammation;autoimmunity;neutrophils.

PD - 059 - **Multiplex assay for the assessment of IgG antibodies in the immunodiagnostic of Canine Visceral Leishmaniasis**

KER, H.G.; FONSECA, J.D.M.; BARCELOS, A.C.A.; RODRIGUES, M.J.; PAIVA, B.C.; SILVA, C.F.; RODRIGUES, F.B.; ROATT, B.M.; REIS, A.B.; SOARES, R.D.D.O.A.. UFOP, UFOP OURO PRETO - MG - BRASIL.

As an attempt to develop more accurate immunodiagnostic assays, flow cytometry is a worldwide trend in the development of high-throughput serodiagnostic tools. This approach allows the discrimination of different particles on the basis of size and fluorescence, and based on these parameters, multiplexing is the process of simultaneously detecting or identifying multiple biomarkers in a single diagnostic test. In this work, it was aimed to coupling potential recombinant antigens to CBA polystyrene beads in order to develop a multiplex diagnosis for Canine Visceral Leishmaniasis (CVL). Certify the functionality of the conjugation of recombinant proteins to CBA functional beads and assess the accuracy in the diagnosis of CVL. The antigens rLci1A and rLci2B were respectively coupled to the CBA beads A4 and E4. Their functionalization and diagnostic accuracy were assessed individually and jointly (multiplexed) in a broad range of canine serum samples, which included dogs infected with *L. infantum* presenting different clinical forms (asymptomatic, oligosymptomatic and symptomatic), dogs infected with other canine pathogens (*L. braziliensis, Ehrlichia canis* and *Babesia canis*), and dogs vaccinated against CVL (Leishmune®, Leish-Tec® and LBSap). The multiplexed immunodiagnostic with A4−rLci1A and E4−rLci2B showed increased diagnostic performance in comparison with the use of each antigenic matrix independently. It presented sensitivity of 85.0% in asymptomatic dogs and 100.0% in the oligosymptomatic and symptomatic. Its specificity was 100.0% in dogs infected with *L. braziliensis*, 70.0% in *E. canis*, and 60.0% concerning *B. canis*. The specificity was also 100.0% in CVL-vaccinated dogs. The multiplex diagnosis showed high accuracy. It was marked by high sensitivity regardless the dog's clinical status and with substantial specificity to avoid cross-reactivity in dogs with other canine pathogens and especially in CVL-vaccinated ones. **Keywords:** Visceral Leishmaniasis;Immunodiagnosis;Multiplex.

PD - 060 - **Role of the IL-1 signaling pathway in the necrotic lesion development induced by SMases D from Loxosceles spider venom**

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Sphingomyelinase D (SMase D), the main toxic component of *Loxosceles* venom, has a well-documented role in the dermonecrotic lesion caused by the envenomation with these species, however, the intracellular mechanisms involved in this event are still poorly known. Through differential transcriptomics of human keratinocytes treated with *L. laeta* or *L. intermedia* SMase D, we identified 323 DEGs common to both treatments, as well as upregulation of molecules involved in the IL-1 signaling. Since this signaling pathway is associated with the activation of the inflammatory process, which could contribute to necrotic lesion development, we investigated the relative expression of some molecules related to this pathway by RT-qPCR after 2 h, 12 h, and 24 h of keratinocytes treatment with the SMases D from *L. laeta* or *L. intermedia*. We observed higher relative expression of IL-1α, CXCL8 and IL-6 only after 2 hours of *L. intermedia* treatment, while with 12 hours, higher relative expression of IL-36γ, IL-1α, IL-1β, CXCL8 and IL-6 was observed after *L. laeta* SMase D treatment. On the other hand, at 24 h, both SMases D induced similar modulation of IL-1 signaling in keratinocytes, showing high expression of ligands and receptors belonging to this pathway, as also lower relative expression of MMP-3. Positive expression correlations of the molecules involved in the IL-1 signaling were just observed after SMases D treatment, but not in the untreated control, confirming the SMase D inflammatory action through IL-1 signaling on the keratinocytes. Herein, we describe for the first time, a cell pathway related to the exacerbation of the inflammatory process, highlighting the contribution of the IL-1 signaling in the development of the dermonecrotic lesion induced by SMases D from *Loxosceles* spider venom, pointing out key molecules as potential new therapeutic targets to control the development of cutaneous loxoscelism. **Keywords:** Cutaneous loxoscelism;Inflammation;IL-1 signaling.

PD - 061 - **Evaluation of the immune profile of chronic Chagas disease patients treated with benznidazole: employing immunoregulatory networks as markers of therapeutic success.**

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Chagas disease (CD), caused by infection with *Trypanosoma cruzi*, leads to chronic Chagas cardiomyopathy, one of the deadliest and most debilitating cardiopathies known. While early treatment is crucial to prevent progression, detection of acute disease is challenging. Some studies have suggested that treatment of chronic Chagas patients is beneficial. However, there are no reliable markers of therapeutic efficacy. The aim of this study was to evaluate the circulating immune profile of chronic CD patients treated with benznidazole (BZ). We collected plasma from 40 patients before and 6 months after treatment and performed: 1- analysis of soluble factors including cytokines, chemokines, and growth factors using Bio-48 Plex Human Cytokine Screening Panel kit; 2- PCR, to detect parasite DNA, and 3- measurement of BZ using mass spectrometry. We found detectable BZ levels in all samples, and 62% had negative PCR results after treatment (PCR-), suggesting parasitological cure. PCR- patients showed increased levels of inflammatory cytokines such as TNF-α, IL-18, and MIF, and decreased levels of the modulatory cytokines IL-10, IL-13, and IL-7. The TNF-α/IL10 ratio also increased, indicating an inflammatory profile post-treatment. We observed increased levels of G-CSF, CTACK, CCL3, and CCL4. Although the combined analysis of these factors did not segregate the "before" and "after" treatment groups, ROC curves suggested that these molecules may be potential markers of efficacy. In addition, using machine learning and multivariate analysis, we found that the segregation of all variables into 3 distinct components allowed to strongly associate PCR- patients to one of the components, with the following variables: IL-16, CCL-2, PDGF, IFNgamma, CXCL-10, IL-1RA and IL-18. These results highlight the potential of immunoregulatory networks as indicators of therapeutic success, and points to immunological mechanisms in chronic CD patients treated with BZ. INCT-DT, FAPEMIG, CNPq, NIH. **Keywords:** Chagas Disease;Bz Treatment;Immunology response.

PD - 062 - **The phenotype of Treg cells that limit immunopathology in dengue and covid19**

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Regulatory T cells (Tregs) are an important control mechanism of the inflammatory response. The suppression of the immune response mediated by Tregs can prevent immunopathology and even death in a variety of conditions. We investigated the presence of Tregs (CD4+CD25+CD127lowFOXP3+) in patients with dengue or covid19 and evaluated their fitness according to the disease presentation. PBMC from mild dengue and dengue with warning signs or severe dengue (ws+/severe), were used to study the phenotype of Tregs. Mild dengue showed higher levels of CD200+ Tregs, during defervescence, and the presence of DENV-specific GITR+IL10+Tregs, during convalescence, which were absent in ws+/severe patients. Also, we found evidence that these are natural Tregs (Helios+). Importantly, IL-10 production by Tregs is greater than those presented by Tr1 cells. These findings associate a better fitness of Tregs with the mild evolution of dengue. To evaluate if these findings were consistent in another viral infection, critical covid19 patients under mechanical ventilation were recruited, PBMC and lung cells were collected during the first 24 hours of intubation and after 7 days. We observed that non-survivors presented lower numbers of Tregs in the blood when compared to survivor patients. In addition, lung Tregs of non-survivors also displayed higher PD1 and lower FOXP3 expressions suggesting a dysfunctional phenotype. Further signs of Treg dysregulation were observed in non-survivors such as limited production of IL-10 in the lungs and higher production of IL-17A in the blood and in the lungs, which were associated with increased PD1 expression. These findings were also associated with lower pulmonary levels of Treg-stimulating factors like TNF and IL-2. Tregs in blood and lungs are profoundly dysfunctional in non-surviving covid19 patients. Our studies show that the presence of functional Treg cells is important to limit the immunopathology found in diseases such as dengue and covid19. **Keywords:** regulatory T cells;dengue;covid19.

PD - 063 - **Serum cytokine levels can predict whether the individual will develop symptomatic or asymptomatic COVID19**

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COVID-19, caused by the novel coronavirus SARS-CoV-2, has emerged as a global pandemic with far-reaching impacts on public health, economies, and societies worldwide. Several known pre-existing factors, such as advanced age, chronic respiratory diseases and cardiovascular conditions, have been identified as contributing to the increased severity of COVID-19, necessitating focused attention on vulnerable populations for improved risk assessment and patient management strategies. Although it is already known that high levels of some cytokines, such as IL-6 and TNF-a, identified during the disease correlate with the severity of the disease, little is known about whether the levels of some cytokines can predict whether the individual will develop the symptomatic or asymptomatic form of the disease. This study evaluated the serum concentration of cytokines in 66 Fiocruz workers before they were infected. They were followed up and, after infection, were divided into groups that developed asymptomatic (44) or asymptomatic (22) COVID-19. Asymptomatic group showed a correlation matrix with different pattern of correlated cytokines than symptomatic group. Some correlations change from positive to negative correlated and vice-versa. Suggesting that the relationships between some cytokines, before infection, can predict the severity of the disease developed. We hope to evaluate the correlations between the studied cytokines using machine learning to be able to predict whether the individual would develop the asymptomatic or symptomatic form of the disease. **Keywords:** COVID19;Cytokine;Severity Prediction.

PD - 064 - **Mycobacterium tuberculosis infection increases the expression of iron transporters in activated and Th1 lymphocytes.**

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Tuberculosis (TB) is one of the diseases caused by a single infectious agent that kills most people in the world. A better understanding of the host response mechanisms that promote protection against *Mycobacterium tuberculosis* (Mtb) infection may help in the development of more effective strategies to cure TB, since there is no effective vaccine for its prevention in adults and its treatment is lengthy with several side effects. Iron plays an important role in both pathogen and host metabolism. In addition, alterations in the intracellular levels of this metal can impact antimicrobial effector pathways in infected cells and play regulatory roles in T lymphocyte differentiation. T cells play a critical role in host resistance against Mtb infection, however, the role played by iron metabolism in the modulation of T cell function in TB is unknown. In order to assess this, we initially infected C57BL/6 mice with H37Rv strain Mtb and evaluated the expression of CD71, DMT1 and ferroportin (the main iron importers and exporter, respectively) in pulmonary $CD4^+$ T lymphocytes by flow cytometry. We found that the expression of all transporters was increased in $CD4^+$ T cells in response to Mtb infection, particularly in $CD44^+$ and in $Tbet^+$ $CD4^+$ T lymphocytes at 4 weeks post-infection, indicating that they are upregulated in Th1 activated lymphocytes. To assess the role of ferroportin in T cells during TB, we infected mice deficient for ferroportin in T cells and assessed their susceptibility to infection in comparison to wild type animals. Our preliminary results indicated that ferroportin deficiency in T cells does not interfere in host resistance to TB. Altogether, our results suggest that iron homeostasis in $CD4^+$ T cells might be impacted by changes in iron transporter expression during Mtb infection, which may play a role in T cell function. However, there seems to be no role for ferroportin in the modulation of T cell responses and /or resistance to TB. **Keywords:** Tuberculosis ;Lymphocytes;Iron metabolism.

PD - 065 - **INCREASED EXPRESSION OF GALR2 IN ORAL SQUAMOUS CELL CARCINOMA (OSCC) DOES NOT AFFECT THE PHENOTYPE OF TUMOR-ASSOCIATED MACROPHAGES**

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Macrophages are antigen-presenting cells important in the activation of adaptive immunity and prototypical phagocytosing/efferocytotic cells. GALR2 may function as an oncogene in OSCC by increasing tumor cell proliferation, survival and by promoting perineural invasion. Increased macrophage infiltration is associated with poor prognosis in various solid tumors, including OSCC, the sixth most common malignancy and a major cause of cancer morbidity and mortality. This study evaluated the infiltration of myeloid cells and macrophage phenotype in an *in vivo* model of OSCC with and without GALR2 overexpression. A xenograft orthotopic model of OSCC was induced in 20 athymic nude Balb/c mice by injecting $5x10^5$ SCC9 cells directly into the floor of the mouth. We used control/empty-vector-transfected (pcDNA, n=10) or GALR2-overexpressing (GR2, n=10) SCC9 cells. Two weeks after tumor induction (when 20% of the initial body weight was lost) animals were euthanized, and tumors were collected, dissected, weighted, and measured. Tumor dissociation was performed using a tumor dissociation kit (Miltenyi Biotec). Flow cytometry analysis was used to study the infiltration of myeloid cells and the phenotype of tumor-associated macrophages (TAMs). Cells were stained with antibodies for CD45, CD11b, F4/80, CD80, and MHCII. There were no significant differences in either tumor weight or size between groups. Compared to SCC9 pcDNA, tumors overexpressing GALR2 (SCC9 GALR2) showed a reduced infiltration of CD45+CD11b+ myeloid cells, albeit no differences in the overall infiltration of CD45+ cells. Infiltration of macrophages (F4/80+ cells) was similar in pcDNA and GALR2 tumors, and MHCII+ macrophages were predominant over CD80+ cells. Overexpression of GALR2 was associated with a reduced infiltration of OSCC by myeloid cells, but did not affect the predominant M2-like phenotype of TAMs. **Keywords:** macrophage phenotype ;myeloid cells ;GALR2 overexpression.

PD - 066 - **Explore Cytometry: A social media platform for scientific dissemination of flow cytometry**

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The lack of professionals in cytometry does not meet the demand of students and professionals who need the technique to develop their work, making Explore Cytometry (EC) a great tool for disseminate the technique and attracting young scientists for their qualification. After a year and a half of the launch of EC, we analyzed the engagement metrics of social medias and asked users to answer a questionnaire aimed at: 1. Knowing better our audience, 2. Mapping our strengths and areas for improvement, 3. Mapping the demands of our public and 4. Assessing the effectiveness of EC in providing user qualification. Our questionnaire was created using an adaptation of the Kirkpatrick evaluation method and divided into Audience profile, Reaction, Learning and Behavior. Audience profile: 106 responses were obtained, representing 21.2% of our audience, 79.3% are between 25-44 years old, 73.6% are female. and 93.4% are residents of Brazil. Most of them are biomedical and biologists (63.6%), scholarship holders (64.2%) and highly qualified (28.3% master's degree, 9.4% doctorate and 26.4 postdoctoral. Reaction: 71.7% said EC delivered relevant content and 75.5% stated EC delivered content they were unaware of. In addition, 98.1% consider the contents to be of high/very high quality. Learning: 48.1% said they had used the knowledge acquired through EC in their work routine and 47.2% declared having reproduced some content learned through EC. Behavior: 91.5% were more confident about their mastery of the technique after following EC and 86.8% had a positive feeling after accessing EC due to the didactic way in which we approached the topic, demystifying the technique and making the experience of following the page positive and motivating. The engagement on Instagram™, our main social media, often exceeds 20%, which is ~4x higher than the average on total Instagram. EC proved to be a powerful tool to attract young people and to generate effective learning in the technique. **Keywords:** Social media;flow cytometry;Scientific dissemination.

PD - 067 - **Purinergic signaling in the development of diet-induced liver cirrhosis**

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Objectives: Chronic hepatic diseases (CHD) represent a serious public health problem worldwide. Among the CHD, cirrhosis has a high prevalence and high mortality. Liver purinergic signaling regulates primary cell functions, such as lipid metabolism and protein synthesis. In addition, this signaling is involved in inflammatory response, cell death, and immunity. P2X and P2Y purinergic receptors were identified in the liver, including P2X7 and P2X4 receptors. Here, we investigated the contribution of purinergic signaling to the development of chronic liver disease. **Methods and results:** Wild-type (WT) and P2X7-deficient ($P2X7^{-/-}$) C57BL6 mice were fed with a modified diet deficient in methionine and choline (DMC) for 6 weeks for the development of liver cirrhosis. Our results showed a significant reduction in body weight in animals from all groups submitted to DMC diet. In the histological analysis, an increase of fat droplets in the hepatic tissue was observed in both groups fed with the modified diet. The cholesterol and triglyceride levels increased in DMC WT and $P2X7^{-/-}$ mice compared to control groups ($p<0.01$). Our qPCR analysis showed that DMC diet increases CD39 mRNA levels compared to animals under a normal diet ($p<0.001$). DMC diet increased TNF-α mRNA levels in both groups submitted to the modified diet ($p<0.01$), while IL1β mRNA levels increased only in DMC $P2X7^{-/-}$ mice ($p<0.01$). In the enzyme-linked immunosorbent assay (ELISA), our results showed that IL1β levels were higher only in WT DMC group compared to the control group. TNF-α levels increased only in DMC $P2X7^{-/-}$ animals. **Conclusion:** Our results suggest a reduced participation of P2X7 receptor in the pathophysiology of liver cirrhosis. Ongoing experiments will help better understand the contribution of purinergic signaling to liver cirrhosis and identify possible therapeutic targets involving the action of receptors and membrane pores related to the purinergic system.Financial support: FAPERJ, CNPq. **Keywords:** Immunology;Purinergic;P2X7.

PD - 068 - **Unveiling Inulin Diet Impact on Pathway Modulation in the Intestinal Epithelium through Enrichment Analysis of Single-Cell Transcriptomics Data**

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The intestinal epithelium (IE) is a crucial interface that protects the body from pathogens and toxins while aiding in nutrient absorption. The IE has complex signaling networks with the immune system, gut microbiota, and diet, which we are just starting to understand. Dietary fibers like inulin play a significant role in shaping the microbial community, influencing the production of metabolites, and the intestinal epithelial barrier function. Here, we used single-cell transcriptomics to explore the diverse gene expression patterns within the colonic epithelium of C57BL/6 mice following a 30-day inulin diet. This analysis revealed distinct subpopulations, including stem cells (SC), cycling transit-amplifying cells (TA), goblet cells (GC), and enterocytes (EC). Specific cell-type differential expression analysis was conducted with MAST, implemented by the Seurat framework, with an adjusted p-value threshold of $p < 0.05$. Enrichment analysis of regulated genes in each subpopulation was conducted with g:profiler and fgsea R package. Despite no change in the abundance of SC and TA subpopulations in inulin-treated mice, these cells exhibited positive modulation of several genes that were associated with chromatin organization, suggesting a potential epigenetic regulation of proliferation after inulin treatment. EC displayed positive modulation of organic acids and lipids metabolism, while GC showed positive modulation of vesicle-mediated transport and lipids biosynthesis. Furthermore, all four subpopulations showed negative modulation of biological processes related to oxidative phosphorylation or aerobic respiration, suggesting that inulin exacerbates the hypoxic microenvironment in the colon. Overall, our study demonstrates the transcriptional specialization of the colonic epithelial subpopulations in response to the inulin diet. **Keywords:** single-cell transcriptomics;colon epithelium;inulin.

PD - 069 - **The Role of NINJ1 in the restriction of intracellular bacteria**

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Legionella pneumophila is a flagellated bacterium that causes Legionnaires' disease. Evolved by replicating within unicellular protozoa in freshwater environments, in the absence of interaction with mammalian hosts. For this reason, ***L. pneumophila*** is an appropriate model of infection for studying innate immune pathways and finding new therapeutic targets. In mammals, upon the infection of macrophages, cytosolic flagellin triggers the activation of NLRC4 inflammasomes, which culminates in the restriction of ***Legionella*** replication. This pathway is characterized by Casp1 activation, leading to pore formation and cell death by pyroptosis. Some works have linked this cell death as the effector mechanism in intracellular bacteria control. It was found that NINJ1 is essential for plasma membrane rupture in lytic types of cell death, however it was never studied the importance of this molecule in the restriction of bacteria. The aim of this work is to understand the importance of NINJ1 in the control of ***L. pneumophila*** infection. Here we used littermate control mice ***Ninj1*$^{+/+}$**, ***Ninj1*$^{+/-}$** and ***Ninj1*$^{-/-}$** to access the effect of this molecule in response to bacterial infection. We observed a defect in the release of LDH in ***Ninj1*$^{+/-}$** and ***Ninj1*$^{-/-}$** BMDMs, treated with Nigericin or infected with ***L. pneumophila***. However, the detection of LDH in ***Ninj1*** deficient cells was higher than in non-infected or ***Asc/Casp1/11*$^{-/-}$** BMDMs, indicating that membrane rupture still occurs in the absence of NINJ1. Additionally, there was a slight reduction in the secretion of IL-1β in the cells infected with WT and flagellin-deficient bacteria. Interestingly, for the first time we found that in the absence of NINJ1, BMDMs can control normally the ***Legionella*** infection. Collectively, these data demonstrate that bacterial control occurs independently of cell membrane rupture, supporting the idea that and alternative process independent of cell lysis operates for restriction of bacteria restriction in macrophages. **Keywords:** Pyroptosis;Ninj1;Legionella pneumophila.

PD - 070 - **LACTOCOCCUS LACTIS NCDO 2118 IMPROVES ORAL TOLERANCE INDUCTION BY MICROBIOTA MODULATION AND LACTATE PRODUCTION**

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Lactococcus lactis subsp. *lactis* NCDO2118 has been shown to present anti-inflammatory properties in mouse models of diseases. Herein, we investigated whether this strain can improve Oral Tolerance (OT) induction to ovalbumin (OVA) in mice and function as a tolerogenic bacteria. The aim of the present study was to evaluate the effect of *L. lactis* administration associated with OVA, in an OT model to investigate its putative “tolerogenic adjuvant effect”. A solution containing bacteria was orally administered through drinking bottles with water for four consecutive days. At day 4, mice received 5 mg OVA by gavage. After 3 days, mice were immunized i.p with OVA + Al(OH)3. Fourteen days later, mice were i.p injected with soluble OVA as a booster. After a week, we evaluated serum levels of anti-OVA IgG1 and IgE, as well as the frequency of innate and T immune cells involved in OT. Tolerogenic dendritic cells, resident macrophage $Cx3CR1^{+}$ and regulatory T cells in mesenteric lymph node (MLN) and lamina propria (LP) were increased after the treatment. Adressing the mechanisms involved, we observed that expression of GPR81 gene (that codes a receptor for lactacte) was higher in gut tissues of *L. lactis* treated mice and administration of GPR81 antagonist abolished the adjuvant effect of *L.lactis* in OT. The microbiota was modulated by *L.lactis* administration, mostly with increase of Akkermansia genera, and short chain fat acids concentrations were modified with emphasis on lactate and propionate that were increased after the treatment. We conclude that *Lactococcus lactis* subsp. *lactis* NCDO2118 has an important adjuvant effect which improves OT and this effect was dependent on binding of GPR81 to lactacte produced by *L.lactis* NCDO2118 and bacteria of gut microbiota that was modulated by its administration. **Keywords:** Oral Tolerance;Lactococcus lactis;GPR81.

PD - 071 - **Therapeutic relevance of pro-resoluting Angiotensin-(1-7) in the model of pneumococcal pneumonia**

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Streptococcus pneumoniae is a major cause of community-acquired pneumonia, leading to high mortality rates. Inflammation caused by pneumococcal infection is necessary for elimination, but must be regulated to prevent further tissue damage and bacterial spread that can lead to sepsis. Angiotensin-(1-7) [Ang-(1-7)] is a pro-resolution mediator that acts on its Mas receptor (MasR) to promote resolution of inflammation. We investigated the effects of Ang-(1-7) and the role of MasR in the context of pneumococcal infection and evaluated lung inflammation, bacterial counts, phagocytosis and lung damage. It was possible to observe that therapeutic treatment with Ang-(1-7) decreased neutrophil recruitment, lung injury, bacterial load in the lung and sepsis. Ang-(1-7) induced phagocytosis of s. pneumoniae and increased expression of lung epithelial barrier genes for bacterial control. In addition, Ang-(1-7) prevented many more deaths when associated with antibiotics after lethal pneumococcal infection. There is no difference in lethality between MasR-deficient mice (MasR-/-) and wild-type (WT) mice after pneumococcal infection. Mediator of resolution of inflammation, such as Ang-(1-7), is a good candidate for the treatment of pneumococcal infections and sepsis caused by *S. pneumoniae*. **Keywords:** infection;Streptococcus pneumoniae;Angiotensin-(1-7).

PD - 072 - **MerTk receptors modulate immune cell populations in the lungs and gut during pneumococcal pneumonia**

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Respiratory infections are among the leading causes of death worldwide. *Streptococcus pneumoniae* has long been one of the most prominent bacterial causes of disease in humans. The inflammatory response against *S. pneumonia* is driven by complex interactions between the infecting pathogen and host immune cells and induces high rates of dead cells. TAM-receptor-mediated efferocytosis, besides clearing dead cells, also inhibits pro-inflammatory pathways through Gas6 or Protein S binding to phosphatidylserine on apoptotic cells by phagocytes. Emerging literature has demonstrated that acute respiratory infections can also impact the gut microbiome, and this lung-gut axis of crosstalk may be a contributing factor in disease worsening. Here we investigated how MerTk regulates the gut-lung axis during *S. pneumoniae* infection. By flow cytometry, we found an increase in the numbers of interstitial macrophages (IMs) and neutrophils and a decrease in the number of monocytes in the lungs of infected MerTk KO mice compared to WT mice. In addition, we observed that infected MerTk KO mice had an increase in a non-classical alveolar macrophages (AMs) population that expresses low levels of SiglecF and CD11c in their BALFs. Interestingly, lung *S. pneumoniae* infection induced a decrease in the numbers of colonic macrophages (cMPs) in the gut of MerTk KO mice compared to WT mice. Together, our findings suggest that the MerTk receptor can control immune cell recruitment and proliferation in the colon as well as in the lungs, emphasizing the significance of taking the lung-gut axis into account while treating pneumonia. **Keywords:** Efferocytosis;pneumonia;TAM receptors.

PD - 073 - **Dietary protein modulates intestinal dendritic and CD4+ T cells transcriptomic landscape in a microbiota-independent manner**

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Dietary proteins are taken up by intestinal dendritic cells (DC), cleaved into peptides, and presented to T cells to generate an immune response. Amino acid (AA)-diets do not have the same effects because AA cannot bind to MHC to activate T cells. Previously, we found that intestinal DC transcriptome is modulated by dietary antigens. However, the microbiota also plays an important role in intestinal mucosal immune regulation. To address this question, we investigated whether the microbiome affects the intestinal DC transcriptomic changes we observed dependent on dietary proteins. Thus, we first treated four-week-old AA and control fed mice with a mixture of antibiotics consisting of vancomycin, ampicillin, neomycin and metronidazole for 4 consecutives days in the drinking water; one day later the microbiome from AA and control diet fed mice were exchanged. Mice were gavaged with a suspension containing cecal contents once a week for 4 consecutive weeks and euthanized one week after the last gavage. We then sequenced the RNA of sorted DCs, CD4+ T and epithelial cells from the upper small intestine and investigated changes in their transcriptome that could be modulated by the microbiome. The analysis demonstrates that intestinal EC transcriptomic changes are influenced by the microbiome whereas transcriptomic changes in DCs and CD4+ T cells are primarily influenced by the lack of protein in the diet. Our findings highlight the importance of dietary proteins for intestinal DC function and mucosal tolerance. **Keywords:** Dietary proteins;Dendritic cells;mucosal immune tolerance.

PD - 074 - **Akkermansia muciniphila induces tolerogenic dendritic cells and CD4+Foxp3+, conferring protection against type 1 diabetes**

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Type 1 diabetes (T1D) is an autoimmune disease with destruction of insulin-producing pancreatic β-cells. Reduction on *Akkermansia muciniphila* has been related in the development of T1D in human and murine models. However, its supplementation effect and action mechanism in this autoimmune disease has not been elucidated. In this context, we investigated whether *A. muciniphila* administration can ameliorate streptozotocin (STZ)-induced T1D. For this propose, the T1D was induced *in vivo* by inoculating 1 daily dose of STZ 40mg/kg for 5 consecutive days, and the viable *A. muciniphila* was administered every other day from the one day before the first dose of STZ until day 15 in C57BL/6 male mice (CEUA 169/2020). Euthanasia was performed 15 days after the first dose of STZ. Our data showed that *in vivo* administration of the *A. muciniphila* was able to control glycemia levels, reduced the incidence of T1D and degree of insulitis in mice with STZ-induced T1D. The modulation of T1D, in mice that ingested the probiotic, was related to the accumulating of $CD11b^{+}CD103^{+}$ dendritic cells (cDC2) in the pancreatic lymph nodes (PLN). We also detected an increased gene expression of Sirpa and Aldh1a2, markers of tolerogenic cDC2, in the colon and pancreas from STZ-Akk group. This group also showed an increase in regulatory T cell (Treg cells) in the PLN and in anti-inflammatory cytokines, like IL10 and TGFb, in the pancreas, when compared those diabetic and non-supplemented mice. Then, we confirmed *in vitro* that *A. muciniphila* is efficient in inducing the differentiation of bone marrow-derived dendritic cells (BMDC with a tolerogenic profile ($CD103^{+}CD11b^{+}PDL\text{-}1^{+}$). In addition, BMDC differentiated in the presence of *A. muciniphila* promoted the Treg differentiation *in vitro*. Thus, the *A. muciniphila* confers immunomodulatory properties able of attenuating T1D in mice, making this probiotic a promising target as new therapeutic tools against this disease. **Keywords:** Akkermansia muciniphila;type 1 diabetes;tolerogenic dendritic cell .

PD - 075 - **Senescent cytotoxic CD4+ T cells cause skin pathology in human cutaneous leishmaniasis**

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Cytotoxic activity constitutes a hallmark contributing to immunopathogenesis in human cutaneous leishmaniasis (CL). In this study, we demonstrated that $CD4^{+}$T cells expressing granzyme B and CD107a accumulate in lesions of CL patients. Furthermore, these cells showed an enhanced expression of activating NK receptors (NKG2D and NKG2C), whereas their ligands (MICAB and HLA-E) were upregulated in lesional macrophages and fibroblasts. Notably, CD4+T cells freshly isolated from the lesional site demonstrated a remarkable capability to kill K562 target cells (lack MHC class I and II), supporting that lesioned environment efficiently activated these cells to exert an MHC class-independent mechanism of cell killing. Phenotypic analyses revealed that lesional $CD4^{+}$T cells encompass both resident and migrating populations, with the predominance of effector ($CD27^{-}CD45RA^{-}$) and highly differentiated-TEMRA ($CD27^{-}CD45RA^{+}$) subsets. Interestingly, the EMRA $CD4^{+}$T compartment exhibited higher expression of granzyme B and CD107a, indicating that they represent the main cytotoxic population in CL lesions. Additionally, immunofluorescence analysis unveilled that cytotoxic-CD4+T cells from lesion express senescence associated markers, p16+ and CD57+, which positively correlated with the lesion size. In order to reproduce the lesional inflammatory milieu, we co-cultured autologous fibroblast with CD4+ T cells in in the presence of the top 5 cytokines expressed in the lesion (IL1-b, IL-18, IL-8, IL-6 and IL-15). Our findings demonstrate that in the presence of these cytokines, $CD4^{+}$T cells were capable of efficiently lysing fibroblasts in vitro. Importantly, the cytotoxicity of $CD4^{+}$T cells was found to be dependent on NKG2D expression, as it could be abrogated by blocking this receptor. Collectively our results provide the first evidence that senescent cytotoxic CD4+ T cells may participate in the skin pathology of human cutaneous leishmaniasis. **Keywords:** Immunosenescence;Immunophatology;cutaneous leishmaniasis.

PD - 076 - **IRF5 promotes Il12b transcription and controls primary infection with Toxoplasma gondii**

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Primary resistance to *Toxoplasma gondii* infection is mediated by interleukin (IL)-12, produced by conventional dendritic cells (DCs), inflammatory monocytes (iMOs) and monocyte-derived DCs (MODCs). Endosomal Toll-Like Receptors (TLRs), the signaling adaptor myeloid differentiation primary response 88 (MyD88) and interleukin-1 receptor associated kinase (IRAK) 4 are essential in initiating IL-12 production and mediating host resistance to protozoan parasites. After recognition of parasite structures by TLRs, signal transduction is mediated by the myddosome, a multiprotein complex containing MyD88, IRAK4, IRAK2 and/or IRAK1. However, many of the signaling events linking endosomal TLR activation and IL-12 production upon exposure to *T. gondii* remains poorly understood, including which transcription factors drive *Il12b* transcription and whether, in this context, the myddosome employs IRAK1 or IRAK2. Here, we report that in conventional DCs exposed to *T. gondii* tachyzoites, IRAK1 promotes activation of the transcription factor interferon regulatory factor 5 (IRF5), which directly binds to the *Il12b* promoter and drive IL-12 expression. Similarly, IRAK2 has a more prominent role on IL-12 release by iMOs and MODCs. As in IRAK4 deficiency, IRF5 deficient DCs show a broad defect on transcription of inflammatory genes, which further suggests that IRF5 is the key transcription factor activated by endosomal TLRs upon *T. gondii* infection. *In vivo*, IRAK1/IRAK2 double knockout (KO), IRAK4 KO and IRF5 KO mice show a profound defect on IL-12 production and were highly susceptible to primary infection with *T. gondii*. Interestingly, either IRAK1 or IRAK2 single KO mice were slightly affected, with small to no deficiency in IL-12, consistent with a redundant role of DCs and iMOs/MODCs. **Keywords:** Cell signaling;Parasitology;Dendritic cells.

PD - 077 - **Platelet proteome reveals features activation and antiviral response in Chikungunya fever**

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Chikungunya fever is a viral disease transmitted by mosquitoes of the genus Aedes. The infection is usually symptomatic and most common symptoms are fever accompanied by joint pain and swelling. In most cases symptoms subside within a week. However, severe prolonged and disabling joint pain, that may persist for several months, even years, are reported. Although the pathogenesis of Chikungunya infection is not fully understood, the evolution to severe disease seems to be associated with the activation of immune mechanisms and the action of inflammatory mediators. Platelets are recognized as inflammatory cells with fundamental activities in the immune response, maintenance of vascular stability and pathogenicity of several inflammatory and infectious diseases. Although the involvement of platelets in the pathogenesis of viral diseases has gained attention in recent years, their activation in Chikungunya has not been explored. In this work, we prospectively included in the proteomics analyzes 9 patients attended at the Quinta D'Or hospital and 9 healthy volunteers during the 2016 epidemic in Rio de Janeiro, Brazil. We explored the platelet proteome of Chikungunya fever patients through a label-free shotgun proteomics approach to identify platelet responses to infection, as well as validation experiments in a larger patient cohort. Exclusively detected proteins (EPs) and differentially expressed proteins (DEPs) were identified in the proteomic dataset and thus classified into biological processes to map pathways correlated with pathogenesis. Significant changes in the expression of proteins related to platelet activation, cell death, and antiviral response through interferon type-I were found in all patients. Since the outcome of chikungunya varies highly among individuals. In summary, platelets play a significant role in Chikungunya fever pathogenesis via platelet activation and antiviral response. **Keywords:** platelets;chikugunya fever;immunometabolism.

PD - 078 - **Identification of lncRNAs produced by Leishmania infantum during in vitro infection of human neutrophils and predicted binding to human coding transcripts**

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Leishmaniasis is a prevalent zoonotic disease in humans. The most severe form of the disease, visceral leishmaniasis (VL), caused mainly by *Leishmania infantum* (syn. *chagasi*) in the Americas, has high fatality rates if untreated. Neutrophil is the first defense cell recruited during *Leishmania* infection, although this cell type can be used for parasite survival and proliferation. Epigenetic mechanisms mediated by long non-coding RNA (lncRNA) have been associated to gene regulation during parasite-host interactions, however, analysis of *L. infantum* produced transcripts during infection of host immune cells are scarce. To understand the role of *L. infantum* lncRNAs in neutrophils during infection, we used bioinformatic analysis to identify parasite lncRNAs in RNA-Seq data derived from *in vitro* infection of primary human neutrophils with *L. infantum*. Data were aligned against the *L. infantum* reference genome (*L. infantum* JPCM5 release 56 - TriTrypDB) and ncRNA IDs were recovered. A total of 136 ncRNAs of *L. infantum* were obtained and were then aligned against transcripts of all other *Leishmania* species available at TriTrypDB database. Alignments with 97% of identity were selected and ncRNAs larger than 200bp were considered as possible lncRNAs. We obtained 28 lncRNAs of *L. infantum* that aligned against *L. donovani* and 13 of these also aligned against *L. aethiopica*, *L. amazonensis*, *L. arabica*, *L. braziliensis*, *L. donovani*, *L. enrietti*, *L. gerbilli*, *L. major*, *L. mexicana*, *L. panamensis*, *L. tropica*, *L. turanica*. Next, the LncTar pipeline was used to calculate the binding potential (ndG<-0.2) of *L. infantum* lncRNAs with mRNAs that were differentially expressed in the infected human neutrophils that were used for parasite lncRNA detection. A total of 11 *L. infantum* lncRNAs with significant binding potential to 12 human mRNAs were obtained for *in vitro* validation of parasite lncRNA effects on human neutrophils. **Keywords:** epigenetics;parasite-host interaction;ncRNAs.

PD - 079 - **Cysteinyl-leukotrienes promote cutaneous Leishmaniasis control**

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Leishmaniasis is a neglected tropical parasitic disease with few approved medications. Cutaneous leishmaniasis (CL) is the most frequent form, responsible for 0.7 - 1.0 million new cases annually worldwide. Leukotrienes are lipid mediators of inflammation produced in response to cell damage or infection. They are subdivided into leukotriene B4 (LTB4) and cysteinyl leukotrienes LTC4 and LTD4 (Cys-LTs), depending on the enzyme responsible for their production. Recently, we showed that LTB 4 could be a target for purinergic signaling controlling Leishmania amazonensis infection; however, the importance of Cys-LTs in the resolution of infection remained unknown. Mice infected with L. amazonensis are a model of CL infection and drug screening. We found that Cys-LTs control L. amazonensis infection in susceptible (BALB/c) and resistant (C57BL/6) mouse strains. In vitro, Cys-LTs significantly diminished the L. amazonensis infection index in peritoneal macrophages of BALB/c and C57BL/6 mice. In vivo, intralesional treatment with CysLTs reduced the lesion size and parasite loads in the infected footpads of C57BL/6 mice. The anti-leishmanial role of Cys-LTs depended on the purinergic P2X7 receptor, as infected cells lacking the receptor did not produce Cys-LTs in response to ATP. These findings suggest the therapeutic potential of LTB4 and Cys-LTs for CL treatment. **Keywords:** Leishmania amazonensis;cysteinyl-leukotrienes;cutaneous leishmaniasis.

PD - 080 - **EVALUATION OF AN RBD-NUCLEOCAPSID FUSION PROTEIN AS A BOOSTER CANDIDATE OF COVID-19 VACCINE**

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The fast spread of the SARS-CoV-2 lead to a pandemic that hurried the scientists to develop vaccines in a record time. Several vaccines that were developed showed great efficacy in diminishing the morbidity and lethality, but none of them offered full protection against the infection due to the ability of SARS-CoV-2 variants to evade immune system, mainly escaping from neutralizing antibodies. Since projections indicate that SARS-CoV-2 will continue among us for a long time the search for more effective vaccines that mitigate infection, disease and transmission remains a priority. Ratifying several studies, we observed that cellular and humoral responses to SARS-CoV-2 antigens tends to drop in vaccinated people 180 days after the first dose, especially in CoronaVac vaccinated volunteers, which led to the need of boost doses and the development of more effective vaccine. In a previous study, we generated a chimeric protein that comprises the receptor-binding domain (RBD) from Spike (S) and the nucleocapsid (N) antigens (SpiN) from SARS-CoV-2. Vaccination with SpiN elicits a protective immune response in rodents, and this formulation is now under phase I/II clinical trial to be evaluated as a boost vaccine. Therefore, our goal in this study is to evaluate the specific anti-SpiN cellular and humoral responses in vaccinated human donors. $CD4^+$ and $CD8^+$ T cells from convalescent and vaccinated individuals produced greater amounts of IFN-g when stimulated with SpiN, compared to SARS-CoV-2 antigens N and S. Also, B cells from these individuals were able to recognize SpiN. When administered as a boost dose in mice previously immunized with ChAdOx1-S, SpiN was able to induce a greater or equivalent immune response to homologous prime/boost. Our data reveal the ability of SpiN to induce cellular and humoral responses in vaccinated human donors, which makes it a good candidate as boost vaccine against COVID-19.**Keywords:** Vaccine;COVID-19;Cellular immunity.

PD - 081 - **Covid-19 but not Flu syndrome is associated with exhaustion and senescence of T cells**

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Risk factors for the development of severe COVID-19 include several comorbidities, and age was the most striking one since elderly people were disproportionately affected by SARS-Cov-2. Among the reasons for this markedly unfavourable response in the elderly, immunosenescence and inflammaging are considered major drivers of this outcome. A finding that was also notable was that hospitalized patients with severe COVID-19 have an accumulation of senescent T cells suggesting that immunosenescence may be aggravated by SARS-CoV-2 infection. The present study was designed to examine the emergence of these changes in a cross-sectional and longitudinal study and determine whether these immunosenescence changes are characteristic of COVID-19 and whether it is dependent on disease severity in cohorts. Our data result that COVID-19 infection increases cellular senescence and exhaustion when compared to other respiratory infection. The results from longitudinal analyses with patients from Portugal and Brazil provided evidence of increased frequencies of senescent and exhausted T cells over a seven-day period in patients with mild to severe COVID-19, suggesting that accelerated immunosenescence in CD4 and especially CD8 T cell compartments may be a common outcome of SARS-CoV2 infection. **Keywords:** COVID-19;immunosenescence;T cell senescence.

PD - 082 - **CORRELATION BETWEEN EXPRESSION OF FOXP3 AND GALANIN/GALR2 IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)**

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Rationale: Regulatory T cells (Tregs) represent the cellular prototype in immune suppression. Tregs constitute a subpopulation of CD4+ T cells, commonly characterized by high expression of FOXP3 (Clin Exp Immunol. 201: 222-230, 2020). The increase in infiltrating Tregs is correlated with poor prognosis in various types of cancer, including Oral Squamous Cell Carcinomas (OSCC) (J Surg Oncol. 116: 1103-1113, 2017; J Exp Clin Cancer Res. 33: 35, 2014). There is disputed evidence indicating that GALR2, the only galanin receptor expressed in leukocytes, is an oncogene in OSCC and also evidence that its ligand, galanin, is immunosuppressive; however the possible relationship between infiltration of Tregs and expression of galanin/GALR2 in OSCC is unknown. ***Objective:*** Investigate the correlation between the FOXP3 and galanin/GALR2 expression in the tumor microenvironment of OSCC. ***Study Design:*** Surgical specimens of 26 OSCC cases (n=11 T1/T2 and n=15 T3/T4) were obtained and processed for analyses. RT-qPCR was performed using RNA extracted by affinity columns. cDNA was synthesized from total RNA using random hexamers as primers and a commercially available system. We assessed the expression of GALR2 (considered an oncogene in HNSCC), galanin (GALR2 ligand) and FOXP3. Spearman correlation test was used to investigate the associations. ***Results:*** FOXP3 expression is positively correlated with GALR2 (ρ=0.018) and galanin (ρ=0.286) in small tumors (T1/T2). In larger tumors (T3/T4), FOPX3 expression was negatively correlated with GALR2 (ρ=-0.486) and positively correlated with galanin (ρ=0.095). Regardless of tumor size, cases with increased GALR2 mRNA had lower expression of FOXP3. ***Conclusion:*** In OSCC, increased GALR2 expression in inversely correlated with FOXP3 expression in the tumor microenvironment. **Keywords:** GALR2 Galanin Receptor;Regulatory T cells;Oral Squamous Cell Carcinoma.

PD - 083 - **Role of autophagy in the immune response to Nontuberculous mycobacteria**

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The nontuberculous mycobacteria (NTM) are considered opportunistic infections, incidence, and prevalence are increasing worldwide becoming a major public health threat. NTM are ubiquitous microbes and are emerging as clinically important strains due to drug-resistant issues. The understanding of the immunopathogenic mechanisms related to NTM infection is of pivotal importance in identifying targets for new therapeutic strategies. The clinical isolates and different species of NTM may differentially modulate the host immune response. Previous studies have demonstrated the capacity of these clinical isolates to subvert immune response and may modulate the autophagic machinery as well as the apoptotic pathway to survive and infect nearby macrophages. The aim of the present study was to investigate the capacity of different clinical isolates of NTM to modulate host immune response. We selected 10 NTM strains, 4 environmental, and 10 clinical isolates. We observed that the strains CCUG, AT21, AT23, and AT24 present a smooth phenotype whereas the isolates 0594, 6814, 0019, AT22, AT25, and AT26 present a rough morphology. We evaluated the percentage of NTM internalization in human M-CSF-differentiated cells (M2 cells) and observed that the 6814, AT23, AT25, and AT26 isolates presented a higher percentage of internalization when compared to other isolates. The clinical isolates reduced cell metabolism by more than 80%, which was reversed using free Heme. Three strains (CCUG, 0019, and AT24) increased the autophagic flux in NTM-infected M2 cells. In 9 clinical strains, M2 cell autophagy flux was impaired suggesting that NTM may modulate this pathway to subvert the innate immune defenses. To sum up, we conclude that the clinical isolates of NTM may be highly virulent, and it can explain why NTM are now commonly infecting seemingly immune-competent children and adults at increasing rates through pulmonary infection. **Keywords:** Nontuberculous Mycobacteria;Macrophage;Autophagy.

PD - 084 - **Ribosomal Proteins for Cross-Species Malaria Defense: Unveiling Cytotoxic T Cell-Mediated Protection Against Blood-Stage**

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Malaria, caused by *Plasmodium falciparum* and *P. vivax*, remains a deadly global mosquito-borne disease. Existing vaccines offer limited protection, necessitating novel candidates for effective, long-lasting defense against multiple *Plasmodium* species. Our previous study reveals a unique protective mechanism involving *P. vivax*-infected reticulocytes (iRetics) activating Cytotoxic $CD8^+$ T Lymphocytes (CTLs) via HLA-I to eliminate infected cells and their intracellular parasites. To identify the HLA-I-associated peptides, we conducted an Immunopeptidomic Analysis and identified 60% of the eluted peptides were derived from Ribosomal proteins conserved across *Plasmodium* stages and species. Using the malaria experimental model, *P. yoelii,* we evaluated ribosomal proteins as cross-species vaccine candidates. The IFN-γ ELISPOT assay, employing splenocytes from *P. yoelii*-infected mice, revealed that 74% of tested ribosomal peptides were immunogenic in the acute infection, with 44% retaining immunogenicity during the convalescent phase, indicating cross-species immunity and memory response. *P. vivax* Antigen-specific (Ag)-CTLs effectively lysed *P. yoelii*-iRetics, while non-specific or naive $CD8^+$ T cells did not. Adoptive transfer of Ag-CTLs reduced *P. yoelii* blood parasitemia and enhanced $CD8^+$ T cell activation, and cytokine production, with no significant effects on $CD4^+$ T cells. Additionally, *P. vivax* Ribosomal proteins immunization induced a protective response against *P. yoelii* challenge, activating $CD4^+$ and $CD8^+$ T cells, eliciting a specific humoral response, and expanding effector and central memory T cell populations while substantially reducing blood parasitemia compared to the control group. In conclusion, our findings highlight Ribosomal proteins as potential candidates for a cross-species malaria vaccine, offering a promising breakthrough for protection against *Plasmodium* infections and advancing malaria eradication efforts. **Keywords:** Cytotoxic T cells;Cross-species defense;Malaria Vaccine.

PD - 085 - **Engineering peptibodies for immunotherapy by repurposing human PD-L1-targeting peptides**

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Peptibodies are biological drugs that combine pharmacologically active peptides with the Fc fragment of an antibody. They offer two main advantages over monoclonal antibodies: 1) significantly extending their bloodstream half-life; and 2) being able to be quickly and inexpensively produced in bacteria. In this project, we aimed to develop engineered peptibodies from PD-L1-interacting peptides, which hold promise as potential therapeutic and diagnostic agents for PD-L1+ tumors. To identify suitable candidates, we conducted a literature search and carefully selected seven peptides, ranging from 12 to 25 amino acids. These peptides have been previously reported to either bind to PD-L1 or disrupt the PD-1/PD-L1 interaction, making them promising targets for our study. We synthesized the corresponding DNA sequences in vitro, optimizing the codon frequencies for efficient expression in *Escherichia coli*. Using overlap extension PCR, we fused the DNA sequences with the Fc portion of IgG1. Following successful assembly, the peptibodies were cloned into the expression vector pet30a and transformed into *E*. *coli* for plasmid expansion and extraction, for subsequent protein expression. Their binding activity was tested by ELISA. This work paves the way to develop the first peptibody targeting PD-L1, potentially opening new possibilities for therapeutic interventions in cancer immunotherapy and lowering costs of antibody-based diagnostic tests, such as immunohistochemistry. **Keywords:** peptibody;anti-PD-L1;blocking peptides.

PD - 086 - **Implementing in silico Strategies for Generating Monoclonal Antibodies Against PD-L1**

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Monoclonal Antibodies (mAbs) are important therapeutic agents with potential for the treatment of several conditions. Today, fully human antibodies can be cloned and expressed in mammal or human cells. Recent development in Artificial Inteligence (AI) has significantly improved in silico prediction of protein structure and protein-protein interaction. In this work, we compare the efficacy of three strategies: one “traditional” in silico approach; one using a neural network; and a third using a language model, to generate mAbs against human programmed death ligand 1 (hPD-L1), a key target in immune checkpoint blockade. In the first approach, the structure of the anti-hPD-L1 Avelumab interacting with hPD-L1 was retrieved from the PDB. Amino acid (AA) positions that were close enough to interact were substituted, and complex stability was calculated using FoldX software. The language model called Efficient Evolution was used with the Avelumab sequence as input. Mutations were suggested that can enhance structure fitness and can impact affinity. Finally, a neural network AI model called RF Diffusion was used to generate a completely novel sequence able to bind hPD-L1. The three newly created mAb sequences were aligned using BLAST for comparison of suggested mutations. The resulting sequences were cloned in the pCI-neo vector and sequenced. Clones were transiently expressed in Expi293 cells and checked in SDS-PAGE gels. Binding and affinity to hPD-L1 are being assessed by ELISA. **Keywords:** mAbs;Immune Checkpoint Blockade;Artificial Inteligence.

PD - 087 - **Previous lung immunization with inactivated-SARS-CoV-2 impacts in the immune response of gastrointestinal infection**

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The interactions between host-pathogens can modulate a functionality of local cells, immune system, and influence on development of diseases. Studies have been shown to impact of this interactions goes far beyond the local environment. There is a crosstalk between the mucosal site in our body, one of these examples is the communication with gut and lung, too named axis gut-lung, and to influence the immune response of both of organs. Although the mechanisms of this crosstalk are not yet well elucidated, studies indicate this occurs through the transit of cells and chemical messengers produced directly by microorganisms, and by the immune response that they direct. These chemical messengers are carried via the blood or lymphatic pathways to regulate immune system functions throughout the body. We evaluated gut-lung axis crosstalk in C57BL/6 mice immunized with inactivated SARS-CoV-2 virus and challenged these animals with *Y. pseudotuberculosis* bacteria. Our hypothesis is how previous pulmonary immunization alters the immune response to gastrointestinal infection. Preliminary data showed, although without statistical significance, animals challenged with bacteria recovered better body weight after infection compared to immunized and infected group, this show that SARS-CoV-2 antigen influences protection against gastrointestinal infection. The analysis of pulmonary inflammatory cells corroborates that data, a decrease in populations of cells such as monocytes and macrophages were observed in the same groups without a statistical difference; however, the IL-6 cytokine production by these cells decreases statistically. Another data observed was the statistical decrease of populations of T CD4+ and T γδ lymphocytes in lungs of this group. The analyzes have pointed to a possible migration of inflammatory cells from lungs to intestines of immunized and challenged group. To corroborate these data, our next steps will be the analysis of the histology of these organs. **Keywords:** SARS-CoV-2;YERSINIA PSEUDOTUBERCULOSIS;IMMUNOLOGY.

PD - 088 - **Acute intestinal infection compromises mesenteric type 2 responses by affecting cholinergic signaling**

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Several neural mechanisms have been studied in the control of the immune response in the mucosal tissues, including the intestinal mucosa. However, little is known about the importance of this type of signaling in adipose tissue immune homeostasis, particularly in the mesenteric adipose tissue adjacent to the gut. Our previous results indicate that acetylcholine (Ach) suppress type 2 responses in the mesentery, as demonstrated by the increased counts of Th2, ILC2 and Eosinophils in both vagotomized or mechamylamine-treated mice. This effect is mediated by IL-33, as it is increased in both models and the effect does not occur in mice that lack IL-33 production. As Yersinia pseudotuberculosis (YP) infection inhibits type 2 responses in the mesentery long after infection resolution, we hypothesized that it could boost Ach production, dampening this immune profile. Therefore we used choline acetyltransferase (ChAT) reporter mice to track Ach production in the model of Immunological scar induced by YP infection. Surprisingly, the remodeling of the mesentery, caused by the infection, did not increase ChAT production in the mesentery nerves. However, immunofluorescence indicates ChAT production colocalizes with the leukocytes (CD45+) in the fat associated lymphoid clusters (FALCs), this shift is confirmed by cytometry which shows a decrease in the frequency of ChAT+ cells in the CD45- compartment, while more ChAT+ CD45+ cells are found after infection. This ChATproducing leukocytes consists mostly of B cells, T cells and γδT cells. Moreover, infection of IL-33 reporter mice indicate a decrease in the number of CD45- cells producing this cytokine. These results suggest leukocytes take on Ach production during YP-induced remodeling, this FALC-centered production could be responsible for decreasing stromal/epithelial IL-33 production and consequent local failure on inducing type 2 responses in the mesentery. Financial support: FAPESP, CNPq, CAPES. **Keywords:** Immunological Scar;acetylcholine;mesentery.

PD - 089 - **Effect of protein deficiency on the homeostatic function of the immune system in the intestinal mucosa**

SANTOS, J.M.; LIMA, G.D.M.; AYUPE, M.C.; GONÇALVES, L.M.; SALGADO, C.L.; MOREIRA, F.; DA SILVA, G.W.; RODRIGUES, G.M.B.; DE ARAUJO, M.V.; DE OLIVEIRA, E.E.; GOMES, E.M.; DA FONSECA, D.M.. ICB/USP, SÃO PAULO - SP - BRASIL.

Protein deficiency is a worldwide health issue, especially in third-world countries. It has negative impacts on several physiological and immunological processes, potentially affecting gut mucosal immunity. The present study aimed to evaluate the gut immune homeostasis in mice receiving a low-protein (LP) or conventional diet (C). C57/BL6 CD45.1+ mice were maintained on either a hypoproteic or conventional diet for 10 weeks. At the end of this period, animals from both groups received transfers of T cells with a specific TCR for Ovalbumin (OVA), previously isolated from the lymph nodes and spleen of RAG-/-OTII CD45.2+ transgenic mice and labeled with cell tracer. After the transfer, C57/BL6 CD45.1+ mice received 3% OVA in their drinking water for one week. At the end, the animals were euthanized, and samples from the mesenteric lymph nodes and the proximal and distal portions of the small intestine were processed and characterized using flow cytometry. No significant differences were observed between the C and LP groups regarding the evaluation of the number of OTII CD4+ and CD4+Foxp3+ cells originating from mesenteric lymph nodes. However, a significantly higher proliferation was observed in OTII cells transferred to the LP group compared to the C, as assessed by cell tracer labeling. Regarding the expression of transcription factors, it was found that LP mice presented a significant increase in OTII CD4+, CD4+Foxp3+, CD4+Foxp3+ GATA3+, CD4+Foxp3+RORgt+, CD4+GATA3+, and CD4+RORgt+ cells in the proximal and distal portions of the small intestine compared to the C group, with a more pronounced effect in the proximal portion of the small intestine. In conclusion, our findings suggest that exposure to food antigens under protein deficiency leads to a conversion of OTII cells into Th2, Th17, and Treg response profiles, but not Th1, in the small intestine of mice. Further studies are necessary to evaluate the functional impact of LP diet against gut pathogens. **Keywords:** Protein deficiency;Homeostatic function;Gut immune homeostasis.

PD - 090 - **MICRONEEDLES: A SMART DELIVERY SYSTEM FOR ANTIBODY-BASED THERAPY**

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Microneedles (MNs) form a safe and minimally-invasive delivery platform of free drugs or nano-formulations. In this study, we propose a combined nanomedicine approach: administration of AuNPs-therapeutic antibodies using polymeric MN for transdermal delivery to increase efficiency of Erbitux®, a commercial anti-EGFR antibody. MN arrays created from an optimized blend of natural and synthetic polymers were developed by LEMN-UFABC and validated for different bio-applications jointly with Microneeds. The mechanical properties were tested on a rheometer. Optical Coherence Tomography (OCT) was employed to visualize the MN's cross-sectional insertion in vitro skin models using OCT. Cytotoxicity was assessed *in vitro* and *in vivo*. Cellular viability in the presence of MNs was performed using tumoral and non-tumoral cells. MN delivery was evaluated in nude mice by flowcytometry and histological analysis. The MNs display favorable dimensions to penetrate the skin and act as drug delivery platform. The OCT showed that MNs are capable to penetrate the skin. The polymer MN arrays showed good mechanical strength, sufficient insertion depth. Toxicity was not detected in FaDu and A431 cell lines. The gross anatomy and histology did not reveal any signs of inflammation following the MNs insertion. The MNs had no systemic toxic effects. Cell viability and binding ability to cell surface EGFR receptor of Erbitux®-AuNPs released by MNs were analyzed by FACS after 6-months of storage at RT. MN mediated successful transdermal delivery of Erbitux®-AuNPs, targeting their biomarker in situ after 7-days of MNs application in the tumor xenograft model. This platform has the theragnostic potential to deliver a range of combination therapies and may be a promising carrier for the transdermal delivery of nanostructured immunotherapeutic, improving the permeation of target therapy through the skin, thus improving efficiency of skin cancer therapy. **Footnote:** CATALISA-ICT, INCT-CNPQ-iii. **Keywords:** ANTIBODY-BASED THERAPY;MICRONEEDLES;SKIN CANCER.

PD - 091 - **Immunological and molecular profile of lung tumors in patients from Porto Alegre/RS – Brazil**

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Lung cancer remains a significant public health concern, with a notable incidence of cases projected for the year 2023 in the state of Rio Grande do Sul, Brazil. Cancer treatment options encompass various modalities, including radiotherapy, chemotherapy, and immunotherapy, with an emphasis on immunological checkpoint inhibitors. To advance our understanding and potentially enhance therapeutic strategies, this study aimed to comprehensively characterize lung tumors in terms of their immunological and molecular features. This project received approval from the Ethics Committee of Santa Casa de Misericórdia de Porto Alegre and Plataforma Brasil, by registration number 52266121.0.0000.5335. Surgical resection provided lung tumor samples, which underwent meticulous macroscopic examination, followed by distinct processing methods. Formalin fixation enabled histological analysis, while enzymatic digestion facilitated the extraction of immune cells for evaluation. RNA later preservation preserved transcript integrity for subsequent assessment. Microscopic analysis of the tumors unveiled diverse pulmonary microarchitectures, highlighting the heterogeneity of the inflammatory infiltrate. Flow cytometry analysis demonstrated variations in CD45+ cell expression within the tumor tissue and the presence of CD4+ and CD8+ T lymphocytes. Furthermore, a transcriptome library was established, enabling the evaluation of crucial genes implicated in lung cancer. Through these findings, a comprehensive immunological and molecular profile of the lung tumors has been established, potentially representing a better therapeutic decision-making for affected patients. **Keywords:** Immunotherapy;Cancer;Histology.

PD - 092 - **Topical application of SCFA accelerates diabetic wound healing in mice**

RIBEIRO, W.R.; DE MENEZES, M.H.T.; FRAGA JUNIOR, V.D.S.; DE OLIVEIRA, A.C.D.S.C.; BENJAMIM, C.F.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Chronic wounds are characterized by a persistent, hyper-inflammatory microenvironment that impairs wound healing progression and are one of the most prominent troublesome complications for diabetic patients worldwide. SCFA are bacterial metabolites produced by microbiota that play essential roles in maintaining homeostasis and have several positive effects on the skin through canonical and epigenetic mechanisms. In this work, we aimed to explore the effect of local applications of SCFA on the wound healing process using a full-thickness excisional skin wound model in type 1 diabetic C57BL/6 mice. Since we did not observe differences in wound closure rate using 1 mM of butyrate, we conducted experiments based on a dose response curve of SCFAs (acetate, propionate and butyrate) individually at 10, 30 and 100 mM which were applied once a day during the first 14 days after wounding. Topical acetate treatment did not accelerate the wound closure at any of the tested doses, while topical propionate and butyrate improved the wound closure rate at 30 mM and 100 mM, respectively ($p<0.05$). On day 7 after wounding, the butyrate-treated group presented a reduced infiltrate of macrophages on the wound compared to the untreated group ($p<0.05$), while no differences were observed for neutrophils. Moreover, butyrate treatment at 100 mM accelerates the wound closure rate in euglycemic C57BL/6 mice ($p<0.05$). To determine if GPR43, a canonical receptor for SCFA, is essential to diabetic wound healing, we performed an experiment using GPR43 knockout (KO) mice, and we found no differences in skin wound closure rate between WT and GPR43 KO mice. In our in vitro approaches, butyrate increased fibroblast migration in a scratch test and did not reduce cell viability at high micromolar to low millimolar concentrations. In conclusion, these preliminary results shows the therapeutic potential of SCFA in diabetic wound management. **Keywords:** short chain fatty acids;chronic wounds;inflammation.

PD - 093 - **BIOIMAGING BRASIL: DEMOCRATIZING INTAVITAL MICROSCOPY ACROSS BRAZIL.**

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Introduction: Intravital microscopy (IVM) is a powerful tool that allows visualization of biological phenomena in vivo. However, widespread use of IVM is hindered by the lack of access to suitable equipment, and unfamiliarity with the technique and development of sophisticated microscopy systems has brought the assumption that IVM is unattainable. Therefore, an expansion in IVM application by Brazilian scientists could be achieved by reversing this logic. We developed a novel logistic strategy to empower institutions to establish IVM regionally: **we bring IVM to them**. **Aim, methods and results:** We plan to visit at least 1 group in each of the Brazilian states. This has been done by locally visiting different groups, which not only are exposed to presential, open-access lecture of ~3h duration (basics of imaging techniques and principles), but also to practical exercises on mouse surgery and imaging procedures using their own microscopes. Also, we design custom-made, low-cost solutions and minor adjustments on their equipment at no cost to them (supported by our grant). Laboratories are selected by our team after registration in a virtual form, and women PIs from under-represented minorities have higher priority. In 12 months of project, we received 36 applications from 19 different states, from which 12 groups were selected. Of these, 5 groups have already been assisted in a period of 4 months, totaling 325 trained scientists and 9000km travelled. More five groups expected in 2023. **Conclusion:** Such unique initiative, supported by CZI Imaging Program, is generating a unprecedent democratization of IVM across Brazil, contributing to improve access to science and enhancing the quality of local projects. Also, based on the testimonials from visited members, we are witnessing a significant increase in scientific enthusiasm and drive for novel techniques. We hope that our initiative paves new roads for similar projects on other areas. **Grant:** Chan Zuckerberg Initiative – CZI. **Keywords:** Intravital microscopy;Immunology;Bioimaging.

PD - 094 - **CLADRIBINE TREATMENT FOR MULTIPLE SCLEROSIS: LONG-TERM EFFECTS ON IMMUNE REGULATION, T-CELL EXHAUSTION AND IMMUNOSENESCENCE**

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Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammation, demyelination, and premature immunosenescence. Cladribine was approved for immune reconstitution therapy in patients with highly active relapsing–remitting MS (RRMS). Here, we investigated whether reconstitution was associated with changes in lymphocyte regulation, exhaustion, and immunosenescence. We recruited 9 RRMS patients treated with oral cladribine. Clinical and immunological assessments were performed over a year of follow-up. Immunophenotyping and cytokine profile (TNF-a, IL-1, IL-6, IL-8 and IL-10) were performed at baseline and 12 months after treatment launch as well as in healthy controls (HC, n=22). Telomere length was evaluated at the T/S ratio by qPCR. Cytokines did not differ between MS and HC. Compared to HC, MS patients exhibit decreased levels of classical and non-classical monocytes, and an increase in intermediate monocytes. We observed an increase in proportions of naïve B and transitional B cells and decreased plasma and memory B cells after cladribine treatment, with Bregs recovered to the levels of HC. Proportions of activated ($CD4^+CD25^+$) T cells reduced significantly after treatment. Increased proportions of immune checkpoint PD1 in $CD8^+$ T cells were noted in MS, with no significant differences in CTLA-4 and LAG on T cells were noted after cladribine treatment. Similarly, no significant changes were observed in the percentage of Treg cells. In contrast, cladribine treatment increased the proportion of immunosenescent ($CD8^+CD28^-CD57^+$) cells. However, MS and HC had lymphocytes with similar telomere lengths. Clinical efficacy might be associated with reducing autoreactive mature circulating B cells, as B-cell depleting therapies (ocrelizumab, ofatumumab) also have high efficacy in RRMS. Altogether, our findings provide new insights into the composition of T, B, and monocytes and the long-term therapeutic effect of cladribine in RRMS. **Keywords:** Multiple Sclerosis;Neuroinflammation;Lymphocytes.

PD - 095 - **Unraveling the Tumor Microenvironment of High-Grade Serous Ovarian Cancer: Single-Nucleus RNA Sequencing Reveals Cellular Subpopulations and Treatment Responses**

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Ovarian cancer (OC) is the most lethal gynecological neoplasm among women, with a five-year survival rate of less than 25%, and the high-grade serous, one subtype of epithelial ovarian cancer, being the most common. OC presents a highly heterogeneous tumor mass, and the interaction between malignant cells and resident and infiltrating cells, such as immune cells, fibroblasts, mesenchymal cells, endothelial cells, and pericytes, as well as components of the extracellular matrix, impacts tumor progression, recurrence, and treatment resistance. The composition and cell interaction inside the tumor microenvironment is important to precision medicine, particularly in its implications for clinical medicine and the development of new methods for diagnosis and treatment. Recent advances in RNA sequencing at single-cell and single-nuclei resolution have allowed a better characterization of cellular subpopulations associated with the tumor. Thus, we aim to characterize these cellular subpopulations of the TME associated with OC at in advance stages in patients who have responded or not responded to chemotherapy through snRNA-Seq analyses. For that, we selected eight patients naive of treatment diagnosed with high-grade serous OC from INCA, and these patients were divided into poor and better response based on type of surgery (R0 -complete surgical resection R1/R2 -microscopic or macroscopic disease). The fresh tumor samples were dissociated and the nuclei recovered and fixed according to the 10X Genomics instruction. The isolated nuclei were resolved by Chromium IX and sequenced using Illumina technology. The raw FASTQ files were processed by the CellRanger pipeline, generating the raw counts matrix. We performed the quality control step and the high-quality nucleus was integrated by scVI. A better understanding of the complex roles of immune cells associated with tumors could provide new insights into the TME and assist in the development of novel therapeutic approaches. **Keywords:** High grade serous ovarian cancer;tumor microenviroment;therapy response.

PD - 096 - **Exploring tumor microenvironment immune subpopulations in high-grade serous ovarian cancer using spatial transcriptomics analysis: implications for treatment response**

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Ovarian cancer, particularly the high grade serous type (HGSOC), is highly lethal among women, with a five-year survival rate below 25%. The majority of patients are diagnosed in advanced stages, leading to tumors with low therapeutic response. The tumor microenvironment (TME) plays a crucial role in cancer progression, metastasis, and treatment resistance, but its underlying mechanisms are not fully understood. Previous studies suggest that resistance to therapy may be associated with increase in non-TME myeloid cells, as specific subpopulations of tumor-associated macrophages (TAMs), which can promote either a pro- or anti- tumor immune response within the TME. Our group has recently identified distinct subpopulations of TAMs, which could potentially be associated with clinical outcomes and prognosis. In light of these findings, our objective was to characterize TME cell subpopulations, particularly TAMs, in responsive and non-responsive ovarian cancer patients using spatial transcriptomics analysis. Spatial transcriptomics is a powerful technique that allows for the mapping of gene expression in its precise tissue location, providing insights into the three-dimensional interactions within different tissue regions. To this, we performed spatial transcriptomics using the 10x Visium Spatial Gene Expression platform on tumor samples from four HGSOC patients from the Banco Nacional de Tumores (INCA-RJ), in stages I and IV who underwent adjuvant chemotherapy and exhibited varying survival rates. These samples were processed according to 10x established protocols and sequenced using Illumina NovaSeq 6000 technology. Our analysis pipeline followed the 10x Space Ranger and bioinformatic tools to identify cell subpopulations distinguishing between these patient groups. This study holds the potential to advance our understanding of TME heterogeneity and its impact on treatment response, contributing to improved clinical management strategies and patient outcomes. **Keywords:** Tumor-associated macrophages;Spatial transcriptomics;High grade serous ovarian cancer.

PD - 097 - **SEPSIS-INDUCED LIPID DROPLETS CONTRIBUTED TO RESISTANCE TO BACTERIAL INFECTION AND TO LIVER DYSFUNCTION**

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Sepsis is a complex syndrome defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. The mechanisms involved in sepsis-induced multiple organ dysfunction (MODS) are multifactorial and still incompletely understood, but disrupted mechanisms of tissue tolerance are believed to be the main causes of tissue damage and mortality. Although pronounced alterations in lipid metabolism and an increased number of lipid droplets (LD) are observed during sepsis, their involvement in the mechanisms of disrupted tissue tolerance and resistance to bacterial infections are poorly understood. In this work, we investigated the role of LDs in the physiopathology of sepsis. In this work, we investigated the role of LDs in the physiopathology of sepsis. Using an experimental model of polymicrobial sepsis, we identified that liver LD overload correlates with increased sepsis severity and acts as a liver dysfunction marker. Concomitantly, LDs increased the content of lipoperoxides, an important component of free radical-mediated injuries during sepsis. The impairment of LD formation by pharmacological inhibition of the DGAT1 enzyme reduces hepatic inflammation and lipid peroxidation markers and improves sepsis-induced liver dysfunction. However, we observed that the inhibition of DGAT-1 temporally anticipates the mortality associated with severe sepsis. To better understand the reason for this outcome, we also evaluated the impact of DGAT-1 inhibition on infection resistance mechanisms. We observed that DGAT-1 inhibition also increased the bacterial load in the peritoneum and blood of septic mice at 6 h and 24 h after sepsis. Moreover, the treatment also inhibited LD accumulation in peritoneal cells and impaired the levels of nitric oxide, PGE_2, LTB_4, CCL2 and IFN-β induced by sepsis. Together, these results support that LD accumulation has a dual role in bacterial sepsis, contributing both to resistance to infection and liver dysfunction. **Keywords:** IMMUNOMETABOLISM;ORGANIC DYSFUNCTION;RESISTANCE TO INFECTION.

PD - 098 - **Evaluation of amphiregulin as a prognostic biomarker of severity and its participation in the pathophysiology of COVID-19**

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Introduction: some patients infected with SARS-CoV2 have massive damage to alveolar cells, resulting from viral replication and exacerbated local inflammation. **Rationale:** By containing infection and repairing injured tissues, the orchestration of immune system cells becomes essential for disease resolution and healing. Amphiregulin (AREG) plays a central role in regulating the host's tolerance mechanisms to infections, so its absence can delay the restoration of lung function in cases of injury. On the other hand, the administration of recombinant AREG enhances the repair of tissues injured by excessive inflammation. **Objective:** Thus, the present study aims to evaluate the role of AREG in the prognosis of severity and resolution resulting from SARS-COV-2 infection. **Methods:** Comparison of body weight, clinical score, oximetry, heart rate and viral load. Assessment of AREG levels and inflammatory mediators such as IL-6 by ELISA, and lung histopathological study during infection of $AREG^{-/-}$ total and $AREG^{fl/fl}$ $LysM^{Cre/0}$ tg hACE-2 mice, by SARS-CoV-2 compared to control (WT). Analysis of the phenotypic profile of lung strains (Calu-3 and A549 tg hACE2) in vitro treated with recombinant AREG and infected with SARS CoV-2. **Results:** We found a lower survival of AREG knockout mice when infected with SARS Cov-2, with exacerbated tissue damage, a slight modulation of viral load and alteration of immune markers such IL-6. Macrophages have a conditional participation during AREG expression. Furthermore, AREG is able to protect lung cells when added throughout the infection. **Conclusion:** Our data indicate the essential role of AREG in the prognosis of the severity of infection by SARS- CoV-2, so that its absence is lethal, having a fine participation in tissue protection and repair. **Keywords:** AREG;SARS-Cov-2;Macrophages.

PD - 099 - **EFFECT OF TRANSPLANTATION OF INTERLEUKIN-4 PROGRAMMED MACROPHAGES IN SEVERE ACUTE RESPIRATORY SYNDROME INDUCED BY MURINE BETACORONAVIRUS**

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Introduction: Alternatively activated macrophages or M2 can be achieved in vitro by several stimuli, including IL-4. This macrophage phenotype possesses anti-inflammatory properties and play a critical role in tissue repair and wound healing. Transplantation of IL-4-programmed macrophages is an immunomodulatory therapy to enhance tissue repair and constitute a putative treatment for inflammatory diseases. Here, our objective was to investigate whether exogenous administration of IL-4-programmed macrophages could mitigate the inflammatory response and serve as a cell transfer treatment for managing lung dysfunction induced by the betacoronavirus (MHV-A59) infection. **Methods**: Mice were intranasally inoculated with MHV-A59 (10^3 PFU), known to induce a robust inflammatory response in the lungs. Bone marrow cells were isolated and differentiated into macrophages, and then polarized toward an M2-like phenotype using recombinant murine IL-4. The IL-4 programmed macrophages ($1x10^6$ cells/mouse) were administered to the mice via intravenous tail vein injection, either in a single dose on day 3 post-infection (pi) or two doses on days 3 and 4 pi. Mice were euthanized on day 5 pi, and bronchoalveolar lavage (BAL) was harvested to evaluate inflammatory leukocytes. The lung tissue was examined to assess chemokine production, histopathological changes, and viral load. Animal Ethics Committee under protocol 123/2023. **Results**: Treatment with IL-4-programmed macrophages reduced lung damage, viral load, and excessive production of the proinflammatory cytokines IFN-Y, IL-6, TNF, and IL-1β induced by MHV-A59 infection. In addition, IL-4-programmed macrophage transplantation reduced total leukocytes and neutrophil numbers in BAL. However, there were no change in protein levels in the BAL and number of circulating leukocytes in the blood. **Conclusion**: Transplantation of IL-4 programmed macrophages show beneficial effects in mitigating the inflammatory response induced by MHV-A59 infection. **Keywords:** Immunotherapy ;Betacoronavirus;Macrophages .

PD - 100 - **INHALED NITRIC OXIDE TREATMENT IMPROVES IMMUNE RESPONSE IN PATIENTS WITH COVID-19**

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The immune response against SARS-CoV-2 depends on the action of different populations of immune cells, exerting a pivotal role on disease progression and clinical outcomes. Nitric oxide (NO), an endogenous molecule, is involved in physiological and pathological processes and has been used in the management of acute respiratory distress syndrome. We participated in a randomized controlled clinical trial to test inhaled NO (iNO) therapy in patients with COVID-19. NO treatment improved clinical scores, shortened days of ventilatory support and reduced the length of hospital stay in COVID-19 patients. Our aim was to investigate the immune profile of PBMC in patients infected with SARS-CoV-2 after treatment with iNO. Patients with positive diagnosis for COVID-19 (RT-qPCR) were randomly enrolled at HSL/PUCRS and assigned to control group with conventional treatment (n=27) or treatment with iNO (n=27). Peripheral blood (5mL) was collected before and 5 days after the intervention. PBMC were isolated and phenotyping was performed to determine immune populations. Our results demonstrated that iNO administration increased $CD56^{dim}CD16^{+}$ NK cells, reduced naïve $CD45RA^{+}CD57^{-}$, and augmented the early differentiated subset $CD45RA^{-}CD57^{-}$ in $TCD4^{+}$ cells. We also detected a reduction in CTLA4 in $TCD8^{+}$ cells, as well as LAG3 and TIM3 in $TCD4^{+}$ cells. $TCD4^{+}$ showed reduced expression of CD28 in patients who received iNO. After adjusting the analysis for the length of hospital stay or days free of ventilatory support, we observed that patients who received iNO showed increased CD38 and PD-1 in $TCD4^{+}$. Our findings suggest that iNO treatment may stimulate the immune response by promoting an increased innate and adaptive immune response by NK cells and lymphocyte activation. Additionally, iNO treatment may inhibit co-inhibitory proteins, which could further enhance the immune response. These effects may contribute to an improvement in clinical outcomes in patients infected with SARS-CoV-2. **Keywords:** COVID-19;Immune profile;Inhaled nitric oxide therapy.

PD - 101 - **Investigating Immune Proteins Linked to Natural Resistance to COVID-19 in Highly Exposed Individuals**

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Worldwide reports have highlighted cases of individuals who were significantly exposed to SARS-CoV-2 but remained uninfected. These observations have led to the hypothesis that certain biological factors may confer protection against COVID-19. Similar to natural immunity to other infectious diseases, it is conceivable that such protection could also exist for COVID-19. To investigate this further, we conducted a unique study focusing on six uninfected women who had been exposed twice to their symptomatic reinfected COVID-19 partners. Our goal was to identify any differential proteins associated with innate and adaptive immune responses, as well as other potential mechanisms of viral entry defense. Plasma proteomics analyses using tandem mass spectrometry were conducted to analyze the data. Quantitative assessment of plasma proteomics revealed that the reinfected men had a higher enrichment of pathways associated to viral entry, acute inflammatory response, and antigen processing and presentation, in resistant women. This suggests the presence of distinct mechanisms of neutralizing SARS-CoV-2 and activating innate immune responses in individuals who show natural resistance despite repeated exposure to the virus. Furthermore, we found that the Ficolins family and mannose binding proteins in lectin pathway, associated with effective pathogen presentation, were more enriched in the resistant women. These proteins might play a crucial role in conferring resistance to SARS-CoV-2. Our findings of specific plasmatic proteins indicate the presence of a unique immune profile in women who exhibit natural resistance to COVID-19 despite repeated exposure to the virus. These protein expression patterns observed between groups could be explained by genetic variants to be investigated more deeply in the next stages of this study. The study was supported by FAPESP (grant numbers 2013/08028-1, 2014/50931-3, and 2020/09702-1) and CNPq (grant numbers 465355/2014-5 and 404134/2020-3). **Keywords:** Global proteomics;Serum-discordant couples;COVID-19.

PD - 102 - **Effects of the treatment with the melanocortin receptor agonist AP1189 in response to SARS-CoV2 infection in vitro.**

TANA, F.; SANTOS, F.R.D.S.; TEIXEIRA, D.C.; DE SOUZA-COSTA,, L.P.; LITWINSKI, V.V.C.; TEIXEIRA, M.M.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Coronavirus infectious disease 19 infection, in analogy to other viral pneumonia like Middle Eastern Respiratory Syndrome, is characterized by an initial viral phase followed by an inflammatory phase which can injure the lung parenchyma. In certain conditions, such as in chronic inflammatory diseases and after severe infections, there is failure to resolve with persistent or exacerbated inflammation, which may lead to tissue damage. Successful resolution requires activation of endogenous programs with switch from production of pro-inflammatory towards pro-resolving molecules and the non-phlogistic elimination of granulocytes by apoptosis with subsequent clearence by surrounding macrophages. Therefore, pro-resolution mediators, or strategies that promote their increase, may regulate inflammation and protect against injury caused by infection, including during COVID-19. Melanocortins are peptides that have anti-inflammatory and pro-resolving actions in humans and elicit their effects by binding to a distinct G protein-coupled receptors known as melanocortin receptors. AP1189 is a melanocortin receptor agonist known to promote resolution of inflammation in arthritis model and various inflammatory conditions. The study was aimed to investigate the role and effect of AP1189 administration in response to SARS-CoV2 infection *in vitro*. For infection experiments *in vitro*, the PBMCs were obtained from whole blood of healthy volunteers. A total of 2x105 PBMCs were plated in 48-well plates and infected with SARS-CoV-2 at MOI of 1. After 1 h of virus adsorption, fresh medium, with or without the AP1189 at different concentrations, were added. Our results showed that treatment with AP1189 did not interfere with cell viability and viral titer. Additionally, AP1189 at different concentrations reduce the production of pro inflammatory cytokines during SARS-CoV-2 infection. These preliminary results suggest that AP1189 may exert pro-resolving effects during Sars-CoV2 infection. **Keywords:** COVID 19;AP1189 ;PBMCs.

PD - 103 - **Consumption of a short-term, high-fat diet protects C57BL/6 mice from developing malaria after Plasmodium berghei ANKA infection**

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It is well known that dietary changes have a significant impact on the immune system, and modifications in lipid balance may contribute to disease progression in a number of cases. Malaria is still a major global health concern, and the development of the disease has already been linked to the host's nutritional status, so it's critical to understand how environmental factors, such as dietary variations, can influence the outcome of infection. We therefore investigated the effect of a short-term diet in a murine model of experimental cerebral malaria. For this, male C57BL/6 mice were fed a high fat diet containing 60% of the calories from lipids for 5 days. Following this period, the animals were infected with Plasmodium berghei ANKA, and parasitemia, survival, and neurological scores were compared. Considering that one of the first elimination routes of the intracellular parasite is oxidative stress, the antioxidant N-acetylcysteine was administered to assess whether the protection would be reversed, but the animals fed a hyperlipidic diet reacted the same way to infection even after NAC administration. Unlike the control group, which died after eight days of infection with roughly 7% parasitized red blood cells, the hyperlipidic diet group was resistant to infection, with no clinical signs and no increase in blood parasitemia. Several proinflammatory cytokines such as TNF-α IFN-γ and IL-6 were increased in the spleen of both infected groups, regardless of their diet. The provision of a high-fat diet to mice for as little as 5 days completely prevents Plasmodium berghei ANKA infection in C57BL/6 mice, while the treatment of an antioxidant failed to reverse the parasite protection. **Keywords:** Malaria;High Fat Diet;Obesity.

PR - 001 - **ROLE OF PLATELET-ACTIVATED FACTOR RECEPTOR IN EXPERIMENTAL ULCERATIVE COLITIS**

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Ulcerative colitis is an inflammatory condition with severe intestinal lesions and dysbiosis can exacerbates the lesions and inflammation. The platelet-activating factor receptor (PAFR) seems to be essential for controlling injury, resolving the inflammatory response in ulcerative colitis, and promoting the clearance of bacterial products. This study aimed to investigates the role of PAFR in ulcerative colitis. WT and PAFR-/- animals were submitted to a DSS-induced colitis model and clinical, histological, and inflammatory parameters were evaluated. The composition of the fecal microbiota was assessed, and the effects of microbiota sharing on the disease were examined through co-housing of WT and PAFR-/- mice. Results showed that PAFR deficiency increased colitis severity, associated with dysbiosis after DSS exposure. On the 15th day, PAFR-deficient mice exhibit higher body weight loss (86 ± 4% and 94 ± 3% of initial weight), increased clinical scores (7 ± 1 and 1.2 ± 0.2 A.U.), and shorter colon length (6.7 ± 0.3 and 8 ± 0.2 cm) versus WT mice. Enterobacteriaceae growth was more pronounced in PAFR-/- mice (7.2 ± 0.6 log CFU/g) than WT mice (5.7 ± 1.5 log CFU/g). Lactic acid bacteria showed similar levels (around 8 CFU/g) in both groups. Notably, antibiotic treatment mitigating DSS-induced bowel disease severity in PAFR-deficient mice. Antibiotic-treated PAFR-deficient mice experienced less weight loss (96 ± 3% of initial weight), lower clinical scores (3.3 ± 0.3 A.U.), and longer colon length (10.3 ± 0.2 cm) compared to non-antibiotic-treated PAFR-deficient mice. Additionally, microbiota sharing between WT and PAFR-/- mice improves disease outcomes in PAFR-/- mice. In conclusion, PAFR plays a crucial role in regulating gut microbiota in DSS-induced bowel disease. These findings suggest novel drug therapy options, potentially utilizing PAFR agonists for inflammatory bowel diseases. The study acknowledges support from FAPEMIG, CAPES, CNPQ, and PROPP/UFJF.
Keywords: INFLAMMATORY BOWEL DISEASES;LIPID MEDIATORS;PLATELET-ACTIVATING FACTOR RECEPTOR.

PR - 002 - **The intricate relationship between melanoma and mast cells: Implications of mast cell accumulation, anti-proliferative effects, and melanin-mediated crosstalk**

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Mast cells (MCs) are hematopoietic immune cells that play a significant role in various physiological and pathological conditions. While they are primarily recognized for their negative effects on allergies, MCs also exhibit a tendency to infiltrate tumors, often in substantial numbers. Melanoma, the most lethal form of skin cancer, arises from the malignant transformation of melanocytes in the skin. In the context of melanoma, MCs tend to accumulate in the tumor stroma, while their presence withing the tumor parenchyma is comparatively lower in numbers. We previously addressed that MCs exert an anti-proliferative effect on melanoma cells and that this effect is dependent on tryptase, a protease stored in MC granules. Conversely, we have also discovered that melanoma conditioned medium exhibits an inhibitory impact on cultured bone marrow-derived mast cells (BMMCs), and this effect can be attributed to melanin, a pigment abundantly produced by melanoma cells. Melanin has been observed to induce the translocation of tryptase to the nucleus of MCs, resulting in significant nuclear remodeling. Furthermore, melanin contributes to MC stabilization and impairs MC activation through the G protein-coupled receptor Mrgprb2. Remarkably, melanin has also been detected within MCs present in the tumor parenchyma. Collectively, these findings indicate a complex interplay between melanoma and MCs, suggesting that melanin may exert an influence on MCs within the tumor microenvironment.
Keywords: Mast cell;Melanoma;Melanin.

PR - 003 - **IFN-γ AND IL-8 ARE ASSOCIATED WITH ASTHMA SEVERITY IN POLYSENSITIZED INDIVIDUALS**

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Introduction: The amount of sensitization to aeroallergen has been associated with a higher severity of asthma. However, there are few studies evaluating the cytokines profile of atopic severe asthmatics, considering the amount of sensitization to the main aeroallergens. **Objective**: To evaluate the serum levels of cytokines in patients with atopic severe asthma, considering the number of sensitization to different aeroallergens. **Methods**: We evaluated 441 subjects with moderate to severe asthma (MSA) and 450 subjects with mild asthma as control group. Sensitization to the main aeroallergens was defined by the presence of specific IgE in the serum, quantified by ImmunoCap™. A panel of four cytokines/chemokines (IL-5, IL-8, IL-17A and IFN-γ) was measured in serum by the Luminex assay. The results are in pg/mL and expressed in median (1st quartile-3rd quartile). **Results**: First, we performed a comparison between atopic (AT) and non-atopic (N-AT) asthmatics, we observed that regardless of the presence or absence of atopy, serum levels of IL-8 were increased in individuals with MSA [N-AT: 2.1 (1.5-3.1); AT: 2.3 (1.5-3.1)] compared to individuals with MA [N-AT: 1.39 (0.9-2.4); AT: 1.7 (1.1-3.0)]. Regarding the number of sensitization, we noted that among atopic asthmatics sensitized to 2 or more aeroallergens, the serum levels of IFN-γ and IL-8 were higher in individuals with MSA [IFN-γ: 1.3 (0.9-1.9); IL-8: 2.3 (1.5-3.2) compared to individuals with MA [IFN-γ: 1.1 (0.8-1.9); IL-8: 1.6 (1.0-2.8], while for IL-5, levels were lower in individuals with MSA compared to individuals with MA ($p<0.05$). We found no significant differences in IL-17A levels between groups, as we also found no differences in cytokine levels in monosensitized asthmatics. **Conclusion**: Asthma severity is associated with elevated levels of IL-8 regardless of atopy and in polysensitized individuals is related to higher levels of IFN-γ and IL-8.
Keywords: severe asthma;atopy;cytokines.

PR - 004 - **Effects of Brassica oleracea Lectin (BOL) on Neutrophil Migration, Surface Marker Expression, and Phagocytosis**

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Neutrophils are vital immune cells involved in pathogen clearance and tissue repair. Understanding the modulation of neutrophil behavior is crucial for developing therapeutic strategies for immune-related disorders. This study investigated the effects of *Brassica oleracea* lectin (BOL) on neutrophil migration, surface marker expression, and phagocytosis. We observed that neutrophils migrating to the peritoneal cavity exhibited reduced CD62L levels compared to tissue-resident neutrophils, suggesting a potential role for CD62L in neutrophil migration. Moreover, neutrophils from circulation showed decreased CD62L expression with BOL treatments of 5 µg/mL and 10 µg/mL, while other treatments showed no significant differences. Quantification of neutrophils in the peritoneal cavity demonstrated that BOL, particularly at concentrations of 1 µg/mL and 2.5 µg/mL, significantly enhanced neutrophil migration compared to the negative control. These findings highlight BOL's potential as a modulator of neutrophil migration. Furthermore, BOL did not alter the production of reactive oxygen species, suggesting that it may not directly impact neutrophil oxidative burst. In terms of phagocytosis, purified neutrophils from Balb/c mice treated with BOL did not show a statistically significant increase. However, there was increased variability at the 1 µg/mL concentration, suggesting a potential trend that warrants further investigation. In conclusion, this study elucidated the effects of BOL on neutrophil migration and CD62L expression, highlighting its potential as an immunomodulatory agent. While BOL did not significantly affect phagocytosis or reactive oxygen species production, its ability to enhance neutrophil migration suggests a potential therapeutic application in immune-related disorders.
Keywords: Lectin;Neutrophil ;immunomodulation.

PR - 005 - **Probiotics improve vaccine efficacy in antibody response**

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Current vaccination protocols raise concerns about the efficacy of immunization and duration of immune protection. Vaccination is important as a prevention for infectious diseases, however many vaccines do not have the ideal efficacy. Numerous factors can interfere with the post-vaccine response. There is evidence that changes in the intestinal microbiota may alter immune response. The formation of the gut microbiota in neonates plays a fundamental role in immunity. Therefore, the microbiota composition may alter the response to vaccines. Probiotics and prebiotics have positive benefits in the immune system of neonates. Thus, supplementation with probiotics and prebiotics may be interesting to improve immunological responses in neonates through intestinal balance. Methods: Newborn mice with single-dose rabies vaccine or single-dose rabies vaccine associated with treatment were used Nuxcell Neo® (2g/animal/week) for up to 3 weeks. Nuxcell Neo® is an immunomodulatory symbiotic and it was gently donated for this essay. Euthanasia and blood collections took place 7, 14 and 21 days after vaccination, blood was used for analysis of inflammation and concentration of circulating antibodies. our results present an increased in antibodies dectation in animals vaccinated with antirabies associated with Nuxcell Neo® treatment in the days 14 and 21 when compared to the group that received only rabies vaccine. In the inflammatory profile analysis, it was possible to observe that there were no relevant and significant changes between the groups, which demonstrates that the health in the animal remains stable. The results of our study confirm the promising impact of the use of Nuxcell Neo® on the immune system by vaccine responses.
Keywords: Vaccine ;Probiotic;Efficacy.

PR - 006 - **Comparative long noncoding RNAs profile of human macrophages in response to Leishmania amazonensis, L. braziliensis, and L. infantum infection**

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The *Leishmania* parasite can subvert macrophage's inflammatory response to survive in this hostile environment, interfering in the mRNAs and long noncoding RNAs (lncRNAs) host-expression. LncRNAs are long noncoding protein RNA (>200 nucleotides) with multifaceted action, involved in the regulation of gene expression via transcriptional and posttranscriptional mechanisms. Also, lncRNA can act as a sponge of miRNAs, regulating the levels of these molecules and cell homeostasis. We performed RNA-seq from THP-1-derived macrophages infected with *Leishmania amazonensis (La), L. braziliensis (Lb), and L. infantum (Li)*, investigating lncRNA and mRNA profiles. We observed a dysregulated expression of lncRNAs in La, Lb and Li-infected macrophages compared to uninfected, with 30% of differentially expressed (DE) RNAs in Leishmania-infected macrophage corresponding to lncRNAs. Interestingly, the lncRNAs expression presented some species-specific modulation, showing a significant exclusively DE lncRNAs in Li-infected macrophages than La or Lb-infected. The major DE lncRNAs were encoded in intergenic and antisense regions and also we found an overlapping transcript and novel transcripts. We identified modulation of some microRNA host genes (miR-HGs), such as the upregulated MIR34AHG, MIR210HG, MIR9-1HG, MIR22HG, and MIR4713HG by all Leishmania species and the exclusively upregulated in Li infection, MIR23AHG. We also performed a coexpression analysis of lncRNA antisense and its related coding protein gene and we found a potential Cis-regulatory network of lncRNA and immune response, highlighting such as the HIF1A/HIF1A-AS3 and IRF1/IRF1-AS1 pairs. Our data suggest a *Leishmania* specie-specific modulation of lncRNA in human macrophages, leading mechanisms of transcriptional and posttranscriptional regulation of genes involved in immune response and metabolism-altering *Leishmania* infection. **Finnancial support: FAPESP, CNPq**
Keywords: Leishmania;macrophage;lncRNA.

PR - 007 - **Deciphering how chronic heat stress alters the immune response induced by a bacterial challenge in Russian sturgeon**

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Sturgeons are critically endangered species due to anthropogenic activities, leading to their farming worldwide. Unfortunately, the increase in water temperatures produced by global warming represents a challenge to develop sturgeon aquaculture in subtropical countries such as Uruguay. Indeed, during the Uruguayan summer, sturgeons face intolerable warmer temperatures, developing a chronic stress response that weakens their defences, favouring infections that increase mortality and produce economic losses. Here, we combined transcriptomic, metabolomic and proteomic approaches to analyse how chronic heat stress affects Russian sturgeon´s immune response to a bacterial challenge. RNA sequencing showed that chronic heat stress altered liver functions, up-regulating genes related to heat-shock response, protein folding, and lipid and protein metabolism, including proteolytic enzymes. In line with these, lipids, lactate, glucose, and several amino acids were predominant serum metabolites in heat-stressed sturgeons, suggesting that chronic heat stress induces anaerobic energy pathways with lactate production, as well as protein degradation to obtain enough energy to maintain energy demands. Besides, exposure to chronic heat stress up-regulated the inflammatory cytokine IL-1β and altered liver structure, which showed perivascular infiltration of leukocytes and foci of necrotic cells. On the other hand, the bacterial challenge with *Aeromonas hydrophila* induced liver expression of IL-1β, lysozymes and acute-phase proteins (APPs) in sturgeons cultured at a tolerable temperature, but not in those exposed to heat stress. Indeed, proteomic analysis revealed that fewer APPs were identified in the serum of chronically heat-stressed sturgeons. Furthermore, the persistence of heat stress increases sturgeon mortality, being higher after bacterial challenge. Overall, our findings give an insight into the impact of chronic heat stress on the anti-bacterial response in Russian sturgeon. **Keywords:** Russian sturgeon;Anti-bacterial response;Chronic stress.

PR - 008 - **MTOR gene variant rs2536 is associated with COVID-19 death in a Brazilian population**

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Introduction: COVID-19 severity and critical outcome are accompanied by a dysregulation in cytokine production known as a cytokine storm, which is involved in the direct cause of death from acute COVID-19 by damaging the lungs and other organs. The mTOR pathway is essential for orchestrating innate immune cell defense, including cytokine production and is dysregulated in severe COVID-19 individuals. The individual genetic background might play a role in the exacerbated immune response. *MTOR* gene variants may influence its functions leading to poor COVID-19 outcomes such as Intensive Care Unit need and death. In this study, we aimed to investigate the association between *MTOR* gene variant rs2536 and COVID-19 severe outcomes. **Methods:** A case-control study was conducted in Brazilian states, where individuals with mild (207) and severe (285) cases of COVID-19 were recruited, with 40 individuals who died. *MTOR* variant rs2536 was genotyped and a logistic regression analysis and Kaplan–Meier survival curves were performed. **Results and Conclusions:** The rs2536 was not associated with the severity of COVID-19. However, death events occurred 3.33-fold faster in individuals with the TT genotype compared with CT or CC genotype patients, and the log-rank test was statistically significant (HR: 3.327; 95% CI:1.33–8.26; p = 0.0097). Individuals with the TT genotype had an approximately 45% lower probability of survival after 20 days of hospitalization compared with those harboring a CT or CC genotype, indicating that the TT genotype may represent a risk for COVID-19 mortality. **Keywords:** mTOR;genetic variant;COVID-19 mortality.

PR - 009 - **PREVALENCE OF AUTOANTIBODIES AGAINST CELL ANTIGENS IN INDIVIDUALS WITH SARS-COV-2 VIRUS INFECTION IN THE METROPOLITAN REGION OF BELÉM**

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Infection with SARS-CoV-2 can lead to the emergence of autoimmune diseases, as verified in research with critically ill patients who may have systemic immune dysfunction, including abnormal levels of several specific autoantibodies. This study was conducted to assess the prevalence of autoantibodies in individuals infected with the SARS-CoV-2 virus in the metropolitan region of Belém during the years 2020 and 2021. To that end, an evaluation was performed in a cohort of 300 individuals, of which 185 were patients with COVID-19 (3 asymptomatic, while in those with symptoms the degree of severity encompassed: 60 mild, 73 moderate, and 72 severe), while 115 were patients without covid-19. Indirect immunofluorescence assays were used in sensitive HEP-2 cells to detect antinuclear antibodies (ANAs) at a dilution of 1:80. 15% (31/208) of the positive covid-19 cases were observed to have ANAs, and only 5.3% (6/115) of the negative ones, demonstrating a significant difference (p=0.0047). The patterns of ANAs detected were of the following types: 35.5% cytoplasmic, 41.9% homogeneous nuclear, 19.4% nucleolar and 3.2% mixed, and there was no significance in the distribution of these patterns between positive and negative cases for covid-19. These ANA patterns also did not differ in relation to the progression of this disease. Notably, the asymptomatic/mild cases were 9.68% cytoplasmic, 6.45% nuclear and 3.23% nucleolar, whereas e in moderate/severe cases the pattern was 25.81% cytoplasmic, 35.48% nuclear, 16.13% nucleolar and 3.23% mixed. Thus, the prevalence of individuals infected with SARS-CoV-2 with autoantibodies was found to be three times higher than in non-infected individuals. Such findings highlight a failure in tolerance mechanisms in the interaction of this virus with the host, with the development of various autoimmune manifestations among patients with covid-19 as a clinical consequence.
Keywords: SARS-CoV-2;Autoantibodies;Antinuclear antibodies.

PR - 010 - **Impacto oxidativo do tratamento prolongado com metilfenidato em crianças com TDAH.**

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O transtorno de déficit de atenção e hiperatividade (TDAH) é um transtorno do neurodesenvolvimento com prevalência aproximada de 5% em crianças. O TDAH apresenta etiologia multifatorial com alterações oxidativas, inflamatórias e neurotróficas. O tratamento com metilfenidato é eficaz e bem tolerado, entretanto estudos em modelo murino demonstram que o uso prolongado pode resultar em alteração dos parâmetros oxidativos. Avaliamos o efeito do tratamento prolongado com metilfenidato nos marcadores estresse oxidativo em pacientes com transtorno de déficit de atenção e hiperatividade. Realizamos um estudo de coorte prospectivo, onde foram incluídas 62 crianças de 6 a 14 anos, de ambos os sexos, sem tratamento prévio. O diagnóstico de TDAH foi realizado por avaliação psiquiátrica. Três coletas sanguíneas foram realizadas, 0, 12 e 24 semanas de uso de metilfenidato (0,65 mg/kg/dia). Dosagens foram realizadas para determinar a capacidade antioxidante total (FRAP), a atividade das enzimas antioxidantes Catalase (Cat) e Superóxido Dismutase (SOD), assim como dois marcadores de dano oxidativo (peroxidação lipídica (MDA) e proteína carbonilada (PC). Não foi observado alteração na FRAP nos dois tempos de tratamento em comparação ao tempo inicial. Entre as enzimas antioxidantes, observou-se redução na atividade de Cat após 12 semanas de tratamento e aumento na atividade de SOD após 24 semanas de tratamento em comparação aos dois tempos iniciais. Em relação aos danos oxidativos, observou-se aumento no MDA após 24 semanas de uso de metilfenidato em comparação a 12 semanas e níveis de PC maiores no primeiro tempo. Nossos achados demonstram que o uso prolongado de Metilfenidato pode modificar os parâmetros oxidativos, promovendo alteração na atividade de enzimas antioxidantes. Embora tenha sido observado um aumento tempo-dependente de MDA, o uso prolongado de metilfenidato resultou em redução de níveis de PC que são marcadores de dano oxidativo permanente.
Keywords: TDAH;estresse oxidativo;metilfenidato.

PR - 011 - **P2X4 receptors signaling pathway modulates chronic inflammatory muscle pain: involvement of macrophage polarization and inflammatory cytokines**

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We have showed that macrophages are involved in chronic inflammatory muscle pain (Brain Behav. Immun. 95:462, 2021). Now, we aimed to test if P2X4 receptors has a role in chronic muscle pain via macrophage polarization and cytokine release. Male Swiss mice (2 months old) from CEMIB/UNICAMP (5244-1/2018) were used. Carrageenan (Cg, 100µg) was injected into gastrocnemius muscle to induce inflammatory pain and, 10 days later, PGE_2 (1µg) was injected at the same site to reveal the chronic-latent pain. Cg and Lipopolysaccharides (LPS) challenged primary culture of peritoneal macrophages and Murine macrophage strain RAW 264.7, respectively. Role of P2X4 was tested by a selective antagonist, 5-BDBD. Mechanical muscle pain was tested by Randall Selitto. Molecular analytical techniques were used - immunofluorescence (P2X4r and the macrophage polarization), ELISA assay (IL-1β) and RT-PCR (*cd86* - pro-inflammatory macrophage phenotype, *il1b, tnf* and *p2rx4* genes). Statistical analysis was performed by One or Two Way ANOVA with Tukey's post-hoc test and the significance level was at $p<0.05$. 5-BDBD (50 µM/muscle) injected previously to Cg reversed the acute and chronic muscle pain and the increase in IL-1β release. Immunofluorescence qualitative analysis confirmed that $P2X4^+$ were co-localized with $F4/80^+$ cells (macrophages). The challenge of primary macrophages with Cg induced an increase in F4/80+ cells co-localized with CD11c+ (pro-inflammatory phenotype), which was reduced by 5-BDBD. LPS-stimulated RAW 264.7 macrophage showed an increased in *cd86*, *il1b*, *tnf* and *p2rx4* genes. These results suggest that inflammatory insults activate P2X4 receptors expressed in muscle macrophages, which trigger their polarization to pro-inflammatory phenotype. Once modulated by the P2X4 receptors, the pro-inflammatory macrophages may contribute to chronic muscle pain by the increase in inflammatory cytokines. CAPES - Finance Code 001. Sao Paulo Research Foundation (2021/02921-2; 2018/13599-1).
Keywords: P2X4;macrophages;muscle pain.

PR - 012 - **Macrophages polarization, in vitro, during Plasmodium berghei infection: the role of curcumin**

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Malaria is a parasitic infection caused by Plasmodium and transmitted to humans through the bite of an infected Anopheles mosquito. The disease is one of the main causes of morbidity and mortality around the world. Malaria presents two distinct phases, the hepatic, and the erythrocytic phase. In the last stage, the importance of macrophages to perform phagocytosis of red blood cells infected with Plasmodium and produce reactive oxygen species (ROS) and nitrogen (RNS), crucial actions for parasite death that regulate parasitemia. Dependent on the activating stimuli in the microenvironment, macrophages can be classically (M1) activated, and are responsible for pro-inflammatory responses such as phagocytosis and microbicidal activity, while the alternatively activated (M2) macrophages act in tissue remodeling and inflammation resolution. Bioactive compounds, such as curcumin, have previously been demonstrated to reduce pro-inflammatory cytokines and chemokines produced by macrophages, as well as the reduction of parasitemia in infected mice. We investigated whether curcumin could change the function of murine macrophages (RAW264.7) and their polarization after infection with *Plasmodium berghei, in vitro*. To evaluate whether curcumin could interfere with these functions observed, we analyzed phagocytic and microbicidal activities three and 24 hours after treatment with curcumin (5 µM), which were added to macrophages previously polarized. In our findings, curcumin increased the phagocytic activity of macrophages when used in non-inflammatory conditions, and reduced iNOS and arginase activities in all macrophage phenotypes infected, suggesting interference in arginine availability by curcumin, and balance promotion in macrophage polarization in M0 neutral phenotype. These results support insight into curcumin treatment in malaria as an adjuvant, promoting equilibrium between pro and anti-inflammatory responses for a better clinical outcome.
Keywords: macrophage;curcumin;malaria.

PR - 013 - **Metabolic reprogramming modulation during experimental sepsis prevents cognitive decline**

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Sepsis is a systemic inflammatory response that leads to metabolic reprogramming of immune cells, triggering hyperinflammation, immunosuppression, and multiple organ failure. During the hyperinflammatory stage of sepsis, energy is preferentially generated through glycolysis instead of oxidative phosphorylation, despite an adequate supply of oxygen, and lipid synthesis is triggered to promote cell proliferation/growth. In the immunosuppressive stage, cellular metabolic pathways change from glycolysis to fatty acid oxidation. Organ failure may be associated with failure in cellular metabolism to restore oxidative phosphorylation and metabolic homeostasis. The central nervous system is one of the first affected organs, leading to sepsis-associated encephalopathy, which is responsible for sequels in survivors, including cognitive damage. In this study, we evaluated the impact of inhibiting triacylglycerol synthesis during the proinflammatory phase of sepsis development and if it could prevent cognitive decline. C57BL6 mice were subjected to cecal ligation and puncture (CLP). At 6, 24, and 48 hours, mice received therapeutic support, with or without diacylglycerol acetyltransferase inhibitor (iDGAT). A sham-operated group was used as a control. We did not observe any prevention of mortality or clinical alterations between septic groups until 72 hours post-surgery. Serum levels of IL-6 and leukotrienes were significantly enhanced at 6 hours in septic mice and reduced by treatment with iDGAT. Lipid droplets were increased in peritoneal leukocytes in septic mice and reduced in those treated with iDGAT. The bacterial load in the peritoneum was higher in animals treated with iDGAT compared to septic mice. On day 15 post-surgery, we evaluated spatial memory in surviving mice recovered from sepsis and we observed cognitive decline, absent in iDGAT ones, suggesting that modulation of metabolic reprogramming due to sepsis could prevent brain dysfunction and its sequels.
Keywords: Sepsis;Immunometabolism;Cognitive Damage.

PR - 014 - **Role of NFAT1 transcription factor in the generation and function of Memory-like CD8+ T lymphocytes in young and aged mice**

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Memory-like cells, also known as Memory-phenotype, are antigen-inexperienced memory T cells present within hosts that have not been exposed to a foreign antigen(s), such as non-immunized/unprimed mice. It is described that this population increases with aging. Knowing that the NFAT1 transcription factor has an important role in “classic” CD8 T lymphocytes, we questioned his part in the generation and function of Memory-like CD8 T cells. When analyzing peripheral immune tissues (peripheral lymph nodes and spleen) from non-immunized mice, we observed that young and aged mice lacking NFAT1 (NFAT1-/-) presented a higher frequency of cells expressing intermediate and high levels of CD44 compared with the control group (C57BL/6). Most of this population does not express CD49d and expresses high levels of CD127, potentially indicating that these are Memory-like cells. It was also noted that this group of cells grows in aged mice when compared with the younger ones. However, in contrast with what has been described for Memory-like cells, this population expresses low levels of CD62L. In aged mice, most of these cells express CD122, matching the Memory-like description, however, in young mice, the majority express intermediate or low levels of CD122. We are still planning on analyzing additional surface markers, like CCR7, so we can better classify these cells. We also going to study the expression of some transcription factors on the thymus, like PLZF and Eomes, to understand the generation of this group of cells. Furthermore, we intend to analyze functional properties *in vitro*, like IFN-gamma and Granzyme B production, proliferation, and cytotoxic capacity.
Financial support: CNPq and FAPERJ.
Keywords: Memory-like;NFAT1;Aging.

PR - 015 - **Interferon-gamma-induced protein 10 (IP-10) and monokine induced by IFN-γ (MIG) as potential biomarkers for tuberculosis diagnosis**

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Laboratory diagnosis of tuberculosis (TB) still has limitations. There is an urgent need to identify biomarkers to differentiate between latent and active TB and monitor treatment efficacy. The objective of this study was to evaluate the potential of interferon-gamma-induced protein 10 (IP-10) and monokine induced by IFN-γ (MIG) chemokines as biomarkers for the diagnosis of different clinical forms of the disease and early identification of therapeutic failure. These chemokines were quantified by flow cytometry in unstimulated blood culture supernatant from the following groups: patients with first-line drug-sensitive TB (TBS) (n=18), patients with first-line drug-resistant TB (DR-TB) (n=31), individuals with latent tuberculosis infection (LTBI) (n=33) and a negative control group (HC) without infection (n=22). IP-10 and MIG have differential levels between the study groups and significantly higher levels in the DR-TB when compared to the others groups. Furthermore, these biomarkers discriminated between LTBI and active TB (IP-10 P=0.0052; MIG P=0.0001). Our results allow us to conclude that the quantification of IP-10 and MIG in the blood is feasible to be used as a new diagnostic alternative for discrimination of active disease from latent infection, as well as as potential biomarkers for early detection of DR-TB.
Keywords: tuberculosis;chemokine;biomarkers.

PR - 016 - **COVID-19 and HIV: Clinical outcomes and inflammatory markers in a cohort from a reference hospital in Rio de Janeiro, Brazil**

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Background: Severe COVID-19 presents a broad spectrum of clinical manifestations associated with a highly inflammatory profile, including cytokine storm, lymphopenia, and deregulation of lymphocyte subsets. Depending on the immunosuppression levels, a higher risk of hospitalization and mortality has been described for COVID-19 in people living with HIV (COVID/PLWH). This study describes inflammatory markers in COVID-19 clinical outcomes with and without HIV. Methods: The study analyzed 116 inpatients of the Hospital Center for COVID-19 (INI/FIOCRUZ), including 22 cases of COVID/PLWH. Plasma samples were tested for a panel of 15 cytokines (ProcartaPlex™ Multiplex Immunoassay_Thermo Fisher) by Luminex™. Sociodemographic, clinical, and laboratory data were obtained from the patient's clinical records. Mann–Whitney U tests were used for numerical continuous variables, and Fisher's exact tests or χ2 tests were used for categorical/nominal variables. Results: Patients were first analyzed according to the COVID-19 outcomes of discharge and death. Older age [(66.61 (IQR=20.25) *vs* (55.11 (IQR=20.37); p=0.001], and cardiac insufficiency (9.3% *vs* 0%; p=0.021), but not the HIV infection (14.8% *vs* 17.5%; p=0.862;), were more frequent in those evolving to death, who also had higher leucocyte counts and levels of Bilirubin, D-dimer, CRP, ESR, Procalcitonin, Ferritin, and the cytokines IP-10, IL-8, and IL-18. The COVID/PLWH group showed CD4 counts of 64 cells/mm^3 (IQR=239), a CD4/CD8 ratio of 0.12, and a viral load of 4.96log_{10} copies/mL (IQR=1.94). Active tuberculosis was more frequent in this group, as well as lower age [44.37 (IQR=17.84) *vs* 62.61 (IQR=19.37); p <0.001), and lower levels of lymphocytes and cytokines IP-10, IL-8, IL-10, TNF-α, IFN-α, IL-17, IL-1β, IL-23, and IL-18 compared to the COVID-19 group. Conclusion: Although a highly immunosuppressive profile was observed in this COVID/PLWH group, no difference in mortality was observed compared to the COVID-19 group.
Keywords: HIV;COVID-19;cytokines.

PR - 017 - **CYTOKINE PROFILE IN HUMAN SCHISTOSOMIASIS AND ITS RELATIONSHIP WITH FIBROSIS AND TREATMENT**

SILVEIRA-NUNES, G.[1]; DE ALMEIDA, M.M.[2]; LEITE, P.M.[1]; FRAGA, L.A.D.O.[1]; MALAQUIAS, L.C.C.[3]; SPEZIALI, E.[4]; MARTINS-FILHO, O.A.[4]; CARVALHO, A.T.[4]; SILVEIRA, A.M.S.[1]. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA/CAMPUS GOVERNADOR VALADARES, UNIVERSIDADE FEDERAL DE JUIZ DE FORA/CAMPUS GOVERNADOR VALADARES GOVERNADOR VALADARES - MG - BRASIL; 2. UNIVERSIDADE VALE DO RIO DOCE - UNIVALE, UNIVERSIDADE VALE DO RIO DOCE - UNIVALE GOVERNADOR VALADARES - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE ALFENAS, UNIVERSIDADE FEDERAL DE ALFENAS ALFENAS - MG - BRASIL; 4. INSTITUTO RENÉ RACHOU-FIOCRUZ MINAS, INSTITUTO RENÉ RACHOU-FIOCRUZ MINAS BELO HORIZONTE - MG - BRASIL.

Introduction: The cytokine response to *S. mansoni* antigens seems to play an important role in pathogenesis of the periportal fibrosis associated with human schistosomiasis. The aim of this study was to investigate whether the cytokine pattern produced by peripheral blood mononuclear cells upon *in vitro S. mansoni* antigen stimulation could be used as a biomarker of periportal fibrosis in schistosomiasis patients and evaluate the impact of Praziquantel treatment in this complex cytokine network. **Methods:** Thirty-one volunteers living in an endemic area were classified into sub-groups according to the presence or absence of fibrosis before (FIB and non-FIB) and after treatment (FIB_T and non-FIB_T). Cytokine (IFN-g, TNF-α, IL-4, IL-5, IL-13, IL-10, IL-17, TGF-β) was measured in PBMC culture supernatants using Cytometric Bead Array (CBA). Cytokine signatures were analyzed using the concept of low and high-cytokine producers. **Results:** Our results demonstrate that FIB presented a decreased cytokine production compared to non-FIB individuals. Furthermore, FIB produced higher proportion of fibrogenic cytokines whereas non-FIB had higher levels of IL-10 and TGF-β, in the non-stimulated cultures. Two years after treatment, a high proportion of fibrogenic cytokines in response to SEA was observed in FIB_T. Those FIB participants who showed a regression of fibrosis after treatment produced high levels of TGF-β e IFN-g, while non-FIB individuals who remained without fibrosis continued producing high levels of IL-10 e TGF-β. **Conclusion:** These data suggest that the concomitant production of high IL-10 e TGF-β levels is associated with protection against fibrosis, and that specific treatment induces a balanced profile of fibrogenic and regulatory cytokine production in individuals with and without fibrosis**.** **Financial Support:** PAPES IV/CNPq; CPqRR; CAPES; FAPEMIG; UNIVALE; UFJF/GV
Keywords: Schistosomiasis;Periportal fibrosis;Cytokines.

PR - 018 - **Breast cancer cell -conditioned media modulate macrophage secretome promoting cancer cell migration**

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In response to the tumor microenvironment secretome, macrophages differentiate into subtypes that influence, by the soluble mediators, the tumor cell behavior. The aim of this study was to elucidate the paracrine effect of the bidirectional communication between the breast cancer cells (MDA-MB 231 e MCF-7), normal mammary epithelial cells (MCF10A), and THP-1 differentiated macrophages by the generation of conditioned media (CM). Tryptophan (Trp) and kynurenine (Kyn) concentrations were determined by high-pressure liquid chromatography. Cytokine levels were evaluated by flow cytometry using Cytometric Bead Array. Mammary cell line conditioned media showed distinct cytokines profiles. MC MDA-MB 231 showed higher interleukine 6 (IL-6), interleukine 1 beta (IL-1b) e interleukine 8 (IL-8). Macrophages cultured in breast cancer cell CM showed an increase in their viability without reactive oxygen species alteration. The secretome from the macrophages previously cultivated with breast cancer cell secretome showed elevated IL-6, IL-1 beta, and tumor necrose factor alfa (TNF-alfa) levels compared to the control. Furthermore, the conditioned media from the macrophages previously cultivated with breast tumor cells MCs promote the indoleamine 2,3-dioxygenase (IDO-1) suppression by reduction of Kyn/Trp ratio upon lipopolysaccharides stimulation. It also increased breast cancer viability, colony formation capacity, and migration rate. Collectively, our data reinforce the crucial role of the paracrine signaling bidirectional between tumor cells and macrophages and their potential for modulation towards aggressiveness.
Keywords: Tumor assocaited macrophages;breast cancer cell line ;conditioned medium .

PR - 019 - **Identification of immune biomarkers and genotyping polymorphism of susceptibility to leprosy disease in patients and household contacts**

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Background: Leprosy is an infectious disease characterized by heterogeneity of clinical manifestations correlated with the host cell-mediated immune response against *M. leprae*. **Objectives:** To evaluate the immune biomarkers and genotype polymorphism of susceptibility to leprosy disease in patients and household contacts. **Methods:** The cytokines (Th1/Th2/Th17) and chemokines were measured by CBA Human Kit in supernatant from PBMC in vitro culture not stimulated (NS) and stimulated by *M. leprae* (ML). Also, genotyping of single nucleotide polymorphisms (SNP) of the TLR4 genes using qPCR assay for DNA amplification was done. These parameters were associated with clinical data on leprosy using Multivariate analysis and Machine Learning. Our sample consisted of patients with leprosy, newly diagnosed (n1=77), individuals without any sign of leprosy (n2=68), and household contacts (HHC n3=93), all of them from a rural and urban area/Minas Gerais, Brazil. **Results:** A model based on Random Forest (RF) was implemented, which allowed us, with about 97% accuracy, to predict the clinical evolution of HHC by recognizing the pattern of their clinical and immunological variables. We found a pattern of chemokines (IL-8 and RANTES) in stimulated cell culture ML. In addition, we verified a 2.3 times greater chance of illness in the carriers of the G allele in the TLR4 gene. The supposed association between the variant rs1927914 in the TLR4 gene and leprosy susceptibility differs from another study, which found no association of this variant with leprosy per se or with leprosy. **Conclusion:** The contrast between cytokine profiles between sick individuals and those supposedly not contaminated with *M. leprae* brings up interesting hypotheses, especially in the IL-8/RANTES duct, that need further studies. Cytokines/Chemokines would be better characterized by component analysis or by multivariate analysis. FINANCIAL SUPPORT: CNPq, FAPEMIG, EMORY UNIVERSITY, UFJF/PROPP/PROEX.
Keywords: Leprosy; immune biomarkers;genotyping polymorphism .

PR - 020 - **Recognition of Plasmodium vivax recombinant antigen by pools of human sera for purification of IgG antibodies from individuals naturally exposed to malaria**

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Antibodies are naturally acquired during *P. vivax* infections and are a crucial component of the immune response against parasitic antigens, including antigenic proteins such as the C-terminal portion of the merozoite surface protein-1 (PvMSP1-19).This protein is highly immunogenic in humans with the acquisition of high levels of IgG antibodies. We analyzed IgG molecules from mixed long-term storage serum samples and the recognition pattern of MSP119. A total of 231 samples were tested in three sera pools, being each with samples from 77 individuals living in a malaria-endemic area in Pará state, Brazil. We used 77 samples from individuals that were not exposed to malaria as the control group. Pools were analyzed by immunoblotting to investigate the structure of the IgG molecule and the recognition of MSP119 after ex vivo storage for at least ten years at 4ºC. Our results showed the presence of bands, revealing a high concentration of IgG antibodies in all pools of sera, and confirmed that the structure of IgG molecules was complete. As expected, the bands corresponded to the light and heavy chains of the IgG class of antibodies. For the detection of IgG specific to PvMSP119, we used a recombinant His6-MSP119 antigen that, due to recognition and band formation, confirmed the high levels of IgG molecules specific to the *P. vivax* MSP119 antigen through immunoblotting. Results also revealed other bands that were not expected for a specific binding with IgG, and are currently under investigation. Our descriptive analyses are relevant as a contribution to the future purification of IgG from these pools of sera containing PvMSP119-specific IgG .Therefore, the stability of anti-PvMSP1-19 IgG molecules allows for their use in subsequent *in vitro* biological assays to investigate aspects of the humoral immune response in *P. vivax* malaria.
Keywords: Malaria ;Plasmodium vivax ;IgG antibodies.

PR - 021 - **Leishmania infantum downregulates canine macrophage iNOS expression via Histone Deacetylase 1 (HDAC1).**

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There is growing evidence that epigenetic regulation of the host genome by intracellular pathogens modulates acute infection. Epigenetic modification is mediated by chromatin remodeling, histone modifications, and DNA methylation. Histone deacetylases (HDACs) remove acetyl groups from lysine residues on histones, thereby leading to chromatin remodeling and gene silencing. In this study we evaluated if infection by *Leishmania (L.) infantum* affects HDAC-1 and iNOS expression in DH82 canine macrophages. Cells were infected with promastigotes of *Leishmania infantum* (1:15) in RPMI-1640 medium (Sigma®, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco®, USA) inactivated by heat, 0.03% L-glutamine (Sigma®, USA), and 100µl/mL of penicillin (Sigma®, USA) and 100mg/mL of streptomycin (Sigma®, USA). Cultures were maintained at 37°C, 5% CO2. For suppression of endogenous HDAC1 expression, cells were treated with sodium butyrate (Sigma-Aldrich, USA) [20mM], or incubated with HDAC1-siRNA (s150968) and HDAC1-siRNA2 (s119558) (Thermo Fisher). Cells were harvested 6h after infection and stained with anti-human HDAC1 antibodies FITC-conjugated (Santa Cruz Biotechnology©), and anti-human iNOS PE-conjugated (BIORBYT). Fluorescence intensity was measured using BD Accuri C6 software (BD Biosciences, CA, USA). Total RNA was extracted from cells, followed by cDNA synthesis; HDAC1 and iNOS mRNA expression was analyzed by qRT-PCR. Parasitic load also was determined by optical microscopy. We observed an increase in HDAC-1 expression in DH82 cells infected with *L. infantum.* After inhibition of HDAC-1, a decrease in parasite load, associated with increased levels and expression of iNOS, were observed in DH82 macrophages infected with *L. Infantum.* In conclusion, epigenetic regulation of iNOS by HDAC1 is associated with a decrease in parasite load, and is an important mechanism modulating the innate immune response involved with the establishment of infection in canine macrophages.
Keywords: Leishmania infantum;macrophage;HDAC-1.

PR - 022 - **Expression of SmATPDases 1 and 2 in Schistosoma mansoni eggs favors IL-10 production in infected individuals**

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Background and objectives: A role of IL-10 is down-regulating T cell responses to schistosome antigens. Since SmATPDases can be correlated to modulation of the immune response, we evaluated the expression of enzymes in *S. mansoni* eggs. **Patients/Methods:** Fecal samples were collected from 40 infected individuals to detect coding regions of the SmATPDases. The cytokines was measured in supernatants of PBMC. The analysis was performed by the global median determination and set up high producers (HP) of cytokines. **Results:** Six individuals expressed SmATPDase1, six expressed SmATPDase2, and six expressed both enzymes. The group who expressed only SmATPDase1 showed a high frequency of IFN-γ, TNF, IL-4 HP, individuals who expressed only SmATPDase2 showed a high frequency of IFN-γ, IL-6, and IL-4 HP and individuals who expressed both enzymes showed a high frequency of IL-10 HP. In the group that showed expression both enzymes was observed lower indices the ratio between IFN-γ/IL-10. The positive correlation between infection intensity and IL-10 levels remained only in the positive SmATPDase group. The IL-10 is the only cytokine induced by the expression of both enzymes. **Conclusions: Our data suggest that t**he expression of both enzymes seems to be a factor that modulates the host immune response by inducing high IL-10 production.
Keywords: Schistosomiasis;SmATPDases;immunomodulation.

PR - 023 - **Functional non-glycosylated recombinant TIM-3_ECD binds to human monocytes and NK cells and activates lymphocytes in vitro.**

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T-cell immunoglobulin and mucin domain 3 (TIM-3) has emerged as an important immune checkpoint receptor in the tumor microenvironment. The TIM-3 pathway holds potential as a therapeutic target for immunotherapy against tumors, autoimmunity, chronic virus infections, and various malignancies, however, many aspects of the biology of this receptor are still incompletely understood, especially regarding its ligands. Previously we showed that recombinant TIM-3_ECD produced in bacteria provided expression gain of lymphocyte activation markers such as CD69, in activated human peripheral blood mononuclear cells (PBMC) showing a promising activation feature. Here we further investigate the source of this lymphocyte activation aiming to determine either if it was direct or indirect. The recombinant human TIM-3_ECD was produced by expression on microbial system, using Escherichia coli BL21 (DE3) host cell, followed by recovery from inclusion bodies by mild denaturating conditions. The purified recombinant TIM-3_ECD was incubated 24 h with PBMC, followed by incubation with an immune cell biomarkers panel (CD4, CD14, CD16 and CD56), as well as with anti-6xHistag FITC (to detect the recombinant protein) and analyzed by flow cytometry. The results showed that recombinant TIM-3_ECD rather binds to monocytes and NK cells than to T and B lymphocytes, even the profile expression of CD14 and CD16 changes with TIM-3_ECD incubation compared to negative control, suggesting that the lymphocytes activation could be driven indirectly by TIM-3_ECD. Further specific monocytes analysis is ongoing to provide more evidence about how TIM-3_ECD interacts with these cells to lead to T lymphocytes activation. These results raise TIM-3_ECD as a promising molecule to be further investigated to anti-tumor immunotherapy.
Keywords: TIM-3;recombinant protein;immunotherapy.

PR - 024 - **The role of microglia in triggering high fat diet-associated cognitive decline**

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Excessive dietary fat is a risk factor not only for metabolic disorders but also for premature cognitive decline and Alzheimer's disease. Recent data from our group show that brain functions are quickly affected by a high-fat diet. We hypothesise that microglia, instead of simply responding to diet-induced damage, acts as a critical trigger to disrupt synaptic homeostasis. To investigate this hypothesis, we first evaluated in C57Bl6J mice the effect of a HFD over time in inducing cognitive impairment and hippocampal microglia activation. After that, we used a chemogenetic approach (Designer Receptors Exclusively Activated by Designer Drugs – DREADD), designed to conditionally inhibit the microglia for evaluation of metabolic and behavioral outcomes induced by HFD. Just 10 days of HFD lead to changes in hippocampal-dependent behavioral tasks (novel object recognition test) in 3 months-old C57Bl6J male mice that persist until 4 and 8 weeks. Sholl analysis and stereology confirmed microglia morphological changes and reactivation in hippocampal CA1 region after 10 days of HFD, while metabolic parameters (adiposity and weight gain) were increased only after 4 and 8 weeks of HFD in C57Bl6J mice. Interestingly, sub chronic chemogenetic microglial inhibition prevented memory impairment, evaluated by novel object recognition and object reallocation tests, in DREADD mice submitted to HDF for 7 days. All together our data support the notion that microglia play a critical role in triggering mechanisms of hippocampal damage induced by HFD.
Keywords: microglia;obesity;cognition.

PR - 025 - **Cooperation between T and B cells reinforce the establishment of bone metastases in a mouse model of breast cancer**

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Immune cells educated by the primary breast tumor and their secreted factors support the formation of bone pre-metastatic niche. Indeed, we showed that 4T1 tumor-specific RANKL$^+$ CD3$^+$ T cells, arrive at the bone marrow (BM) before metastatic cells and set the pre-metastatic niche. In the absence of RANKL expressed by T cells, there is no pre-metastatic osteolytic disease and bone metastases (BoMet) are completely blocked. Adding to the role of T cells, we have recently demonstrated that DCs assist RANKL$^+$ T cells activities at bone pre-metastatic niche, by differentiation into potent bone resorbing osteoclast-like cells, keeping their APC properties, providing a positive feedback loop to the osteolytic profile. Here we are showing that BM-derived CD19$^+$ B cells, from 4T1 tumor-bearing mice, also express RANKL. Analysis of trabecular bone mineral density by conventional histomorphometry and X-ray microtomography (micro-CT) demonstrated that RANKL$^+$ B cells cooperate with 4T1-primed CD3$^+$ T cells to induce bone loss. Moreover, RANKL expression by B cells depends on T cells activity, since experiments performed with B cells derived from 4T1 tumor-bearing nude BALB/c mice resulted in the maintenance of trabecular bone mass instead of bone loss. Altogether, we believe that 4T1-primed RANKL$^+$ B cells alone are not central mediators of bone loss *in vivo* but when associated with T cells induce a strong decrease in bone mass, accelerating both breast cancer progression and bone metastases establishment. Although several studies performed in different pathological settings, showed that B cells, positively and negatively impact on osteoclastogenesis, due to their capacity to secret pro or anti-osteoclastogenic cytokines, as far as we know, this is the first report showing the role of RANKL expression by B cells on breast cancer-derived bone metastases scenario.
Keywords: bone metastases;T cells;RANKL.

PR - 026 - **Immunomodulatory nanosystems with active targeting to phagocytes promote the production of neutralizing antibodies against the SARCoV2 virus in cows' colostrum**

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Biocompatible immunomodulatory nanosystems active targeted to phagocytes (NIBDAF) were developed by Embrapa based on green nanotechnology approach aiming to improve the efficacy of existing vaccines for cattle. Cows produce a significant amount of immunoglobulin in the colostrum and nutraceutical products with potential usage in viral disease prevention and control have become available for humans recently. The objective of our work was to develop a protocol for immunizing cows in order to produce hyperimmune colostrum with neutralizing activity against SARSCoV2.The recombinant RBD protein was obtained using the Expi293F™ expression system and purified by Ni-affinity chromatography. Pregnant cows were immunized with RBD-Alum adjuvant (InjectAlum®), RBD-NIBDAF or saline buffer (n=5 per group). The study was approved by the Ethics Committee on Animal Use (1915290721). Immunizations and serum sample collections were performed 45, 30, and 15 days before the expected birth date and on the day of parturition, along with colostrum. Production of IgG, IgG1, IgG2 anti-RBD, and viral neutralization were evaluated by ELISA in all samples. Cows immunized with recombinant RBD-Alum adjuvant produced higher amounts of all subclasses of antibodies compared to the RBD-NIBDAF group ($p<0.05$), except for IgG2. Moreover, no significant differences were observed in viral neutralization in serum samples between these two groups (32% in RBD-NIBDAF and 43% in RBD-Alum). In colostrum, NIBDAF provided 66% neutralization compared to 91% in the Alum group. These are promising results since RBD-NIBDAF without any other adjuvant have induced the production of neutralizing antibodies, especially the IgG2 subclasses known to be more effective in complement activation than other IgGs in bovine species. These results also support the use of cows as biofactories of neutralizing antibodies, providing an alternative for preventing future emerging and re-emerging diseases.
Keywords: Colostrum;bovine;nanosystems.

PR - 027 - **Schistosoma-induced regulatory pathways in human infections**

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It has been known that *Schistosoma* infections induce immunoregulatory pathways as a survival mechanism. Regulatory $CD4^+$ T lymphocytes (T reg) are the main cells that participate in this response. We aim of this study was to evaluate the regulatory signature mediated by T reg lymphocytes from individuals infected with *S. mansoni.* **MATERIALS AND METHODS**: Of the 19 participants, 12 (63,1%) had *S. mansoni* infection and 7 (36,8%) had no infection. Regulatory lymphocytes were obtained from PBMC and stimulated with the *S. mansoni* egg antigen (SEA) to phenotypic and intracellular cytokine evaluation performed by flow cytometry. T reg cells were then isolated with magnetic beads for use in T reg lymphocyte-depleted cultures. In these cultures, the levels of IL-10, IL-13, Il-17 and IFN-γ were evaluated by ELISA. **RESULTS**: We observed higher frequencies of Treg lymphocytes ($TCD4^+CD25^+FoxP3^+$), as well as higher expression of IL-10 and TGF-β by these cells. It was also observed in infected individuals a higher frequency of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ T cells, while there was no difference in the expression of CTLA-4 by these lymphocytes. Increased PD-1 and TGF-β expression were also observed in non-regulatory T lymphocyte populations ($CD4^+CD25^{Low}FOXP3neg$). Individuals infected with *S. mansoni* have higher levels of IL-10 and IL-13 in the PBMC supernatant cultures and lower levels of IFN-g compared to non-infected individuals. T reg cell depletion led to a reduction of IL-10 and IL-13 levels, whereas it did not alter IFN-g levels in the group of infected individuals. **CONCLUSION**: In individuals infected with *S. mansoni* the regulatory mechanisms seem to involve IL-10, TGF-β, and PD-1 pathways, but not CTLA-4. Additionally, the expression of TGF-β, PD-1, is not restricted to regulatory populations. These data contribute to increase our knowledge of pathways explored by these parasites favoring the survival of *S. mansoni*.
Keywords: Regulatory CD4+T lymphocytes;Schistosoma mansoni;Schistosomiasis.

PR - 028 - **In the search of cross-reacting human monoclonal antibodies between SARS-CoV-2 variants**

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Since the beginning of COVID-19 pandemics, neutralizing monoclonal antibodies (mAbs) have been sought for use in the infection control, especially for vulnerable individuals, such as immunocompromised and elderly people. The authorization that had been granted to some mAbs was later revoked as new virus variants presenting mutations in the spike protein escaped the original protection. New variants within the omicron strain have evaded the pattern of mAb binding and neutralizing efficacy. The search of mAbs capable of binding to the new epitopes available in the mutant strains continue to be considered valuable for the therapeutics of COVID-19. We report the discovery of human mAbs displaying characteristics that makes us evaluate them as worth of consideration for further development. Recently it was proposed that antibody screening at acidic pH can guide neutralizing antibody selection due to the RBD's up/down conformational change (10.1002/aic.17440). In order to relate the effects of pH with neutralizing antibody selection, our group performed antibody binding experiments by ELISA with BA 2.75 and BA 5 variants of the Sars-COV-2 Receptor Binding Domain (RBD) in PBS (pH 7.4) and pH-adjusted in acetate-phosphate buffer (pH 6, 5 and pH 4.5). Three antibodies recognized the BA 2.75 variant. Only mAb S.11.1.E10 was able to recognize the BA 5 variant, under all experimental conditions. This particular antibody presents a recombination VDJ IGHV3-9, IGHD3-9 and IGHJ6 and lambda light chain. The three antibodies presented here are potential candidates for cross-reacting human monoclonal antibodies between SARS-CoV-2 variants.
To elucidate the region of antigen-antibody interaction, molecular docking was performed with a monomer of the SARS-CoV-2 S Omicron Spike XBB that has the up-form RBD. It was observed that the analyzed antibodies show interaction with the RBD, particularly in the region of the Receptor Binding Motif (RBM).
Funding: FAPESP, MCTI, CNPq, F.Butantan
Keywords: omicron;neutralizing antibody;COVID19.

PR - 029 - **Ursolic acid-rich Cecropia pachystachya extract controls parasitemia in C57BL6 mice with acute experimental Chagas disease**

NICOLAU, S.T.; BARRETO, J.J.; FABRINI, C.D.; STEFANELLO, A.; FERREIRA, C.Z.P.; BORRERO, V.M.S.; AYALA, T.S.; MENOLLI, R.A.. UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ, CASCAVEL - PR - BRASIL.

Chagas disease is a neglected tropical disease with only two drugs available, and extracts from the *Cecropia pachystachya* have already described several compounds with antimicrobial and anti-inflammatory activities. This study evaluated the anti-*Trypanosoma cruzi* action and the immunomodulation exerted by an extract of *C. pachystachya* leaves rich in ursolic acid on experimental acute Chagas disease in C57BL/6 mice. Supercritical extraction with CO_2 was used to obtain the extract (CPE). C57BL/6 mice were infected with a non-lethal amount of blood trypomastigotes of *Trypanosoma cruzi* (Y strain) and treated orally for ten days with CPE, benznidazole (BZN) or both (CPE/BZN). A control group received no treatment. At the end of 20 days of infection, survival, weight, blood leukocytes, NO dosage and IL-4 of splenic cells, and NO of peritoneal cells were evaluated. Animals treated only with CPE showed a reduction in parasitemia compared to the non-treated group, with the survival of 100% of CPE-treated animals, while those in control showed 80%. The animals in the two groups treated with BZN did not have parasitemia detected from the sixth day of counting. The weight of the animals showed no significant difference between the beginning and the end of the experiment. The animals treated with BZN (with or without CPE) showed increased blood leukocytes between the infection's beginning and the fourteenth day, different from the other two groups. Also, the IL-4 dosage of the splenic cells of CPE-treated (with or without BZN) showed a significantly higher concentration than the other groups, while NO secretion from splenic cells was higher in the CPE/BZN-treated animals than the three other groups. It is concluded that treatment with CPE was able to control parasitemia and attenuate the effects of acute experimental *T. cruzi* infection in C57BL/6 mice, which the recognized anti-inflammatory activity of ursolic acid may cause. Financial support: CAPES and Fundação Araucária.
Keywords: treatment;Trypanosoma cruzi;antiparasitic.

PR - 030 - **Deciphering cardiac transcriptional dynamics of chronic chagasic cardiomyopathy in rhesus macaques infected for 20 years**

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Chagas disease, caused by the *Trypanosoma cruzi* protozoan, is an endemic parasitic disease of Latin America, affecting 7 million people. Although most patients are asymptomatic, 30% develop complications, including the often-fatal chronic chagasic cardiomyopathy (CCC). Here we described genes associated with heart injury, parasitemia, and NOS2 expression in rhesus monkeys (RM) using transcriptomic analysis by RNA sequencing (RNA-seq). Briefly, animals were inoculated subcutaneously with 10e4 trypomastigotes/kg of the Colombia strain, followed for up to 26 years, and euthanized. Samples from the ventricle were taken, and total mRNA was extracted, and reverse transcribed and processed to RNA-seq. Two healthy and four *T. cruzi*-infected RM were analyzed. Cardiac gene expression profiling allowed us to identify 602 differentially expressed genes (DEGs); 11 downregulated and 591 upregulated genes were identified in the infected animals. From the downregulated genes, SDH, *FBGF-11*, *CXCL1, CXCL3,* and *MBPC* were noteworthy. From the up-regulated genes, 399 were related to Ig-like domain containing proteins (*IGLV, IGKV, IGHV*; ex. IGHM, FDR= 2.22e-21; IGKC, FDR=7.84e-12; IGLV5-45, FDR=7.89e-07) and BCR genes. Among the 80 most highly expressed genes all except *CXCL9, cathepsin* and *SH2D1A* were related to the Ig family. Immune related genes that were upregulated were related to regulatory functions of T, B, NK, and macrophage. Next, gene expression were correlated with clinical data. *IGKV-1, IGLV9-49,* and *NPR3* were related to fibrosis; *RAB3B, IGLV-10-54,* and *IGLV3-27* with cardiac *NOS2* expression; and *CELA2B, RAB17*, *IGLV3-10* and *SSPO* with heart parasitemia. Biological processes affected by CCC are mainly involved in the regulation of immunoglobulins and B cells. Those processes are important in determining disease outcome. Understanding genes associated with disease may lead to improved insight into CCC pathogenesis and the identification of prognostic factors for CCC progression.
Keywords: Heart transcriptome;Chagas disease;rhesus macaques.

PR - 031 - **Evaluation of immunomodulation of adipose tissue infected with Trypanosoma cruzi in indirect co-culture with peripheral blood mononuclear cells treated with Benznidazole**

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Adipose tissue (AT) plays a crucial role in energy homeostasis, but it is also associated with the immune response and acts as a reservoir of infection for parasites. *Trypanosoma cruzi* can use AT as a possible mechanism to evade the host's immune response. Benznidazole (BZ), a drug used to treat Chagas disease, may reduce its effectiveness if the AT works as a barrier. Therefore, we propose an indirect co-culture model that evaluates the immunomodulation in the microenvironment of human AT infected by *T. cruzi* in indirect contact with peripheral blood mononuclear cells (PBMC) and treatment with BZ. For this, we used stem cells derived from human AT that were differentiated into adipocytes. After differentiation, adipocytes were infected with *T. cruzi* (T) for 3 hours. Then, we added PBMC from volunteer individuals (n = 5) to the upper insert of the culture plate and after 48 hours the cells were treated with BZ. After 72 hours of treatment, the culture supernatant was collected for cytokine and chemokine dosage through the cytometric bead array. In addition, infected adipocytes were quantified for parasite load assessment using the TcSAT-IAM system. Our results showed a high parasite load in the infected culture conditions. However, for PBMC+AT+T+BZ there was a decrease when compared to PBMC+AT+T. We observed higher levels of IL-2 and IL-6 in the PBMC+AT condition compared to the PBMC (p=0.0014; p=0.0369). In contrast, we found decreased production of TNF and IL-8 in culture conditions where AT is present (PBMC+AT) compared to PBMC (p=0.0332; p=0.0208). We also observed an increase in MCP-1 in the PBMC+AT condition compared to the PBMC+AT+T culture conditions (p=0.0106). Therefore, BZ did not induce immunomodulation in the experimental conditions. However, we believe that the interaction between PBMC and AT promoted immunomodulation through increased expression of receptors that may lead to decreased or increased cytokines and chemokines, among others. **Keywords:** Immunomodulation;Adipose Tissue;Trypanosoma cruzi.

PR - 032 - **Potential of Mygalin as a regulator of the inflammatory response**

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Introduction: Mygalin is a synthetic acylpolyamine analogue of spermidine, isolated from hemocytes of the spider Acanthoscurria gomesiana. This molecule is bactericidal activity and acts by regulating the inflammatory response induced by LPS in macrophages. Molecular docking analysis has been widely used to evaluate the interaction of new compounds and molecules involved in the immune response. In this study we will analyze, through molecular docking analysis, the ability of Mygalin to interact with immune response products to better understand its immunomodulatory role. Further, evaluate its antioxidant activity. Methods: The analysis of the interaction of Mygalin with the COX-2 and iNOS proteins was performed by molecular docking using the Autodock Vina program, and the receptor-ligand binding interactions were visualized and analyzed using the Discovery Studio Visualizer software. In vitro, analysis was performed with Raw 264.1 lineage pre-treated with Mygalin (50-450 µM) and subsequently stimulated with LPS (100 ng/mL) for 6 hours. The total RNA extraction was performed and then converted to cDNA which was used as a template for amplification of the genes for COX-2, iNOS and β-actin, as an internal control, using specific primers. The antioxidant activity of Mygalin (0-1000 µM) was evaluated using DPPH essay. Results: In silico analyzes showed that Mygalin has interactions with key amino acid residues for COX-2 (ARG121 and TYR356) and iNOS (GLN257 and GLU371), and these key amino acids are those that interact with their specific substrates. In the in vitro essay, the addition of Mygalin significantly reduced the expression of iNOS and COX-2 induced by LPS and had antioxidant activity. Conclusion: The in silico and in vitro assays, confirm the anti-inflammatory and antioxidant activity of Mygalin.
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Keywords: Mygalin;Acilpolyamine;Inflammation.

PR - 033 - **Flavonoid agatisflavone regulates miR146a and miR155 and the neuroinflammatory response in β-amyloid-stimulated human microglia**

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INTRODUCTION: The control of microglia activation and the neuroinflammatory process are strategies that have been investigated in the development of new therapeutic approaches for neurodegenerative diseases (NDD). Agathisflavone, a biflavonoid purified from the leaves of Cenostigma pyramidale (Tul.), has demonstrated anti-inflammatory and neuroprotective properties in in vitro and ex vivo models of NDD. **OBJECTIVES:** to investigate the anti-inflammatory mechanisms of agatisflavone in human microglial cells in terms of modulation of the expression of microRNAs and inflammatory mediators and signaling after an inflammatory stimulus associated with Alzheimer's disease. **METHODS:** Cultures of human microglia of the C20 lineage were exposed to oligomers of the β-amyloid peptide (AB, 500 nM) for 4 h or to lipopolysaccharide (LPS, 1 µg/mL) for 24 h and then treated or not with agatisflavone (1 µM) for 24 h.**RESULTS:** AB and LPS induced microglia cultures to assume an activated inflammatory state, with increased expression of miR-146a and miR-155 and inflammatory mediators IL1-β, IL-6 and NOS2. However, in cells exposed to inflammatory damage and treated with agatisflavone, we observed a significant reduction in the concentration of miR146a and miR-155, as well as the evaluated inflammatory cytokines. We also observed in cells stimulated only with AB, an increase in the p-STAT3/STAT3 signaling protein ratio, and a reduction in the p-STAT3/STAT3 ratio in microglia cells stimulated with AB and treated with the flavonoid. **CONCLUSION:** Thus, these data reinforce the anti-inflammatory effect of agatisflavone, highlighting its potential in the regulation of miRNAs associated with neuroinflammation, and its potential as a promising molecule for the adjuvant treatment or prevention of NDD. **SUPPORT:** CNPq; INNT; CAPES;UFBA;FIOCRUZ;IDOR.
Keywords: agathisflavone;human microglia;anti-inflammatory effect.

PR - 034 - **Effect of herbal preparation from Euphorbia tirucalli (avelós) and Synadenieum grantii (janaúba) in in vitro growth and chemotherapy of human breast cancer cells (MCF-7)**

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Introduction: Herbal preparation from *Euphorbia tirucalli* (avelós) and *Synadenieum grantii* (janaúba) is popularly used to treat different types of cancer in Brazil. The latex in these preparations is composed of a complex mixture of biologically active molecules. However, there is no scientific evidence of the beneficial effect of these preparations in the treatment of cancer. **Objective**: To investigate the *in vitro* effect of herbal preparation in both the proliferation of breast cancer cells (MCF-7) and these cells submitted to chemotherapy with doxorubicin. **Material and methods**: We used two-dimensional (2D) and three-dimensional (3D) cell culture to evaluated morphological alterations, gene expression, and migration capability of MCF-7 cells treated with natural and filtered preparation of avelós (NA, FA) and janaúba (NJ, FJ). The 3D cultures were generated in a methylcellulose solution (5%). The cells were deposited in a U-bottom non-adherent plate, at a concentration of $1x10^4$cell/ 100mL/well. The spheroids (after 4 days of production) were treated with NA, FA, NJ, FJ, and doxorubicin solutions. **Results**: The results demonstrated that most concentrations of natural or filtered latex solution significantly stimulated (*$p<0.05$) the *in vitro* growth of MCF-7 compared to untreated cells (control). The effect of chemotherapy was evaluated when the cells were exposed to herbal solutions after treatment with doxorubicin (2.5 mg/ml). The results showed that latex reduced the effects of anti-neoplastic agents in 2D and 3D culture. Moreover, MCF-7 spheroids increased the invasiveness after latex exposure. The latex induced the relative expression of cytokine's genes such as IL-1, IL-6, TNFa, IL-10 and TGF-b. **Conclusions**: Latex of Avelós and Janaúba preparations exhibited important pro-tumor activity when test *in vitro* in human breast cancer cells (MCF-7).
Financial support: FAPESP and CNPq.
Keywords: Herbal preparation;MCF-7;3D cell culture.

PR - 035 - **IMMUNOMODULATORY PROPERTIES OF FIBER-DERIVED SHORT CHAIN FATTY ACIDS ON MURINE PERITONITIS AND SEPSIS**

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Dietary fiber fuels the inflammatory response during peritonitis induced by the commensal bacteria *Bacteroides fragilis* and sterile cecal contents (SCC), through activation of the NLRP3 inflammasome and induction of IL-1β, acting as a danger sign in extraintestinal sites. However, the role of soluble fiber as a source of short-chain fatty acids (SCFAs) in the gut was not explored in this model, although its beneficial effects have already been demonstrated in several inflammatory scenarios. SCFAs, as acetate and butyrate, are produced by microbiota fermentation of fiber and have immunomodulatory properties, acting both through histone deacetylase (HDAC) inhibition and metabolite-sensing receptors, as GPR43. Here, we set out to assess whether dietary fiber intake, as well as GPR43 receptor signaling, impacts on inflammatory responses during murine peritonitis and sepsis and affects the disease outcome. GPR43-deficient mice are more susceptible to peritonitis induced by *B. fragilis* and SCC, showing a higher score of abscesses and greater cellularity in the peritoneal cavity, more specifically in the polymorphonuclear population, accompanied by higher production of IL-1β and lower IL-10 secretion, when compared to wild-type mice. Adoptive transfer of peritoneal macrophages from C57BL/6 mice to GPR43-deficient animals was able to revert the phenotype. C57BL/6 mice fed a soluble fiber-deprived diet showed a higher score of abscesses, reproducing the phenotype observed in GPR43-deficient mice. Using the model of polymicrobial sepsis induced by CLP (cecal ligation and puncture), the absence of GPR43 resulted in increased cellularity (and polymorphonuclear cells) in the peritoneal cavity. Our results suggest that fiber-derived metabolites, as SCFAs, present immunomodulatory functions via GPR43 during peritonitis and sepsis, attenuating the acute inflammation and, possibly, preventing the long-term commitments.
Keywords: SCFAs;Peritonitis and Sepsis;inflammation.

PR - 036 - **Is there any difference in the in situ immune response in active localized cutaneous leishmaniasis that respond well or poorly to meglumine antimoniate treatment or spontaneously heal?**

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Localized cutaneous leishmaniasis caused by *Leishmania braziliensis* can either respond well or poorly to the treatment or heal spontaneously; It`s seems to be dependent on the parasite and/or host factors, but the mechanisms are not fully understood. We evaluated the in situ immune response in eighty-two active lesions from fifty-eight patients prior to treatment classified as: early spontaneous regression (SRL-n=14); treatment responders (GRL-n=20); and non-responders (initial/relapse, PRL1/PRL2-n=24 each). Immunohistochemistry was used to identify cell/functional markers which were correlated with the clinical characteristics. PRL showed significant differences in the lesion number/size, clinical evolution, and positive parasitological examinations, when compared with the other groups. SRL presented a more efficient immune response than GRL and PRL, with higher IFN-γ and NOS2 and lower expression of macrophages, neutrophils, NK, B cells, Ki-67+ cells. Compared to SRL, PRL had fewer CD4+T cells and more CD163+ macrophages. PRL1 had more CD68+ macrophages and Ki-67+ cells but less IFN-γ than GRL. PRL present a less efficient immune profile, which could explain the poor treatment response; while SRL had a more balanced immune response profile for lesion healing. Altogether, these evaluations suggest a differentiated profile of the organization of the inflammatory process for lesions of different TL evolution.
Keywords: host-parasite interaction;cutaneous leishmaniasis;immunopathology.

PR - 037 - **EFFECT OF DIETARY SUPPLEMENTATION WITH DHA-RICH FISH OIL ON PULMONARY IMMUNE RESPONSE OF C57BL/6 MICE INFECTED WITH PLASMODIUM BERGHEI ANKA**

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The malaria-associated acute respiratory distress syndrome (ARDS) occurs more frequently in adults than in children and is considered an important complication of malarial infection, which can lead to death. Among the factors that contribute to pulmonary involvement, the secretion of inflammatory mediators, endothelial vascular permeability and adherence of parasitized red blood cells to endothelial capillaries stand out. Fish oil has been widely studied for its potential to modulate the immune response, being indicated as a possible alternative in the treatment of ARDS. Thus, this study aims to evaluate the protective role of fish oil rich in DHA at the pulmonary level during experimental malaria, for this purpose, C57BL/6 mice were prophylactically treated with 3.0 and 6.0 g/Kg of DHA in fish oil for 15 days, followed by infection with *Plasmodium berghei* ANKA. The animals were evaluated daily for the clinical profile, being euthanized on the 7th day post infection for evaluation of the histopathological profile of the lungs, evaluation of the cytokine profile, occurrence of vascular permeability, edema and cellular profile. Treated animals showed a significant increase in their survival, as well as a preservation of clinically evaluated motor activities. Likewise, blood-alveolar barriers were preserved and pulmonary edema was reduced. All these data are corroborated by histopathological analyses. When analyzing cytokines profile, the treated group showed reduced level of proinflammatory cytokines (TNF-α and IFN-g) when compared to the only infected group and an increase in IL-10 level in treated groups. Analysis of the cellular phenotypic profile showed a decrease in total leukocytes as well as a decrease in CD8 T lymphocytes in previously treated animals, as well as a modulation in the activation of macrophages and dendritic cells.
Keywords: malaria;ARDS;immune response.

PR - 038 - **Analogues of Caulerpin alkaloid, N-Methyl and N-Methyl O-Ethyl, inhibit inflammatory response in a murine model of Zymosan-induced peritonitis**

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Caulerpin (CLP) is an alkaloid extracted from the seaweed *Caulerpa racemosa* with anti-inflammatory activity. In our laboratory, previous studies showed anti-inflammatory activity of CLP in murine models of ear edema, peritonitis, acute lung injury and colitis, through the inhibition of leukocyte migration and the production of pro-inflammatory cytokines. Therefore, the aim of this study was to generate analogues of the classic CLP molecule, in order to increase its lipophilicity and consequent increase in permeability in biological membranes, aiming to produce an increase in its anti-inflammatory effect, which were evaluated in a murine model of zymosan-induced peritonitis. Therefore, structural modifications were carried out in the indole nucleus and in the ester group of CLP, to get the two analogues, N-methyl and N-methyl-O-ethyl. Then, male *Swiss* mice were treated orally with the two different analogues (4 and 2 mg/kg) -, classic CLP (2 mg/kg) or Dexamethasone (1 mg/kg) and one hour later, the animals received zymosan intraperitoneally (40 mg/kg). After 24 hours, the animals were euthanized, the peritoneal exudate collected, centrifuged, the supernatant separated for IL-6, MPO and NO measurement and the pellet used for infiltrating leukocyte count. The data obtained showed that CLP analogues were more effective in decreasing cell migration than classical CLP. Regarding IL-6, except for the N-methyl-O-ethyl group at a dose of 2 mg/kg, all doses of classic CLP and analogues were effective in decreasing the levels of this cytokine in peritoneal lavage. No significant levels of NO or MPO were detected in the studied exudate. Consequently, CLP and analogues were effective in reducing the inflammatory process in a murine model of Zymosan-induced peritonitis.
Keywords: Peritonitis;Analogues;Caulerpin.

PR - 039 - **Computational Biotechnology application to promote better antigen-antibody response**

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The antigen-antibody interaction is applied in many scientific and laboratory tests. The specificity and affinity of the binding is an important factor in the selection of antibodies. Traditional methods of antibody production were incremented based on molecular knowledge and recent works report the sequencing and recombinant production of antibody chains or fragments (Fab, scFv) allowing their reproducibility. This highlights ways of development and editing antibodies sequence using biotechnological tools. In this context, the use of in silico methods that consider the three-dimensional conformation and the physicochemical properties related to the amino acid sequence can direct the in vitro tests, reducing expenses and time spent. The present work aims to design and analyse antibodies, testing their affinity to antigens from structural bioinformatics, directing changes in the amino acid antibody sequence to increase affinity to the target. The interaction of 100 single-domain antibodies (sdAb) with their specific and also non-specific antigens was tested using three-dimensional models to identify patterns of molecular docking and interactions. According to results, modifications were made in the CDR-H1, H2 and H3 loops of the sdAbs by homology modelling, altering the amino acid sequence to increase the affinity for the new targets. Different patterns were observed in the simulations involving specific and nonspecific antigens, demonstrating the accuracy of the methodology used, with 100% of the antibodies having better binding patterns with their targets. In addition, the incorporation of modifications according to the observed binding patterns for nonspecific antigens allowed an increase in the number of interactions and bindings in all cases. The research reinforces the use of computational methods to guide the antibodies design and improve their affinity with target molecules, potentially reducing the time and costs associated with their development.
Keywords: IN SILICO;ANTIBODY;ANTIGEN.

PR - 040 - **Early transcriptional immune signature predicts chronic arthralgia post-Chikungunya infection**

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Introduction: Chikungunya virus (CHIKV) is an arthritogenic alphavirus that can frequently cause post-acute viral syndrome (PAVS), with painful symptoms as in Long COVID. Chronic Chikungunya is mainly characterized by chronic arthralgia (CA) and can persist for months and years, significantly impacting quality of life. Currently, there is no vaccine or therapy. Identifying mechanisms behind disease progression can reveal potential diagnosis and therapeutic targets to mitigate Chikungunya and other PAVS.Methods: We analyzed bulk transcriptome of PBMC obtained during acute CHIKV infection of patients that further recovered (n=11) or evolved to CA persisting for one year (n=24). Healthy controls (n=9) were also included. Total RNA extracted from PBMC was used for library preparation with TruSeq Stranded Total RNA Library Prep and sequenced with NextSeq(Illumina). DESeq2 was used to identify differentially expressed genes (DEG). RT-PCR of the selected differentially expressed genes was used to validate the results in another group of CA (n=21) and recovered (n=11) cases. Results: Blood samples were collected in a median of 1 (1-3) days after disease onset for both groups, with more females in the CA group (66% versus 36%). Seven genes (PTGER3, IKZF2, ACKR3, TMEM176B, TMEM176A, NCS1, ST8SIA1) were differentially expressed between CA and recovered (Log2FC ≥|1| and FDR ≤0.1) and further validated by RT-PCR. CA group presented higher expression of IKZF2, which codes the Helios transcription factor produced by T regulatory cells. Among the three genes downregulated in the chronic group, ACKR3 acts as a scavenger for CXCL12 and is expressed by antiviral patrolling monocytes. Expression of ACKR3 negatively correlated with viral load. In conclusion, our findings revealed potential prognosis biomarkers for chronic chikungunya. In addition, results suggested early regulation of immune response can be involved in the progression to PAVS, revealing potential targets for therapy.
Keywords: chikungunya;rna-seq;biomarker.

PR - 041 - **LEARNING JOURNAL (LJ): EMPOWERING STUDENTS THROUGH COMPETENCY-BASED LEARNING**

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The Learning Journal (LJ) emerged in 2022 as a facilitating learning strategy. To create it, the professor needs to have a clear understanding of the cognitive, skills, and attitudes competencies they want to develop in their students by the end of the course. It is crucial to reference the National Curriculum Guidelines, the Teaching Plan, and consider technological trends, the job market, and the post-pandemic scenario. The development of LJ occurs during the planning of the classes, listing macro-competencies such as "understanding the immune system" and "comprehending immunological tolerance," for example. However, these are broad and abstract for students. It is essential to break them down into small, hierarchical parts using the revised Bloom's Taxonomy and Miller's pyramid. For instance, for the macro-competency "understand the immune system," micro-competencies are listed: listing primary organs, listing secondary organs. The appropriate choice of verbs (actions) is essential for self-directed study, helping students with attention difficulties or other special situations. Advanced students benefit as the LJ allows them to go beyond the classroom content. Assessments should be for learning (AFL) and described in the LJ. AFL must be the driving force for students to be protagonists of their learning and pursue competencies. They should be planned to evaluate macro-competencies directly related to the profession and assess the micro-competencies listed in the LJ. The LJ is a tool for self-assessment and peer assessment. The professor can randomly request LJ verification, engaging in a friendly conversation with the student about their individual performance. The design of the LJ facilitates the self-assessment process with emojis reflecting the student's perception of their learning, providing feedback, and enabling formative assessment. In higher education, the LJ is innovative, allowing the professor to conduct classes in any format.
Keywords: Competency-based Learning;AFL;higher education.

PR - 042 - **DISTINCT CD105 (ENDOGLIN) EXPRESSION ON NORMAL BONE MARROW B CELLS AND LEUKEMIA BLASTS IN CHILDHOOD B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA**

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Introduction: B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL) corresponds to about 80% of ALL, mainly affecting children and adolescents. Relapses affect about 20% of patients, with 40-70% overall survival after recurrence, highlighting the need for new therapeutic approaches. The CD105 molecule (endoglin) is a TGF-β co-receptor, its expression in BCP-ALL blast cells is related to a poor prognosis. In this context, the analysis of CD105 expression in normal and neoplastic lymphoid precursors can be an important tool to understand the role of this molecule. In addition, it can contribute to monitoring Measurable Residual Disease (MRD) analysis. **Methods and Results:** Bone marrow (BM) samples from patients diagnosed with BCP-ALL, aged between 0 and 18 years, treated at Hospital Aristides Maltez (HAM) were analyzed. Samples were previously evaluated by flow cytometry for diagnostic purposes (D0) and MRD follow-up (D33). Panels of antibodies that meet the classification of the World Health Organization (WHO) were used, with the inclusion of the anti-CD105 antibody. We evaluated CD105 expression in normal B cell precursors, mature B cells, and leukemic blasts from pediatric patients with BCP-ALL. CD105, at diagnosis, was expressed in 73.33% of patients with BCP-ALL, with the highest expression in leukemic blasts. When compared with DRM, the highest expression was in the hematogonia in negative DRM (67.28%), median fluorescence intensity (MFI) of 87.26. When analyzed individually (monitoring from D0 to relapse of one patient), the blasts had a higher expression and MFI, 21.56% and 26.14, respectively. **Conclusion:** We observed positive CD105 expression in 33% of the included patients, with higher population expression and high MFI. Furthermore, we observed a higher expression of CD105 in more immature B cells, suggesting a correlation with the maturation process of the B lineage.
Keywords: BCP-ALL;Maturative curves;Endoglin.

PR - 043 - **Profile of programmed death receptor 1 and ligands in lepromatous leprosy**

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O receptor de morte programada 1 (PD-1) é expresso durante a ativação dos linfócitos, prevenindo a resposta efetora exacerbada e proporcionando tolerância celular. Quando esses gatilhos são atenuados, as células perdem esse receptor, porém em condições crônicas essa molécula permanece constitutiva causando a exaustão celular. Assim, as células perdem progressivamente a capacidade de produzir IL-2, TNF e IFN-γ, respectivamente, tornando-se hipofuncionais e não responsivas aos antígenos. Assim, nossa hipótese é que a ativação e a manutenção de PD-1 podem ser importantes no desenvolvimento do perfil supressivo em pacientes com hanseníase lepromatosa (LL). Assim, coletamos amostras de sangue periférico de 8 pacientes LL e 13 borderline tuberculoide (BT), antes da poliquimioterapia, e também de 11 voluntários saudáveis de área endêmica para a doença. Então, no presente trabalho avaliamos as frequências das subpopulações de linfócitos T PD-1+ e monócitos apresentando ligantes de PD-1 na superfície celular por meio de citometria de fluxo, bem como a concentração de PD-1 solúvel (sPD-1) em todos os participantes por ELISA. Nossos resultados revelaram uma frequência aumentada de linfócitos T $CD3^{+}CD4^{+}$ $PD\text{-}1^{+}$ e $CD3^{+}CD8^{+}PD\text{-}1^{+}$ em pacientes LL em comparação com BT. Também identificamos um aumento de $CD14^{+}CD16^{neg}$, $CD14^{+}CD16^{+}$ e $CD14^{neg}CD16^{+}$monócitos expressando PD-L1 e PD-L2 em pacientes lepromatosos em contraste com BT. Além disso, pacientes LL têm uma concentração maior de sPD-1 em comparação com pacientes BT e voluntários saudáveis. E esse parâmetro, em pacientes LL, foi inversamente correlacionado com a idade. Em conjunto, nossos dados sugerem que pacientes LL apresentam maior chance de desenvolver exaustão celular do que indivíduos BT, e esse perfil celular pode contribuir para o perfil supressivo, tipicamente apresentado nessa forma clínica.
Keywords: Programmed death receptor 1 ;Cell exhaustion; Lepromatous leprosy.

PR - 044 - **Role of Tumor-infiltrating immune cells and systemic immune mediators in the overall outcome role and as biomarkers in cervical cancer patients submitted to chemoradiation therapy**

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Cervical cancer (CC) shows variable response rates to the standard chemoradiation therapy protocols and high mortality. The patient's immune status can affect cancer progression, response to therapy, and disease recurrence. Here, locally advanced CC patients were evaluated before treatment and classified as responders (R) and non-responders (NR) according to standard chemoradiation therapy response. First, our results showed a higher percentage of tumor-infiltrating lymphocytes (TILs) in R patients, while NR patients presented higher numbers of $CD8^{+}$ and $PD\text{-}L2^{+}$ TILs as well as the PD-L1 IR. In addition, NR patients have higher levels of several systemic soluble mediators and correlation between them and TILs immune markers, suggesting a role of functional polarization of CD4 T cells toward the Th1, Th2, Th17, and T-reg subset in R patients while correlations indicate $CD8^{+}$ and $CD68^{+}$ role in the NR group. ROC curves analysis showed some immune markers and cytokines, such as PD-L1-IR area (Total and intra-tumoral region), PD-L2 (Total and stromal region), CD8, FGF-basic, IL-7, IL-8, IL-12p40, IL-15, and TNF-a that are promising candidates as predictors of the CC patient's response to chemoradiation. Taking all these results together, we suggest that the dysfunctional state of TILs and imbalance in soluble immune mediators' production may be associated with therapeutic failure. We also bring new mechanistic insights suggesting cooperation between local and systemic immune responses. In summary, these findings may help establish immunological patterns associated with predicting response to conventional treatment and also assist in CC prognosis.
Keywords: Tumor-Infiltrating Lymphocytes (TILs);cytokines;cervical cancer.

PR - 045 - **Characterization of the immune profile in patients with oral squamous cell carcinoma (OSCC)**

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Background: Oral squamous cell carcinoma (OSCC) causes over 350,000 cases annually and particularly impacts populations in developing countries. In Brazil, this cancer affects 15,000 people per year with about six thousand deaths. The major risk factors are smoking and alcohol consumption. Determining the immune profile in patients we can elucidate immune mechanisms behind disease progression and develop more effective precision immunotherapeutic regimens. **Methods:** We performed a prospective cohort of 23 OSCC, peripheral blood samples were collected to analyze the soluble and cellular immune profile using bioplex platform and multiparametric flow cytometry, respectively. **Results:** Casuistry characteristics included tumors sites: Tongue (39.1%); Buccal mucosa (21.7%); Hard palate (4.3%); Gengive (21.7%) and Buccal floor (13.0%). Regarding risk factors: 56.52 % are no smoking and 47.83% are alcohol consumption. When we separated the patients for clinical stage, the major patients were in stage 4 of disease (39.1%). Based on these clinical characteristics, we evaluated the soluble and cellular factors and observed that patients in quarter stage had higher expression of cytokines IL1-ra, IL8 and MIG. In addition, we correlated the tumor size (pT) with the expression of cytokines, observed greater expression of cytokines IL-6, IL-12p40 and IP10 in patients with more advanced tumors. We also observed cell markers in the groups of patients that were studied through flow cytometry that corroborate the findings mentioned above **Conclusion:** Our preliminary data show that these patients have an exhausted immune profile. Thus, future studies may better clarify the role of exhaustion and whether these patients would benefit from any immunotherapy.
Keywords: Oral squamous cell carcinoma;Oral Cavity ;immune profile.

PR - 046 - **Extracellular microvesicles contribute to a specific immune response related to the antimonial treatment of Cutaneous Leishmaniasis patients**

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Human cutaneous leishmaniasis (CL) caused by *L. braziliensis* is the most common form of leishmaniasis, causing skin lesions, progressing to severe lesions in the mucous membranes. Its clinical evolution depends on the interaction between the parasite and the cellular immune response. In CL an efficient immune response are in charge to a beneficial resolution of lesions. This interaction may be performed through releasing of cytokines and extracellular vesicles (EVs). EVs, comprising exosomes, microvesicles (MVs) and apoptotic bodies, are formed and released by most eukaryotic cells under different physiological and pathological conditions and having important contributions in the modulation of immune responses in several diseases. **Aim:** to investigate the MVs contribution in the CL pathogenesis, through quantifying and phenotyping the MVs using nano-flow cytometry. **Methods**: Plasma and supernatant of antigen-stimulated-PBMC-cultures were obtained from before, during and at the end treatment patients and submitted nano-flow cytometry. **Results:** we observed higher frequencies and concentration of MVs in supernatant of antigen-stimulated-PBMC samples from patients during and after treatment, comparing to patients before treatment and healthy subjects. We also observed high frequencies of $CD14^{+-}$- and $CD4^{+}$-originated MVs in supernatants samples from patients before treatment, comparing to healthy individuals. **Conclusion:** these data confirm the effectiveness of nano-flow cytometry in the MVs quantification and phenotyping in the supernatants of PBMC-culture samples, to investigate the MVs contributions in the pathophysiology of CL, suggesting an important modulator role in the effective specific immune response against parasite. Besides MVs may also be used as biomarkers to prognosis of the active stages of the disease and under therapeutic protocols.
Keywords: extracellular microvesicles;flow cytometry;human cutaneous leishmaniasis.

PR - 047 - **Impact on parasitemia and mortality mediated by IFN-γ gene depletion in an experimental model of Chagas disease**

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Introduction: The TH1 response is crucial for protection against parasitic propagation, observed in *Trypanosoma cruzi* infection, mainly due to macrophage potentiation mediated by IFN-γ concentrations. In the absence of IFN-γ, other routes are activated and trigger an alternative response. In addition, reports indicate a variation for the pathogenicity of the disease according to the concentration of the inoculum. Objectives: The association between different routes for mortality rates and parasitological behavior against different inoculum concentrations for *T. cruzi* were evaluated. Methods: The study was previously assessed and approved by the institutional CEUA (UFTM). Wild-type (n = 10) and IFN-γ knock-out (n = 10) mice were infected with different inoculums ($3x10^3$ and $3x10^4$ blood trypomastigotes forms of the Y strain of *T. cruzi*) for parasitemia and mortality rate after 12 days of infection. Results: Among infected animals there were no differences in mortality rates ($p>0.05$), on the other hand the number of blood parasites and parasitemia peak were higher and faster in wild-type animals and infected with the highest inoculum from *T. cruzi*. In IFN-/- animals, the peak of parasitemia was also faster, with no difference in the number of parasites when compared to animals that received the lowest inoculum. Conclusions: In addition to IFN-mediated modulation, parasite load was a factor that mobilized IFN modulation by different mechanisms. Thus, studies that consider antigenic rates should be considered in evaluations for disease mechanisms.
Keywords: Trypanosoma cruzi;Parasite load;Imune response.

PR - 048 - **Sorting with the CytoFLEX SRT: is there a limit?**

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Cell sorting is highly demanded and demanding. The need to isolate exact phenotypes is on the rise. This applies to basic research, clinical research and in the field of cell therapy. For the latter, it is for instance crucial to test the functionality of cells and be able to enrich the functional population before infusion into the patients. In other settings, sorting enables to characterize unwanted events from a solution (eg. isolating bacteria from water), or to look at the very small particles such as extracellular vesicles that are abundant in cell cultures and in biological fluids. In this poster, we will illustrate the multiple applications of sorting using the CytoFLEX SRT. We will cover from the very common to the most uncommon applications, to highlight the array of capabilities and discuss the potential for future applications. Alongside with the capabilities of the CytoFLEX SRT will be described how this cell sorter has been designed to facilitate its operation. This will enable to showcase the CytoFLEX SRT paradox: achieve more by doing less.
Keywords: Cell Sorting;Extracellualr Vesicles;NanoFlow;.

PR - 049 - **Metabolomics profiling in People Living with HIV**

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Introduction: Among all people living with HIV (PLWHIV), Elite Controllers (EC) stand out, once this group of individuals is capable of controlling the viremia with CD4+ T cells count maintenance for 10 years or more. The metabolomic profile investigation of these individuals may provide an insight about the factors that lead to this phenotype. **Methods:** To investigate a differential metabolic profile of ECs, we performed an untargeted metabolomics of plasma from 62 PLHIV, classified as Viremic (VR), Successfully Treated (ST), Long Term Non-Progressor (LTNP) and EC, twenty Healthy Donors (HD) were also included. Using plasma samples, an untargeted metabolomic analysis was performed using a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). A subsequent targeted metabolomic analysis was performed as validation. Further analyses were developed using Jurkat cell lines infected with HIV and treated with metabolites. **Results:** All groups of PLWIH displayed different metabolic profiles. EC showed a higher number of altered metabolite abundance, with a downregulated profile when compared to HD. Sphinganine showed an inverse correlation with the viremia, which was corroborated by the *in vitro* results, where the treatment with the metabolite reduced the viremia 48 hours after the treatment. Glutamate was reduced in EC in comparison to all other groups and, *in vitro*, a higher concentration of the metabolite was responsible to enhance the HIV infection in Jurkat cells. Betaine was demonstrated to have an inverse correlation to the viremia, with higher levels observed in VR compared to EC. *In vitro*, lower concentrations of the metabolite were associated with a lower infection. **Conclusion:** The metabolite profile of PLWHIV may be involved in the course of the infection in PLWHIV. These finds reinforce the importance of a better understanding of the metabolomic profile, to a better comprehension of the mechanisms of spontaneous control of HIV infection.

Keywords: HIV;metabolites;reservoir.

PR - 050 - **Decreased expression of CD314 by NK cells correlates with their ability to produce IFN-γ after BCG Moscow vaccination and is associated with distinct early immune responses**

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The immune response to vaccines is complex, with unpredictable variables, and results in various outcomes. BCG vaccination induces innate and specific responses that can lead to protection against tuberculosis and cross-protection against other infections. NK cells have been associated with BCG-induced protection. We hypothesize that differences in NK cell status or phenotype before BCG vaccination may have a role in the ability of BCG to activate the immune response. Participants of the phase II BCG-COVID-19 clinical trial were evaluated prior to and 15 days after BCG vaccination. The participants were assigned to three different groups according to variation in IFN-γ expression (median fluorescence intensity, MFI) by NK cells between days one and 15 after BCG vaccination. The main difference in NK phenotypes among the groups prior to BCG vaccination (day 1) was reduced CD314 expression in group 2 individuals. A negative correlation between expression of CD314 at day one and that of IFN-γ by NK cells after BCG vaccination was observed. Immune responses after BCG vaccination were compared between groups. Group 1 presented an increase in the cytotoxic NK subpopulation ($CD56^{dim}CD16^{-}$) and in CD63 MFI of neutrophils and induction of specific CD4 T-cells producing IFN-γ, IL-17 and IL-22. Group 2 presented an increase in inflammatory NK cells ($CD56^{bright}CD16^{+}$) and CD4 T-cell expression of IL-17. Group 3 showed increased $CD56^{dim}CD16^{-}$ NK cells, expression of CD49 and CD63 by neutrophils and specific CD4 T-cells producing IL-17 and IL-22. In conclusion, decreased CD314 expression by NK cells correlated with the ability of NK cells to produce IFN-γ in response to BCG. Individuals who presented an increase in NK cell IFN-γ responses did not show enhanced neutrophil activation or the same heterogeneity of specific T-cell responses as in other groups; thus, the NK cell IFN-γ response to BCG vaccination influences the immune response at 15 days after vaccination.

Keywords: vaccine;innate immunity;heterogeneity response.

PR - 051 - **Platelet-Granulocyte aggregates and inflammasome activation in vaccine induced thrombotic thrombocytopenia (VITT) after immunization with adenovector SARS-CoV-2 vaccines**

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although rare, cases of Vaccine Induced Thrombotic Thrombocytopenia (VITT) associated SARS-CoV-2 adenovector vaccines became concerning. Vaccine components aggregate with platelet factor 4 (PF4), exposing neoepitopes, leading to high titers of anti-PF4 antibodies, forming immunocomplexes that activates platelets through FCγRIIa. Here, we investigated inflammasome activation and platelet-leukocyte aggregates (PLAs) formation as contributors to VITT development. Individuals with post-vaccine thrombosis (PVT) (n=57) were classified according to guidelines by the likelihood that these events are VITT related (NEJM.385:1680-1689, 2021) into 20 unlikely, 7 possible, 18 probable and 12 definite VITT (DV) cases. Age/sex matched unvaccinated (UV) individuals (n=28) were also included (CAAE-48532621.8.0000.5262/52396621.0.0000.5262). PVT cases presented higher circulating tissue factor, p-selectin, IL-1beta and IL-18 when compared to UV. Moreover, DV plasma presented higher levels of active caspase-1 and of the inflammasome derived cytokines IL-1beta and IL-18 compared to unlikely VITT PVTs. To investigate PLA formation and inflammasome activation, we performed ex- vivo plasma exchange assays, substituting the platelet pour plasma (PPP) from healthy volunteers' whole blood with PPP from DV or UV for 1, 3 and 6 hours, and evaluating through flow cytometry. At all-time points, a greater percentage of granulocytes (CD15 +) displayed the active form of caspase-1 when incubated with DV plasma, that also induced increased formation of platelet-granulocyte aggregates (PGAs) at 3 and 6 hours. Moreover, the percentage of PGAs expressing active caspase-1 was elevated when incubated with DV plasma, indicating that PGA formation could participate in inflammasome activation, and possibly contribute to VITT pathology. As NLRP3 inhibitors are under clinical trials, a better understanding of inflammasome activation in the development of VITT could be beneficial for future clinical management

Keywords: 1;2;3.

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