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FAIC
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4TH FRANCO ARGENTINE IMMUNOLOGY CONGRESS

LXXIII SAI CONGRESS

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Discurso de Apertura – LXXIII Reunión Anual de la Sociedad Argentina de Inmunología y 4ª Reunión Franco-Argentina de Inmunología (SAI–FAIC 2025)

Ana Rosa Pérez.
Presidente de la Sociedad Argentina de Inmunología.

Estimadas y estimados colegas, es un gran honor darles la bienvenida a la LXXIII (Septuagésima tercera) Reunión Anual de la Sociedad Argentina de Inmunología (SAI) y 4ª Reunión Franco-Argentina de Inmunología (FAIC), organizada conjuntamente por nuestra sociedad y por nuestros queridos colegas franceses: Eliane Piaggio, José Cohen y Olivier Boyer. Rosario no albergaba un Congreso de Inmunología desde hacía tiempo, y este regreso se da de manera muy especial, coincidiendo con el 300° aniversario de nuestra ciudad, un hecho que nos llena de alegría y orgullo por poder celebrarlo en este marco científico y académico.

En esta edición nos honran con su participación distinguidos investigadores de Francia y también engalanan este evento reconocidos investigadores radicados en Chile, Brasil, Alemania, Australia, Reino Unido, Estados Unidos y Portugal. A todos ellos, agradecemos profundamente su generosidad y compromiso con esta Sociedad, así como también su entusiasmo por compartir su trabajo y conocimiento, contribuyendo al prestigio de este encuentro.

La inmunología se ha consolidado como una de las disciplinas más transformadoras de la medicina actual, ciencia clave para entender y tratar las enfermedades del siglo XXI. Su importancia creciente se refleja en dos recientes Premios Nobel de Fisiología o Medicina, uno de ellos otorgado a quienes descubrieron los "*Immune checkpoints*" o puntos de control inmunológicos y, hace sólo apenas unas semanas, a los investigadores que identificaron las células T reguladoras, pilares fundamentales del equilibrio inmunológico y de nuevas estrategias terapéuticas.

El programa científico de este Congreso abarca un amplio espectro de temas: desde la inmunología básica y clínica, hasta la traslacional. Con un total de 175 trabajos científicos, 13 simposios -incluidos dos organizados junto a nuestra sociedad hermana la Asociación de Inmunólogos Clínicos de Argentina -AINCA-, y con sesiones especialmente dedicadas a doctorandos, posdoctorandos y a investigadores en etapas iniciales de su carrera, aspiramos a que este encuentro sea, como siempre, un espacio fértil de intercambio entre generaciones y una oportunidad para el surgimiento de nuevas colaboraciones. Esperamos, asimismo, que este Congreso refleje la vitalidad y diversidad de la inmunología actual, que se manifiesta en la participación activa de socias y socios de todo el país, fortaleciendo el carácter federal de nuestra comunidad científica.

No obstante, en esta ocasión no podemos soslayar el contexto actual. La ciencia enfrenta, no solo en nuestro país, sino en el mundo, un escenario desafiante. En muchos países, incluida la Argentina, la investigación y la educación —pilares de cualquier sociedad desarrollada— se ven amenazadas por la desvalorización del conocimiento, la desinformación y la reducción de los recursos públicos destinados a la ciencia. En particular, nuestro país ha presenciado estos 2 últimos años, la disminución de ingresos a becas y a la carrera de investigador, la pérdida del poder adquisitivo de docentes y científicos, y la subejecución -por no decir parálisis- de los fondos de investigación previamente asignados por la Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación. Todo esto configura un panorama sumamente preocupante que pone en riesgo la continuidad de líneas de trabajo estratégicas y estimula la emigración de nuestros jóvenes talentos. Nuestro país fue pionero en América Latina en la construcción de un sistema científico sólido, basado en la educación pública y en el sostenimiento de la investigación. Hoy, debemos reafirmar ese modelo. La ciencia no es un gasto, es una inversión en soberanía, y en desarrollo humano.

Ha llegado el momento de mostrar con convicción al mundo, qué investigamos y por qué lo hacemos, cuál es el impacto de nuestros aportes y por qué la ciencia es la base del progreso humano, en particular en relación a aquello que nos convoca: **la salud**, pilar fundamental del bienestar de las personas y por extensión, de la prosperidad y desarrollo de las naciones.

En este contexto, la organización de este Congreso representó un verdadero desafío, marcado por la escasez de recursos y diversas dificultades logísticas. Por ello, queremos expresar nuestro profundo agradecimiento a la Agencia Santafesina de Ciencia, Tecnología e Innovación (ASACTEI), a la Fundación Williams, al CONICET y a The Company of Biologists, por los subsidios que hicieron posible la realización de este encuentro. Extendemos también nuestro reconocimiento al Instituto Curie y, de manera especial, a la Embajada de Francia en la Argentina, por el apoyo brindado a través del Institut Français d'Argentine. Asimismo, destacamos muy especialmente el respaldo de

la Universidad Nacional de Rosario, cuya tradición en docencia, investigación y extensión se refleja en su permanente compromiso con la ciencia y la salud pública. Nuestro sincero agradecimiento a la Facultad de Ciencias Médicas por su hospitalidad y acompañamiento institucional. Agradecemos también los valiosos aportes de la Fundación Ciencias Médicas, la Fundación Prats, la Fundación Federada y el Colegio de Médicos, así como, de manera muy especial a Hemoderivados de la UNC, cuyo generoso aporte permitirá financiar los Premios SATZ y de Investigación Clínica. Finalmente, hacemos extensivo nuestro reconocimiento a la Municipalidad de Rosario y a las empresas auspiciantes, cuya colaboración ha contribuido a la concreción de este Congreso.

Deseo expresar mi más sincero agradecimiento al Consejo Directivo de la SAI, por su dedicación y compromiso. En particular, Rossana Ramhorst, vicepresidenta de la SAI, por su apoyo incondicional. A Natalia Santucci y Florencia Quiroga, secretaria y tesorera respectivamente, sobre quienes recayó gran parte de la tarea organizativa. Y al resto de los miembros, por su activa y entusiasta participación en la organización de este Congreso y en las múltiples actividades desarrolladas a lo largo del año.

Mi reconocimiento también por su labor rigurosa y generosa, a los evaluadores de los 175 trabajos presentados a este Congreso, a los coordinadores de sesiones y de pósters, y a los jurados de los Premios SATZ e Investigación Clínica. Agradezco también a los integrantes de las Comisiones de Docencia y Clínica, quienes organizaron distintas actividades y respondieron a numerosas demandas.

Deseo que esta reunión tan esperada, nos inspire a un intercambio científico y humano enriquecedor, que fortalezca nuestras redes de colaboración y reafirme el valor insustituible del encuentro presencial. Con esta convicción y orgullo, declaro formalmente inaugurada la LXXIII (Septuagésima tercera) Reunión Anual de la Sociedad Argentina de Inmunología y 4ª Reunión Franco-Argentina de Inmunología.

Muchas gracias y bienvenidos a Rosario.

Keynote Lecture

mRNA splicing generates tumor-specific antigens and new protein isoforms

Sebastián Amigorena
Institut Curie, PSL University, Immunity and Cancer,Paris, France.

Session 1: Immunotherapy and Precision Medicine

CD38 Blockade to Controls GVHD: Clinical Relevance in Humans and Preclinical Validation in Mice

José Cohen
Université Paris Est Créteil, Créteil, France; AP-HP, Groupe hospitalo-universitaire Chenevier Mondor, Centre d'Investigation Clinique, Biothérapie, Fédération hospitalo-Universitaire TRUE, Créteil, France.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents a curative approach for several hematologic malignancies; however, acute graft-versus-host disease (aGVHD) remains a major cause of morbidity and mortality. Through comprehensive immunological profiling, we identified CD38 expression on distinct T cell subpopulations as a key feature associated with the onset of aGVHD. Patients with aGVHD exhibited a specific immune signature characterized by an expansion of $CD38^+$ central and effector memory T cells, implicating these cells in disease pathophysiology. In parallel, the administration of a clinical-grade anti-CD38 monoclonal antibody in a xenogeneic mouse model effectively controlled GVHD manifestations. These findings provide a strong rationale for the clinical evaluation of CD38-targeted therapies as a novel strategy to prevent or treat aGVHD in allo-HSCT recipients.

Bioartificial Platforms: Bridging Immunology and Precision Medicine

Dr. Eduardo Chuluyán
Centro de Estudios Farmacológicos y Botánicos (CEFYBO), facultad de Medicina, Universidad de BuenosAires.

Peripheral blood analyses and 2D cell culture models are commonly used to study immune cell parameters; however, they fail to accurately reflect the behavior of tissue-resident cells involved in autoimmune diseases and organ transplantation. To address this limitation, we developed and validated 3D fibrin hydrogels derived from healthy individuals and immunosuppressed kidney transplant recipients as a model to investigate immune cell dynamics within a tissue-like environment. Using this platform, we identified distinct immune signatures not only between healthy controls and transplant patients, but also among subgroups within the latter. Notably, this approach enabled us to recognize patients who, despite being under immunosuppressive therapy, exhibited immune profiles suggestive of insufficient immunosuppression—an observation that brings us closer to precision medicine. Integration of these findings with renal function trajectories underscores the potential predictive value of the model, which provides an ex vivo tool to evaluate immune dynamics with direct relevance to graft outcomes.

Cryptic transcripts as a source of non-canonical tumor antigens

Joshua Waterfall
Translational Research Department, Institut Curie, Paris, France

The ability of CD8+ T cells to distinguish cancer cells from healthy tissues is essential for effec-

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tive anti-cancer immunity and immunotherapy approaches. We show that altered localization and function of mutated oncogenic transcription factors, such as EWS-FLI1 in Ewing sarcoma, drives the expression of recurrent, abundant, processed mRNA species not expressed in healthy tissues. A subset of these transcripts are translated, processed, and presented by HLA complexes on the cancer cell surface and serve as attractive targets for immunotherapy approaches including TCR-T and cancer vaccines.

Session 2: B-Lymphocytes and Humoral Immunity

Age-associated B cells: from aging to autoimmunity

Dra. Virginia Rivero
Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba and Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Córdoba, Argentina.

Aging profoundly remodels the immune system, promoting chronic inflammation and loss of tolerance. Among B cell subsets, age-associated B cells (ABCs), defined by CD11c and T-bet expression, expand with age and under autoimmune conditions. We found that ABC accumulation, activation, and differentiation occur in both sexes but are markedly enhanced in aged females, within a proinflammatory milieu that supports their survival and autoreactive potential. Sorted ABCs from both aged males and females secreted anti-dsDNA and anti-histone autoantibodies upon TLR7 stimulation, yet only aged females displayed circulating autoantibodies in vivo, suggesting that the female environment promotes ABC activation and differentiation into antibody-secreting cells. In a TLR7-driven lupus-like model induced by topical imiquimod administration, ABCs represented the main source of autoantibodies and infiltrated the kidney before overt nephritis. Treatment with the A2A receptor agonist CGS21680 reduced ABC frequencies and serum autoantibodies, highlighting ABCs as key mediators and potential therapeutic targets in lupus-like disease.

Monoclonal antibodies in the fight against infectious diseases: from therapeutic agents to tools for antigen discovery and vaccine design.

Pascal Poignard
Université Grenoble Alpes

Session 3: Inborn Errors of immunity

New monigenic predisposition to autoimmunity

Friédéric Rieux-Laucat
Université Paris Cité, Laboratoire d'immunogénétique des maladies auto-immunes pédiatriques, Institut Imagine, Paris, France.

Autoimmune lymphoproliferative disease: the importance of seeing the whole picture.

Dr. María Virginia Paolini
Unidad de Medicina de Precisión. Hospital Oncológico María Curie - AINCA

Chronic non-malignant lymphoproliferation and autoimmune cytopenia are frequent initial manifestations of inborn errors of immunity (IEI) and are expressions of immune dysregulation. Correct diagnosis is challenging but important for therapy. Autoimmune lymphoproliferative syn-

drome (ALPS) is a genetically defined IEI that combines these manifestations. The term “autoimmune-lymphoproliferative immunodeficiency” (ALPID) describes a group of patients with a similar disease phenotype, but who are more likely to have other IEI or IEI mimics (caused by somatic mutations), for which targeted therapies are available. This case report describes an adult patient with a history of autoimmune cytopenia and benign lymphoproliferation who was referred with a strong clinical suspicion of ALPS. During the clinical evaluation, progressive hypogammaglobulinaemia and respiratory infections developed, so treatment with gammaglobulin was initiated. Pre-test biomarkers for ALPS (sFASLG, vitamin B12 and CD3+ TCRαβ+ CD4− CD8− double-negative T cells) were investigated to rule out this genetic condition. A targeted exome was requested.

Complement system and Immune dysregulation: the piece that completes the puzzle

Carolina Bouso
Unidad de Inmunología Hospital Clínico San Borja Arriarán and Clínica Las Condes, Santiago, Chile - AINCA

Inherited complement deficiencies encompass an intriguingly heterogeneous group of genetic disorders with a wide spectrum of clinical manifestations. Depending on the affected factor, patients may present with severe infections caused by encapsulated organisms, atypical hemolytic uremic syndrome, hereditary angioedema, or systemic lupus erythematosus, among other manifestations of immune dysregulation. The role of the complement system in the regulation of the immune response will be discussed.

Session 4: Neuro-immuno-endocrinology and Immuno-Metabolism

Exploring the molecular mechanisms of intestinal Host-Microbiota interactions

Prof. Dr. Marco Aurélio R. Vinolo.
Institute of Biology, University of Campinas, Brazil.

Short-chain fatty acids (SCFAs), key metabolites of the gut microbiota, play a central role in shaping intestinal homeostasis and immune function. Using integrative approaches, including transcriptomics, microbiome profiling, gnotobiotic, and genetically engineered mice models, our group has uncovered previously unrecognized effects of SCFAs on intestinal epithelial cells and neutrophils. These findings will be discussed during the presentation and reveal novel mechanisms through which SCFAs drive host adaptation to the microbiota and modulate responses under inflammatory conditions.

γδ T cells in glioblastoma: Immune response and tumor-derived components

Carolina Jancic
Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

Glioblastoma (GBM) is a highly aggressive brain tumor associated with poor prognosis. γδ T cells play a pivotal role in host defense and tumor surveillance. We found that GBM cells and their secreted factors induce a Th1-like response in γδ T cells. Notably, GBM-derived extracellular vesicles boost γδ T cell activation via MIC-dependent mechanism. These results underscore the potential of γδ T cells for development of immunotherapeutic strategies against GBM.

Mini-Orals

TSLP as a novel therapeutic axis in Glioblastoma: modulation of the tumor and immune microenvironment through interplay with the cholinergic system.

Alejandra Infante
Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

Functionalized Extracellular Vesicles: Reprogramming Inflammation through Adenosine Production and Targeted Delivery

Tomás Grosso
Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

Session 5: Translational immunology

Single Cell Profiling of Tregs: Implications for Tumor Immunity and Cancer Therapy

Dra Eliane Piaggio
Translational Immunotherapy. Unit Immunity and Cancer (U932), Institute Curie, Paris, France

We studied CD4+ T cells in the tumor and draining lymph nodes of NSCLC patients and identified that the tumor-reactive T cells showed a follicular and tissue resident transcriptome and shared TCRs across tissues. We generated a translational platform for the identification of tumor-associated Treg targets, enabling the development of novel therapeutic strategies now under clinical testing. Our findings provide mechanistic insights and opportunities for targeting $CD4^+$ T cell subsets in cancer.

Dissecting the crossroads of circulating and tissue-resident T cells in solid tumors

Álvaro Lladser
Centro Ciencia & Vida, Santiago, Chile.
Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile

CD8 + T cells have the potential to eliminate cancer cells. Immunotherapies that exploit this ability have become a standard of care across different cancers. Emerging evidence indicates that effective and long-lasting antitumor protection requires the concerted action of both circulating and tissue-resident memory CD8 + T cells. However, the interplay among circulating and tissue-resident compartments in solid tumors remains poorly understood. Therefore, we are comprehensively studying memory CD8 + T cells in human solid tumors and mouse models using functional assays, multidimensional flow cytometry analyses and single-cell transcriptomics. Our findings reveal new insights into the differentiation pathways and clinical impact of tumor-specific memory CD8 + T cells infiltrating human solid tumors.

The dual role of inflammation in cancer immunity

Dr. Santiago Zelenay
The Lydia Becker Institute of Immunology and Inflammation, The University of Manchester, Manchester, United Kingdom.

Immunotherapies based on antibodies targeting T cell immune checkpoints have transformed the landscape of cancer treatment across multiple tumour types. These therapies can induce long-lasting responses in patients with late-stage cancers as well as in (neo)adjuvant settings. However, most patients experience only transient benefit or none at all, and many suffer harmful side effects. Solutions to these clinical problems require an improved fundamental understanding of the principles that govern anti-cancer immune responses. Our group at the Cancer Research UK Manchester Institute investigates the signals and pathways that dictate the establishment of tumour inflammatory environments that either promote or restrain the anti-tumour function of the immune system. By combining genetically engineered pre-clinical models with the analysis of cancer patient samples, we aim to identify the underlying mechanisms that enable immune escape and drive progressive tumour growth and spread. In this talk, I will present our recent findings uncovering a surprising role for glucocorticoids in triggering CD8 + T cell-dependent tumour control in immunotherapy-resistant melanoma.

Session 6: Infection and Immunity

From C. difficile to M. tuberculosis infection: a gut lung tale

Virginia Pasquinelli
Centro de Investigaciones Básicas y Aplicadas (CIBA), Universidad Nacional del Noroeste de la Provincia de Buenos Aires (UNNOBA).

The gut-lung axis involves host-microbe and microbe-microbe interactions that shape immune responses and maintain host homeostasis. Alterations in gut microbiota communities could have a profound impact on lung physiology, contributing to disease progression. Our research focuses on how antibiotic-induced intestinal dysbiosis and *C. difficile* infection affect pulmonary immune responses. We found that changes in intestinal homeostasis compromise the distal lung structure and modulate innate immune responses against respiratory infections such as Tuberculosis, highlighting trained immunity as a potential mechanism within the gut–lung axis.

Coupling constitutive fluid uptake with intrinsic viral resistance in human macrophages.

Philippe Benaroch
Institut Curie, PSL, Research University, Paris, France

Macrophages survey tissues via micropinocytosis, yet must avoid becoming viral entry points. We identify GAS7 as a cytoskeletal regulator that couples fluid uptake with resistance to diverse DNA and RNA viruses. GAS7 promotes membrane ruffling while restraining protein translation, creating a cellular state unfavorable for viral replication. By balancing the activity of small GTPases, GAS7 allows macrophages to efficiently sample their environment without increasing susceptibility to infection.

Session 7: Vaccines

Advances in Rational Vaccine Design: The Role of Adjuvants in Modulating Immunity Using Staphylococcus aureus Proteins as a Model.

Carolina Veaute
Laboratorio de Inmunología Experimental. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral, Santa Fe-Argentina.

Adjuvants are key modulators of immune responses, enhancing memory and vaccine efficacy.

We developed and evaluated a series of delivery vehicles and immunostimulant formulations based on liposomes and lipopeptides for immunisation against Staphylococcus aureus proteins in both murine and bovine models. While all formulations enhanced antigen-specific responses, they elicited distinct immune profiles. These findings provide valuable insights into the rational design of tailored adjuvant formulations for vaccine development.

Development of vaccine prototypes for resurgent diseases: lessons learned and consolidation of a pertussis platform based on OMVs

Daniela Hozbor
Laboratorio VacSal. Instituto de Biotecnología y Biología Molecular. Facultad de Ciencias Exactas. Universidad Nacional de La Plata. CONICET. La Plata. Buenos Aires. Argentina

Outer Membrane Vesicles (OMVs) represent a promising platform for developing vaccines against resurgent and antimicrobial-resistant pathogens. Their natural composition of antigens and pathogen-associated molecular patterns (PAMPs) facilitates efficient uptake by antigen-presenting cells, eliciting robust humoral and cellular immunity. Using Bordetella pertussis as a model, we have consolidated an OMV-based vaccine prototype (patented in the US and Brazil) that demonstrates an excellent safety profile and potent protective efficacy in preclinical studies. This platform functions dually as a versatile immunogen and adjuvant, presenting antigens in their native conformation and effectively modulating the immune response. The lessons learned from developing this pertussis vaccine underscore the extensive potential of OMV-based strategies for addressing pressing challenges in both human and veterinary medicine.

Session 8: Innate Immunity

Macrophages and T cell immunity in Cancer

Julie Helft
Institut Cochin, Phagocytes and Cancer Immunology Laboratory, Université Paris Cité, Paris, France.

Macrophage infiltration is a hallmark of solid cancers, and overall macrophage infiltration correlates with lower patient survival and resistance to therapy. Tumor-associated macrophages, however, are phenotypically and functionally heterogeneous. We have recently identified a discrete population of FOLR2+ tissue-resident macrophages in healthy mammary gland and breast cancer primary tumors. In human, FOLR2+ macrophages localize in perivascular areas in the tumor stroma, where they interact with CD8+ T cells. The density of FOLR2+ macrophages in tumors positively correlates with better patient survival. In mice, FOLR2+ macrophages efficiently prime effector CD8+ T cells ex vivo. In vivo, absence of FOLR2+ macrophages in the mammary gland correlates with faster tumor progression and is associated with altered T cell landscape. This study highlights specific roles for tumor-associated macrophage subsets and paves the way for subset-targeted therapeutic interventions in macrophages-based cancer therapies.

Dendritic cells infiltrating tumors: challenges and opportunities

Pierre Guermonprez
Dendritic cells and adaptive immunity, Immunology department, Pasteur Institute, Paris, France. Département Biologie du Développement et Cellules Souches, Institut Pasteur, Université Paris Cité, Paris, France.

Dendritic cells infiltrating the tumor microenvironment can play a major role in the activation of the immune system. Here we highlight case studies in which infiltration or terminal differentiation are limited by immunosuppressive cues of the tumor micro-environment. We discuss novel interventions purposed to circumvent these limitation and unleash the immunogenic activity of intra-tumoral dendritic cells

Neutrophil extracellular vesicles in Hemolytic Uremic Syndrome development

Analía Trevani
Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

Hemolytic Uremic Syndrome (HUS) develops as a consequence of infections with Shiga toxin (Stx)-producing Escherichia coli strains, predominantly in children under five years of age. It is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. Our in vitro and in vivo findings support that extracellular vesicles released by neutrophils in response to Stx contribute to HUS development.

Session 9: Host-Pathogen Interactions

Integrated systems immunology approach for the identification of immune responses predicting progression to severe dengue fever.

Diana S Hansen
Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, VIC, Australia.

Typical symptoms of uncomplicated dengue (DF) include fever, headache, muscle pains, rash cough, and vomiting. A proportion of cases progress to severe dengue haemorrhagic fever (DHF), associated with vascular permeability, thrombocytopenia, and haemorrhages. Progression to DHF is difficult to diagnose at the onset of fever, which complicates patient triage, posing a socio-economic burden on health systems.

To identify parameters associated with protection and susceptibility to DHF, we pursued a systems immunology approach integrating plasma chemokine profiling, high-dimensional mass cytometry and peripheral blood mononuclear cell (PBMC) transcriptomic analysis at the onset of fever in a prospective study conducted in Indonesia. Progression to uncomplicated dengue featured an expansion of ICOS + CD4 + and CD8 + effector memory T cells. These responses were virtually absent in cases progressing to severe DHF, that instead mounted an innate-like response, with high frequencies of CD4 + non-classical monocytes predicting increased risk of severe disease.

Our results provide proof of concept for the potential of system biology approaches to identify discrete populations in the blood predicting reduced or increased risk of DHF to develop diagnostic tools for detection of complicated cases.

MHC-I down-modulation by bacterial RNA: from an immune evasion strategy to a potential therapeutic approach.

Paula Barrionuevo
Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.

In our lab, we have investigated for years the strategies employed by Brucella abortus to evade the immune system and persist chronically in the host. We have demonstrated that a key strategy in combating this infection is the reduction in MHC-I surface expression by B. abortus RNA in macrophages and other infected cells. Furthermore, other bacterial RNAs can mimic this phenomenon. Given that downregulation of MHC-I by bacterial RNA can activate NK cells, pivotal components against multiple tumors, we are investigating the therapeutic potential of using bacterial RNA to modulate MHC-I as a strategy to stimulate an anti-tumor response.

Session 10: Oncoimmunology

Impact of endocrine therapy on immunotherapy response in luminal breast cancer

Mariana Salatino, PhD
Laboratory of Glycomedicine, IBYME-CONICET, Buenos Aires, Argentina

Hormone-receptor-positive luminal breast cancers exhibit limited immunotherapy response. We found that Mifepristone, a progesterone receptor antagonist, can regulate several immunoregulatory programs, including those associated with T cell exclusion and immunotherapy resistance in luminal breast cancer. These changes led to an altered immune infiltrate and a downregulation of the TIM-3 immune checkpoint ligand, Galectin-9, reshaping the TME towards an immunologically active state. Our findings suggest that Mifepristone may open the gate for immunotherapy-based treatments for this prevalent breast cancer subtype.

Targeting the splicing machinery to modulate tumour immunogenicity

Marianne Burbage
Institut Curie, Immunity and Cancer, Paris, France.
[2]PSL Research University, Paris, France.

Defining sources of shared, immunogenic, cancer antigens (Ags) as well as strategies to boost tumour immunogenicity are long-standing goals. Cancer mutations can give rise to neo-Ags that elicit an immune response but are often private to each patient. It is therefore critical to identify alternative sources of tumour Ags. We and others have recently described a key contribution of the "non-coding" genome, in particular transposable elements (TEs) to tumour antigenic landscape. Here, we will discuss how non-canonical splice junctions between exons and TEs (JETs) contribute to anti-tumour immunity and how to target their formation to improve immune detection of cancer cells.

Dendritic cell heterogeneity: subsets and cell-fate-switching

Juliana Idoyaga, PhD
University of California, la Jolla, San Diego, USA.

Our lab is driven by one central question: how do dendritic cells (DCs)—the immune system's decision-makers—shape lymphocyte responses? DCs can guide T cells to launch strong immune defenses against diverse threats or, alternatively, to promote tolerance when a restrained response is needed. This makes DCs central players in immunity and attractive targets for therapies in cancer, infection, and autoimmunity. Yet many mysteries remain—what truly makes DCs unique, and how is their function regulated? Our lab tackles these questions by studying DCs across species and contexts to uncover the principles that guide their identity and behavior. We are finding that DCs are far more diverse and adaptable than once thought, with the surprising ability to switch fates under certain conditions. In this talk, I will highlight our current understanding of DC heterogeneity, show how viral infections help reveal these principles, and discuss how fate-switching enables DCs to change roles in response to stimulation.

Session 11: Clinical Immunology

Immune-mediated necrotizing myopathies from mice to humans and vice versa

Olivier Boyer
Department of Immunology and Biotherapy, Univ Rouen Normandie, CHU de Rouen, Rouen, France.

Autoantibodies (aAbs) typically target tissue-specific, membrane-bound autoantigens (aAgs). Myositis is a remarkable exception because aAgs are ubiquitous and intracellular. Notably, in immune-mediated necrotizing myopathies (IMNM), pathogenic aAbs target two ubiquitous and intracellular proteins: the signal recognition particle (SRP) or the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). This raises fundamental questions about the cause of the organ specificity of this autoimmune disease, and how circulating aAbs can access their targets within the cytoplasm. This presentation will summarize IMNM pathomechanisms, and will discuss the possibility of functional inhibition of their antigenic targets via non-conventional mechanisms of cellular penetration.

Diagnosing the Undiagnosed: New Variants and Functional Genomics in Inborn Errors of Immunity

Dr. María Belén Almejún
IQUIBICEN-CONICET/DQB-Fac. de Cs. Exactas y Naturales-UBA

Inborn errors of immunity (IEI) are a heterogeneous group of genetic disorders with clinical manifestations ranging from infections to autoimmunity, autoinflammation, allergy, and cancer predisposition. Early diagnosis is crucial for guiding therapy and improving outcomes. This presentation will explore the diagnostic pathway from initial laboratory findings to advanced molecular and functional analyses. Real patient cases will illustrate how collaborative networks and functional genomics contribute to the identification of novel variants, strengthening regional diagnostic capacity and promoting equitable access to advanced technologies.

Cancer-associated fibroblasts and control of T cell infiltration in lung and head & neck cancer

Helene Salmon
Institut Curie, Equipe Leader Fondation ARC, PSL University, Paris, France.

Growing evidence shows that cancer-associated fibroblasts (CAFs) are essential players in regulating tumor fate and immune responses in solid tumors. I will present our recent study showing that CAFs can restrict intratumor T cell infiltration, notably through forming an organized and dense fibrillar capsule around tumor nests. We will also discuss the impact of targeting both TGFb-PDL1 on the tumor stroma and T cell infiltration in head and neck cancer patients, and recent findings on the role of CAFs in promoting tumor invasion and cancer progression in bladder cancer.

Session 12: Mucosal and Reproductive Immunology

Infection-Induced Treg Dysfunction: A Double-Edged Sword for Tissue-specific Immunity

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Acute infectious challenges are a frequent occurrence worldwide and have been proposed as initiating factors for tumors, chronic inflammatory and metabolic disorders. However, identifying specific mechanisms mediating the association between defined infectious agents and the initiation of chronic disease has remained elusive. The major question to be addressed in this talk is how changes in gut homeostasis, including infection, tumor development and dietary restriction, affect the gut and lung immune homeostasis and predispose to disease.

An Altered Glycome Shapes IgA B-Cell Responses and Gut Immunity During Intestinal Inflammation

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A healthy gut immune system exquisitely balances defenses against pathogens and tolerance to beneficial microbes; in this sense, B cells are central to intestinal homeostasis, and secretory Immunoglobulin A (SIgA) is a key modulator of the microbiome. In ulcerative colitis (UC), defective tolerogenic mechanisms lead to heightened immune responses resulting in tissue damage. Notably, and even though both dysbiosis and basal plasmacytosis (a dense infiltration of plasma cells in the lamina propria) are typical features of UC, the role of B cells in this pathology is still unclear.

Sialylation (the terminal modification of glycans with sialic acid, a negatively-charged monosaccharide) plays a crucial role in B cell function; however, the relevance of sialylation in intestinal IgA B-cell response has been scarcely explored. In this work, we show that SIgA presents an inflammation-dependent, transient decrease in α(2,6)-sialylation in active UC. This abnormal glycosylation was also observed in dextran sodium sulfate (DSS)-induced colitis, where lamina propria IgA+ plasma cells and B cells mirrored this glycopattern. Experimental models of colitis demonstrated that B cells lacking α(2,6)-sialic acid in N-glycans exhibit defective differentiation into IgA+ plasma cells and an impaired capacity to suppress inflammation, leading to increased neutrophil infiltration within the colon. Ultimately, analysis of single-cell transcriptomics data from UC patients showed a potential imbalance in sialic acid metabolism that can be associated to SIgA desialylation.

In summary, our research reveals an abnormal glycosylation profile in SIgA, IgA+ plasma cells, and B cells during intestinal inflammation that, in a retrofeedback loop, may amplify inflammation in the gut.

How Immune Cells Adapt to Pregnancy and the Microbial Landscap

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Pregnancy success depends greatly on a dynamic immune balance. This interplay between pro- and anti-inflammatory mediators is crucial for fetal tolerance, placentation and immune defence. The discovery of a microbiome in the upper reproductive tract, once believed to be sterile, has reshaped our understanding of maternal tolerance and highlighted the need to explore how bacterial tolerance is orchestrated during pregnancy.

Session 13: Adaptive Immunity

Regulation of humoral responses in human autoimmunity

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It has long been known that CD4 T cells are necessary for effective antibody responses to provide help to B cells, triggering a germinal centre (GC) reaction where affinity maturation and isotype switching occur. However, the nature of the dedicated CD4 helper T cells, known today as T follicular helper (Tfh), was only recently described. A population of Foxp3+ regulatory cells with unique access to the follicle – T follicular regulatory (Tfr) cells – represent the counterpart of Tfh cells. In addition, Tfh cells also specialize according to the inflammatory environment, acquiring different properties under type-1 or type-2 conditions. The interplay between different specialized subsets of Tfh and Tfr cells can determine the outcome of humoral responses, including the for-

mation of tertiary lymphoid structures that can occur in chronic inflammatory conditions and cancer. The detailed knowledge of regulatory mechanisms controlling T cell- B cell interactions offer an opportunity for therapeutic modulation, namely in autoimmune diseases or to achieve more effective vaccination.

A Tale of Changing Personalities: Treg Cell Heterogeneity and Its Stage-Dependent Impact in T. cruzi Infection

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Regulatory T (Treg) cells are key modulators of immune homeostasis whose function adapts to the inflammatory environment. In Trypanosoma cruzi infection, we found that Treg cells undergo dynamic, stage-dependent changes reflecting progressive specialization. During acute infection, they acquire an activated Th1-suppressive phenotype, whereas in the chronic phase they adopt features associated with tissue repair and local regulation. This transition involves redistribution from lymphoid to peripheral tissues such as skeletal muscle, where Treg cells display a mixed Th1/tissue-repair program. Functional depletion experiments revealed that systemic and local loss of Treg cells had distinct effects on inflammation, tissue injury, and parasite control. Together, these findings show that Treg cells adapt their "personality" to temporal and spatial cues of infection, balancing immunity and pathology through context-dependent regulatory programs.

Real-World Research: Translating Science into Impact Talking with experts - I

Bridging basic research, cancer immunotherapy and precision medicine: the journey of an anti-MICA monoclonal antibody through storms of technology transfer

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MICA (MHC class I chain–related protein A) is a promising target for precision medicine, while its soluble form (sMICA) may serve as a diagnostic and prognostic biomarker. This presentation will summarize two complementary developments: a therapeutic anti-MICA monoclonal antibody and a FRET-based immunoassay to validate sMICA as a biomarker in liquid biopsies. Both initiatives illustrate technology transfer pathways, each with distinct scientific and operational challenges. The presentation will outline the journey from basic research to translational application, emphasizing the achievements and hurdles encountered, and the interactions withCONICET and biopharmaceutical partners throughout this process.

From the Research Institute to the Hospital: The Journey of an Academic Start-Up

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The *Unidad de Conocimiento Traslacional Hospitalaria* (UCT-H) at the Dr. Arturo Oñativia Hospital in Salta, Argentina, represents an academic initiative designed to bridge biomedical research and clinical practice within the public health system. Led by Dr. Marta A. Toscano, Independent

Investigator of CONICET, the program integrates expertise in immunology, obesity and cancer to foster translational research with societal impact. Through strong multidisciplinary collaboration, team building, and institutional partnerships, the UCT-H has evolved into a dynamic academic start-up embedded in a hospital setting. This experience highlights the challenges and opportunities of creating sustainable research structures that connect discovery with patient care.

Talks with Experts - II:

Exploring Dendritic Cell Immunometabolism: Toward a Rational Strategy for Enhancing BCG Vaccine Efficacy

Luciana Balboa
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Tuberculosis remains the world's deadliest infectious disease. The BCG vaccine, our only available option, fails to provide consistent protection in adults, primarily because it inadequately activates key host immune responses. A major obstacle in the field is the lack of known correlates of protection.

In this talk, we propose a novel and unexplored predictor: the speed of the immune response initiation. We hypothesize that accelerating the initial events following vaccination is critical for achieving durable immunity.

Our strategy is grounded in our discovery that HIF1A-mediated glycolysis is essential for dendritic cell (DC) migration to lymph nodes during M. *tuberculosis* infection. Since DCs are responsible for transporting BCG to these sites to prime adaptive immunity, we asked: can we enhance this process?

We will present our hypothesis that stabilizing HIF1A in BCG-infected DCs will accelerate BCG trafficking to the lymph nodes, facilitating earlier onset of the adaptive immune response. We posit that this fundamental shift in timing will not only strengthen the immune response but also improve its quality and durability, forging a more robust defense from the very moment of vaccination.

The ARVAC journey: from formula design, preclinical and clinical evaluation to its regulatory approval as a booster vaccine for adults.

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We developed ARVAC, the first recombinant subunit SARS-CoV-2 vaccine approved in South America, designed to adapt to emerging variants and serve as a booster. This collaborative project, carried out with the pharmaceutical company Cassará, involved more than 500 professionals from 20 public and private institutions. Phase I–III clinical trials showed that the vaccine was safe and effectively boosted neutralizing antibodies against multiple and very distant variants, including Omicron, regardless of prior vaccination platform. It also increased antigen-specific IgG in serum, IgA in saliva, and IFN-γ cellular responses. ARVAC was approved in Argentina and is now being commercialized, available in pharmacies and vaccination centers.

Early Career Researchers Session 1

The Case of Glucocorticoids in the Context of Immunoendocrine Imbalance in Human Tuberculosis

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Tuberculosis (TB) remains a major global health problem. Like other chronic infections, it is characterized by dysregulation of homeostatic systems in which the hypothalamic–pituitary–adrenal (HPA) axis and cortisol play central roles. Our findings in different forms of TB and related comorbidities indicate that chronic activation of the HPA axis leads to elevated cortisol levels and altered glucocorticoid signaling, potentially contributing to glucocorticoid resistance, particularly in progressive disease. In this context, additional endocrine disturbances and increased levels of immunomodulators appear to represent an insufficient attempt to limit the tissue damage driven by the persistent immune response. Understanding these mechanisms may offer new perspectives for developing targeted immunomodulatory therapies in chronic infectious diseases such as TB.

Dendritic Cells at the Crossroads: Tolerance or Inflammation during Embryo Implantation

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Successful implantation relies on a finely tuned crosstalk between embryo, endometrium, and maternal immune cells. Dendritic cells (DCs) are central in determining tolerance or inflammation at the maternal-fetal interface, yet how they are shaped by the human endometrium remains unclear. In recent years, our group has initiated a pioneering line of research investigating how different endometrial microenvironments condition monocyte-derived DCs into distinct regulatory or lytic-inflammatory subsets, including HLA-G^{+} DC-10. DCs effectively act as biosensors of the stromal secretome, impacting embryo implantation. Building on our findings, we propose ER stress transmission as an emergent immunomodulatory mechanism shaping DCs within the embryo–endometrium–immune cell 'iron triangle' of implantation.

Understanding chronic intestinal inflammation progression: fibroblasts in focus!

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Persistent inflammation in Inflammatory Bowel Disease (IBD) patients induces severe complications, such as fibrosis and increased risk of colorectal cancer (CRC) development. Preventive and therapeutic strategies of these long-term consequences are still challenging. Given that fibroblasts are key stromal cells involved in tissue damage, wound healing, and the tumour microenvironment composition, they have gain relevance as potential diagnostic and therapeutic target cells. We found that miR-21 overexpression in chronic inflamed mucosa induces fibroblast activation. We propose that detecting miRNAs and its target genes differential expression on stromal cells, could be used as biomarkers of IBD and CRC progression.

Beyond the Pandemic: Macrophage Hyporesponsiveness to Mycobacterium tuberculosis After SARS-CoV-2 Infection

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The post-COVID-19 era has seen an increased tuberculosis burden. To investigate whether prior SARS-CoV-2 infection alters macrophage function, we established an in vitro model using prima-

ry macrophages exposed to abortive viral infection, followed by a resting period to allow them to return to a pseudo-basal state. Afterward, cells were infected with virulent H37Rv M. tuberculosis (Mtb). Virus-primed macrophages exhibited reduced HLA-DR expression and impaired cytokine production upon Mtb challenge. These results suggest that SARS-CoV-2 induces a state of macrophage hyporesponsiveness, which may contribute to enhanced TB susceptibility and pathogenesis in the post-pandemic context.

Beyond T Cells: egc Superantigens as Modulators of Innate Immunity

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Superantigens (SAgs) are potent immunomodulatory toxins best known for their ability to induce massive T-cell activation and cytokine release. However, egc-encoded SAgs - SEI, SEO, SEG, and SEM - exhibit a broader spectrum of action, extending their influence well beyond the adaptive immune compartment. We found that these toxins not only drive oxidative burst, cytokine release, and NET formation in neutrophils, but also activate NK and γδ T cells, eliciting IFN-γ and TNF-α responses with distinctive profiles. Their effects further extend to myeloid-derived suppressor cells (MDSCs), uncovering new layers of immune modulation. Altogether, egc superantigens reshape innate immunity, revealing an underappreciated dimension of their immunomodulatory potential.

Early Career Researchers Session 2

Assessment and reclassification of variant of uncertain significance in a patient with hemophagocytic lymphohistiocytosis (HLH)

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We evaluated a patient with a clinical picture of hemophagocytic lymphohistiocytosis (HLH) with a variant of uncertain significance (VUS) in XIAP. Loss-of-function variants in XIAP are a cause of XLP-2 (X-liked) and the patient's phenotype correlates with described alterations in XIAP. But the p. Cys203del variant has not been previously reported. The variant cosegregates with the XL inheritance pattern and is not present in healthy relatives. In-silico studies reveal that cysteine 203 is highly conserved. Multiple lines of computational evidence predict deleterious effect of this variant. Also, we could demonstrate that the patient's PBMCs do not expressed XIAP protein. Conclusion: Through clinical, biochemical, and bioinformatic analyses the variant could be reclassified to likely pathogenic.

Empowering immuno-oncology through sustainable data to reveal predictors of immune checkpoint blockade response

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In this work, we leveraged a sustainable data approach to identify predictive biomarkers of response and survival in melanoma patients treated with immune checkpoint blockers (ICB). Most of the data analyzed, circulating proteomics and mass cytometry, were originally generated by our group for different purposes, and were now integrated with transcriptomic data from tumors and PBMCs to explore immune mechanisms underlying ICB response. Responders exhibited higher levels of TRANCE and specific NK/B cell subsets, whereas non-responders showed increased lev-

els of 4EBP1, CDCP1, CCL4, and CCL3. A combined protein–cell signature involving CDCP1 and NK cells emerged as a promising predictor. Transcriptomic analyses suggested the tumor as a potential source of these circulating biomarkers. Altogether, this study illustrates how sustainable data use can empower immuno-oncology by uncovering new predictive insights into ICB response.

Galectin1/glycan axis as a novel glyco-checkpoint linking immunosuppression and angiogenesis in cancer

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MDSCs constitute an immune suppressive population that promotes tumor immune escape and angiogenesis and mediate anti-cancer therapy resistance. We found that Galectin-1/glycan interaction enhances MDSC function and its blockade fosters MDSC reprogramming and tumor growth inhibition.

Liver stage P. falciparum epitopes to advance the study of CD4⁺ T cell responses in childhood malaria

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The CD4+ T cell response against liver stage Plasmodium is critically important for immune protection against reinfection, but its target antigens remain largely unknown. Identifying these antigens is not only important for vaccine design but would also afford new ways to investigate the immune determinants of protection. We deployed a combination of data mining, epitope prediction, in vitro immunoassays, and a novel molecular peptide-MHC binding screening method to identify liver and blood stage P. falciparum epitopes. Preliminary results show that T cells from malaria-exposed Ugandan children responding against antigens from different parasite life cycle stages are functionally different.

Early Career Researchers Session 3

Combined glyphosate and chlorpyrifos-based pesticides impair innate and adaptive immune functions: an in vitro approach.

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Growing evidence links pesticide exposure to cancer development. Here, we assessed the impact of environmentally relevant doses of a glyphosate-based herbicide (Roundup®, R) and a chlorpyrifos-based insecticide (Clorpi48®, C), or their combination (R+C) on immune cell functions. The combination of R+C, but not the individual formulations, significantly impaired NK cell cytotoxicity, IFN-g production, and immune synapse formation. In T cells, R+C exposure inhibited proliferation, Th1 differentiation, IL-2 signaling, and IFN-γ secretion by CD8⁺ T cells, all key functions for effective antitumor responses. Mechanistically, oxidative stress contributed to the antiproliferative effect, as scavenging of H 2 O 2 by catalase addition restored T cell proliferation.

Probiotics and respiratory Allergies: past progress and future prospects

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The WHO reveals an allergy prevalence of more than 20% of the global population, with an estimated increase of 50% by 2050. Most publications between 2017 and 2024 related to allergies and probiotics focus on clinical trials, meta-analyses, and reviews (with mixed results), with basic research contributing the least to the area. It is critical to strengthen studies in this area that provide accurate information on the mechanisms of probiotics in the prevention and/or treatment of allergies, thus bolstering their use, either alone or in combination with existing therapies, as enhancers of current treatments' efficacy.

The dark side of serotonin: Arthritis aggravation by serotonylation in a TNFR1 KO murine model

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Serotonin, also known as "the happiness hormone", has emerging roles beyond neurotransmission, including immune regulation. Here, we show that protein serotonylation, a transglutaminase-dependent modification, exacerbates arthritis in TNFR1 knockout mice infected with Yersinia enterocolitica. These mice displayed elevated serum serotonin levels and increased serotonylation, whereas pharmacological inhibition reduced joint inflammation. Our findings reveal a novel pro-inflammatory role of serotonylation in arthritis and identify this pathway as a potential therapeutic target in immune-mediated arthropathies.

Development of bivalent RBD adapted COVID-19 vaccines for broad sarbecovirus immunity

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COVID-19 vaccine adaptation is critical to face emerging SARS-CoV-2 variants with immune evasion. We adapted the ARVAC vaccine to XBB.1.5 and JN.1 as monovalent and bivalent formulations. Monovalent vaccines showed strong homologous neutralizing responses but limited breadth, whereas Gamma-containing bivalent formulations achieved broad cross-neutralization from Ancestral to JN.1 and even SARS-CoV-1, with robust CD4+ T cell activation. These findings support Gamma-based bivalents as a strategy toward pan-sarbecovirus protection.

Oral Presentations – Selected Abstracts

Oral Session 1

IAI-073

Type 2 Diabetes induces CD8$^+$ T Cell Dysfunction in Trypanosoma cruzi Infection

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Type 2 diabetes mellitus (T2DM) is characterized by chronic low-grade inflammation and an increased risk of infection. We investigated the impact of T2DM on $CD8^+$ T-cell function during *Trypanosoma cruzi* (*T. cruzi*) infection, comparing results from an experimental mouse model with peripheral blood mononuclear cells (PBMCs) from T2DM patients. C57BL/6 mice were fed a 34.5% kcal fat diet and 20% v/v fructose in drinking water for 20 weeks. At week 8, a single intraperitoneal streptozotocin dose (100 mg/kg) was administered to establish experimental diabetes (D+T). Diabetic (D+Ti) and non-diabetic (Ni) mice were infected with 1000 trypomastigotes. D+T animals exhibited increased glycemia compared with controls (p=0.01). D+Ti mice showed higher parasitemia and $CD8^+$ T cells with reduced circulating (p=0.001) and splenic (p=0.01) granzyme $B^+CD44^+CD8^+$ T cells, decreased activation markers, and increased IFN-γ (p=0.02), IL-6 (p=0.01), and IL-10 (p=0.01) upon stimulation compared with Ni mice. Functional assays revealed that $CD8^+$ T cells from the D+Ti group co-cultured with infected macrophages decreased macrophage apoptosis (p=0.001) and allowed higher parasite loads (p=0.02) than Ni-derived cells. Consistently, $CD8^+$ T cells from T2DM PBMCs showed a lower frequency of granzyme B^+ cells (p=0.04) and a higher frequency of IFN-γ^+ cells (p=0.02) compared with non-diabetic controls after PMA-ionomycin stimulation. Analysis of the purinergic pathway as a key regulator of immune responses, revealed that activated $CD8^+$ T cells from PBMCs displayed a higher frequency of $CD39^-CD73^+$ cells (p=0.03), with similar findings in mice. Notably, blocking IL-6 and IL-10 or adding ATP to CD3/CD28-activated $CD8^+$ T cells from D+T mice partially restored granzyme B production, whereas adenosine abrogated this effect. Moreover, infected CD73KO mice under the T2DM model exhibited reduced circulating cytokines (p=0.01). In summary, T2DM impairs $CD8^+$ T-cell effector functions during *T. cruzi* infection, reducing cytotoxicity and enhancing cytokine production. Modulation of purinergic signaling partially reverses this dysfunction, suggesting a potential therapeutic target to improve immune responses in diabetic patients.

IAI-187

A war on two fronts: How Clostridioides difficile could reshape the macrophage response against Mycobacterium tuberculosis

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The gut–lung axis has emerged as a critical interface in host defence, yet the influence of intestinal pathogens on pulmonary immunity remains poorly understood. Here, we investigate whether the training of human macrophages (MØs) with heat-inactivated *Clostridioides difficile* (CDH), a major cause of antibiotic-associated diarrhoea and a significant opportunistic pathogen, modulates MØs subsequent interaction with *Mycobacterium tuberculosis* (Mtb), the leading cause of mortality from a single infectious agent. Monocytes from healthy donors were isolated by Ficoll–Hypaque density gradient and CD14-positive magnetic selection, differentiated into monocyte-derived MØs, and cul-

tured with or without CDH (NAP1/BI/027 strain) for 1, 2, 5, or 7 days. MØs were then stimulated with γ-irradiated whole-cell Mtb (WCMtb; H37Rv strain) for 1 h or 24 h. Endocytic uptake of CDH and WCMtb, SLAMF1 (Mtb receptor) and LAMP2 (lysosomes) expression, and morphological features were assessed by flow cytometry and fluorescence microscopy. MØs viability remained ~90% at day 7 without media replenishment. CDH uptake peaked at day 2 (~50% of the MØs) and remained stable thereafter. CDH training induced distinct morphological adaptations in MØs. CDH-trained cells retained an isolated, spherical phenotype. Notwithstanding, upon challenged with WCMtb, MØs showed an increased surface area and clustering, specifically those MØs that had internalized only WCMtb ($p<0.05$ for day 7). Notably, CDH training reduced WCMtb endocytosis by MØs, with maximal suppression observed at day 7. Regarding SLAMF1, WCMtb upregulated its expression on day 5; however, this induction was abrogated by CDH training, which also suppressed LAMP2 expression in MØs. Additionally, CDH-trained MØs displayed reduced formation of tunneling nanotubes. This work provides the first experimental evidence of direct crosstalk between *C. difficile*–*M. tuberculosis* and macrophages. We evidenced that a prior exposure to *C. difficile* can attenuate macrophage endocytic responses to *M. tuberculosis*. This implies that intestinal pathogens may modulate innate immune responses against respiratory infections and emphasize trained immunity as a potential mechanism within the gut–lung axis.

VM-084

Co-encapsulation of antigens and U-Omp19 adjuvant in polymeric microparticles containing nanoparticles for the development of oral vaccine formulations.

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Oral vaccines are easy to administer but their development still faces many challenges, such as low stomach pH and proteolytic digestion by gastrointestinal proteases. We previously demonstrated that U-Omp19 from Brucella spp. is an oral vaccine adjuvant that protects co-delivered antigens from proteolysis in the gastrointestinal tract and increases antigen (Ag) specific adaptive immune responses. In this work, we aim to protect vaccine formulation by encapsulating it in microparticles containing nanoparticles (micro/nanoparticles). Thus, poly- (lactic-co glycolic)-acid (PLGA) nanoparticles encapsulating RBD (Gamma variant) as Ag and U-Omp19 as adjuvant were synthesized using the double emulsion- solvent evaporation (DE-SE) method. To deliver the vaccine to the large intestine, where adaptive immune responses can be triggered, nanoparticles were covered with Eudragit (Eu). Encapsulation efficiency was determined by SDS-PAGE. BALB/c mice were orally immunized with i) RBD and U-Omp19 encapsulated in micro/nanoparticles Eu-PLGA(RBD + U-Omp19), ii) microparticles Eu(RBD + U-Omp19) iii) non-encapsulated RBD+U-Omp19 and iv) non-encapsulated RBD+CT. RBD-specific IgA responses were determined in feces and bronchoalveolar lavage (BAL) by ELISA. Four weeks after the last immunization, adaptive immune responses were evaluated in Payer's Patches and lungs by flow cytometry. Mice immunized with vaccine formulations encapsulated in micro/nanoparticles showed a significant increase in both Ag-specific IgA responses at feces and BAL when compared to the control group that received the same non-encapsulated vaccine. In Peyer's Patches the frequency of RBD specific IgA+ plasmablasts (B220+ CD19+ CD138+ IgA+ RBD+) was higher in mice immunized with microparticles than in the non-encapsulated group. An increase in follicular helper T cells (CD4+ PD1+ CXCR5+) was observed in mice immunized with micro/nanoparticle encapsulated vaccine. Mice immunized with either particle formulation showed an increase in total Ag-specific IgA B cell population (B220+ CD19+ IgA+ RBD+) at the lung. In conclusion, polymeric particles that encapsulate both the antigen and the adjuvant U-Omp19 increase Ag specific IgA in feces and BAL and produced an increase the frequency of B cells in the Payer's Patches and lungs in comparison with non-encapsulated vaccine.

VM-167

A novel strategy to improve offspring protection against Bordetella pertussis

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Pertussis is a vaccine-preventable respiratory disease caused by Bordetella pertussis (Bp) that affects individuals of all ages and has resurged in recent decades. Infants, particularly those under 2 months of age who have not received any specific vaccines, are the most vulnerable group. To protect them, a strategy that has emerged with available vaccines is immunization during pregnancy. In this approach, acellular pertussis (aP) vaccines are employed, and it has proven effective in reducing infant mortality through antibody transfer. However, aP vaccines present important challenges, as they provide only short-term protection, fail to induce adequate protection against some currently circulating bacteria, and to prevent disease transmission. We developed a Bp OMV-based vaccine prototype to overcome current aP limitations, first tested in a two-dose mice model and now evaluated as a maternal booster. This approach is expected to counteract the Th2 bias characteristic of neonates, promoting a more robust and long-lasting immune response and thereby improving disease control in this vulnerable group. In this study, using the murine pregnancy model established in our laboratory, we compared OMV-based boosters administered during pregnancy (OMVPreg) with the commercial aP booster (aPPreg). We assessed humoral immunity transfer (HIT) by measuring anti-Bp total IgG and isotypes in offspring (neonates and infants) born at different time points following maternal immunization. Offspring from OMVPreg dams (OMVpups) exhibited significantly higher levels of total IgG and IgG3 compared to offspring from aPPreg dams (aPpups, $p<0.0001$). This trend persisted across successive litters, with OMVpups also showing increased IgG1 levels relative to aPpups ($p<0.01$). To evaluate the impact of HIT on disease control, offspring were challenged with B. pertussis. While pups from both immunized groups were equally protected against lung colonization in early and later litters, a notable finding was that adult offspring (60 days old) from OMVPreg dams displayed greater protection than their aPpups counterparts ($p<0.05$). Interestingly, IL-22, a cytokine previously associated with protection, was found at higher levels in OMVpups. Collectively, these findings support OMV as a promising booster for pregnancy, capable of inducing a more balanced immune response in offspring and providing protection comparable to, or potentially exceeding, to the licensed aP vaccine over the long term.

VM-169

Enhanced Immunogenicity through Co-administration of COVID-19 and Pertussis Vaccines

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The neonatal immune system exhibits a strongly regulatory profile, limiting the induction of protective immunity through direct vaccination early in life. Consequently, immunization during pregnancy has become a cornerstone strategy to protect both mothers and newborns. Currently, four vaccines are recommended for pregnant women: diphtheria-tetanus-pertussis (Tdap), seasonal influenza, COVID-19, and respiratory syncytial virus. These vaccines have proven safe for both mother and fetus, inducing robust maternal immune responses that are transferred to the neonate via transplacental and lactational routes. As maternal immunization expands to include new vaccines, evaluating potential interactions when multiple formulations are co-administered is essential. This study provides an initial evaluation of co-administering Tdap with either a protein-based COVID-19 vaccine (CoVacPro) or an mRNA-based vaccine (CoVacmRNA) in a murine

model, using 2 intramuscular doses followed by pregnancy immunization. Maternal antibody responses were significantly enhanced by co-administration when Tdap was given with CoVacPro, total IgG levels against B. pertussis, diphtheria, and tetanus were significantly higher than with Tdap alone ($p<0.001$). For specific COVID-19 antibodies (anti-Spike), CoVacPro+Tdap did not alter IgG levels compared to CoVacPro alone, whereas co-administration of CoVacmRNA+Tdap induced significantly higher IgG levels than CoVacmRNA alone ($p<0.0001$). Notably, although co-administration of CoVacmRNA+Tdap did not increase total IgG levels against Tdap antigens, it induced a clear Th1-biased response, as reflected by increased IgG2a levels. Antibody transfer to offspring mirrored these findings. Offspring from CoVacPro+Tdap-immunized dams exhibited higher anti-Tdap IgG levels in comparison with pups born to Tdap dams. Offspring from CoVacmRNA+Tdap dams showed slightly lower but persistent anti-Tdap IgG titers. Critically, offspring from both co-administered vaccine groups were protected against pertussis since they showed dramatically reduced bacterial loads recovered from lungs when compared to controls (log CFU/lungs: ~1 vs. ~6). In summary, maternal co-administration of Tdap with COVID-19 vaccines (protein- or mRNA-based) is safe, enhances maternal and neonatal antibody responses, and confers robust protection against B. pertussis infection. These results support the consideration of co-administration strategies in future maternal immunization programs.

VM-184

Phase 4 observational study on the immunogenicity of the ARVAC Gamma–Omicron BA.4/5 bivalent COVID-19 vaccine

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COVID-19 remains a threat due to the continuous emergence of SARS-CoV-2 variants that evade the protection generated by vaccines and prior infections, highlighting the critical need for vaccine strategies capable of addressing emerging viral threats. ARVAC is a protein subunit vaccine based on the receptor-binding domain (RBD) of the Spike protein, developed entirely in Argentina, and approved for use as a booster vaccine in adults. In this work, we evaluated cellular and humoral immune responses elicited by ARVAC bivalent formulation (Gamma–Omicron BA.4/5) in an observational study. Sixty adults with prior COVID-19 vaccination were enrolled and received one ARVAC bivalent boost dose. Blood samples were collected before vaccination and 28 days post-vaccination and plasma and PBMCs were obtained. Antigen-specific T cell responses were measured by ELISPOT to assess cellular immunity. At day 28 post-vaccination, there was a significant increase in the frequency of IFN-γ–and IL-4–producing specific T cells compared with baseline, in response to both Omicron BA.4/5 RBD and Omicron JN.1 RBD peptides. This response remained significant regardless of the number of previous booster doses received. Humoral response was assessed by determining anti-RBD IgG titers and neutralizing antibody titers. ARVAC bivalent Gamma/Omicron BA.4/5 vaccine induced a significant increase in specific IgG levels at 28 days post vaccination, independent of sex, age (<60 or ≥60 years), and number of previous booster doses. A significant increase in neutralizing antibody titers against both ancestral SARS-CoV-2 and Omicron BA.4/5 variant of concern at 28 days post-vaccination was observed. In conclusion, ARVAC bivalent Gamma–Omicron BA.4/5 vaccine induces a broad antigen-specific immune response, characterized by IFN-γ and IL-4 T cell production, increased IgG titers, and neutralizing activity against ancestral and Omicron variants. These results support the use of bivalent vaccines containing the Gamma antigen to provide cross-variant protection and contribute to broad population immunity against current and emerging SARS-CoV-2 variants.

Oral Session 2

MR-182

Chronic exposure to glyphosate-based herbicides reduces sperm quality, disrupts seminal fluid-induced immunomodulation in the female reproductive tract, and impairs fetoplacental development.

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Although glyphosate-based herbicides (GBH) are widely used, particularly in Argentina, their potential impact on male fertility and reproductive health has not been investigated. Despite being identified as endocrine-disrupting chemicals, the effects of paternal GBH exposure on male fertility, pregnancy outcomes, and offspring remain unexplored. Thus, using an animal model in mice, we herein investigated the effects of male chronic exposure to GBH on sperm quality, the immunomodulation induced within the female genital tract during pregnancy, male fertility parameters, and fetoplacental development. C57BL/6 male mice were administered an aqueous solution of GBH (400 mg/kg/day, orally) for 35 consecutive days. After that, mating experiments were conducted with unexposed BALB/c females. On day 40, the males were euthanized and analyses were performed on body weight, testicular histology, sperm quality, serum testosterone levels, and different splenic immune cell populations. In mated females, uterine immune cell infiltration was evaluated during the peri-implantation window. Moreover, different fertility parameters and fetoplacental development were assessed on gestational day 19. Although GBH exposure did not affect body weight, exposed males showed significantly reduced testicular weight (despite preserved histology), as well as impaired sperm quality and decreased serum testosterone levels. Besides, no differences were observed in the frequencies of different splenic immune cell subpopulations between GBH-exposed and unexposed males. Interestingly, females mated with exposed males showed significantly increased infiltration of different leukocyte subsets in the uterus during the peri-implantation period than females mated with control males. Furthermore, pups sired by GBH-exposed males displayed marked alterations in fetoplacental development. Finally, multivariate analysis revealed a clear distinction between pups sired by control versus exposed males, further indicating that paternal GBH-exposure disrupts fetoplacental development. Our data indicate that chronic GBH exposure in males reduces serum testosterone levels and impairs sperm quality without altering systemic immune cell populations. These changes are associated with disrupted immunomodulation in the female reproductive tract and impaired fetoplacental development. Together, these findings highlight the critical role of paternal factors in reproductive risks associated with GBH exposure.

IC-113

IL-22 as a Modulator of Intestinal Transplant Rejection: Regenerative and Protective Potential

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Background. Acute cellular rejection (ACR) remains the major barrier to long-term success after intestinal transplantation (ITx), compromising mucosal integrity and driving graft loss. The IL-22/IL-22BP/IL-22R axis has been implicated in epithelial repair and mucosal homeostasis. Methods. Intestinal grafts from Sprague-Dawley rats were transplanted into Wistar recipients under suboptimal immunosuppression with tacrolimus (0.6 mg/day). Animals were divided into two groups: controls receiving vehicle plus tacrolimus, and the experimental group receiving recombinant IL-22 (rIL-22, 30 µg/day on 11, 13 and 15 post-transplant days). On day 21, grafts were collected for histological analysis, immune profiling, epithelial proliferation (Ki-67), apoptosis (TUNEL), and goblet cell quantification. Clinical outcomes included survival and weight recovery. In parallel, IL-22, IL-22R, and IL-22BP mRNA levels were quantified in human intestinal biopsies from non-rejectors (n=9), rejectors (n=7), and non-ITx controls (n=8). Results. Human biopsies showed significant downregulation of IL-22 and IL-22BP during ACR ($p<0.05$), whereas IL-22R expression tended to increase. In rats, rIL-22 did not prolong survival but promoted earlier weight recovery ($p<0.05$) and preserved mucosal structure. Severe ACR occurred in 50% of controls but was completely prevented in rIL-22–treated grafts ($p<0.05$). Villus-to-crypt ratio, goblet cell counts, and epithelial proliferation were maintained, while apoptosis was significantly reduced ($p<0.05$). Conclusion. Recombinant IL-22 mitigates severe rejection and enhances epithelial regeneration in experimental ITx. These findings highlight IL-22 analogues as promising adjunct therapies to protect the graft and improve outcomes in intestinal transplantation. Moreover, the regenerative and protective effects of IL-22 could potentially be extended to other intestinal disorders characterized by epithelial barrier damage.

OI-024

Adaptive NK cell infiltration is associated with improved overall survival in breast cancer

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Adaptive natural killer (adNK) cells are a specialized subset of NK cells that emerge after HCMV infection and exhibit enhanced functions vs conventional NK (cNK) cells. They include increased IFNγ production and degranulation, especially via CD16, with persistence and distinct phenotype. While studied in peripheral blood (PB), little is known about their presence, phenotype, and role within the tumor microenvironment (TME) of solid cancers. We investigated the distribution, phenotype, and functionality of adNK cells in breast cancer from 41 patients using paired PB, tumor, and adjacent tissue samples. Flow cytometry showed that while the proportion of adNK cells is similar between tumor and PB, in some patients it is higher in the TME ($p<0.05$). Tumor adNK cells are phenotypically similar to those from PB. Compared with cNK, they retain a higher capacity to produce IFNγ ($p<0.05$) and to degranulate after stimulation with PMA/ionomycin. Interestingly, IFNγ production in tumors did not significantly differ from PB, suggesting partial preservation of function despite the TME. Considering their functionality, we tested whether intratumoral adNK cells associate with the status of CD8+ T cells. We found a positive correlation between adNK cells and PD-1+TIGIT+ CD8+ lymphocytes, a phenotype linked to activation and exhaustion. This subset displayed increased degranulation after PMA/ionomycin, indicating they may retain effector capacity despite inhibitory receptors.To assess the impact of adNK infiltration on survival, we analyzed TCGA

BC datasets using the GEPIA2 tool and an adNK gene signature as a surrogate of infiltration. Kaplan-Meier analysis showed that higher adNK infiltration correlated with better overall survival (Logrank=0.033; HR (high)=0.7; p(HR)=0.034), with no difference in disease-free survival. Our findings reveal that adNK cells not only infiltrate breast tumors but also maintain functional features that could contribute to shaping the intratumoral immune landscape. The observed association with a distinct subset of CD8+ T cells highlights a potential interplay between these two lymphocyte populations, raising the possibility that adNK cells may contribute to the control of breast tumors.

OI-029

Endogenous galectin-1 Links CD8+ T Cell Exhaustion and Immune Checkpoint Receptor Pathways

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Antitumour immune responses are held in check by a plethora of inhibitory signals that promote tumour-immune escape. Galectin-1 (Gal1), a glycan-binding protein that recognizes N-acetyllactosamine residues in complex N- and O-glycans promotes immunosuppression by triggering expansion of regulatory T cells, inducing tolerogenic dendritic cells and reprogramming myeloid cells. Through single-cell RNA sequencing analysis, we found that Gal1 expression was elevated in exhausted CD8+ tumour-infiltrating T cells (Tex) compared to their effector counterparts, suggesting a potential role of this lectin in modulating CD8+ T cell exhaustion. To test this hypothesis in vivo, we used CRISPR-Cas9 editing on OVA-restricted OT-1 CD8+ T cells to generate Gal1-deficient antigen-specific CD8+ T cells (sg*Lgals1*). We injected control and sg*Lgals1* OT-1 CD8+ T cells into B16-OVA-bearing *Rag2*-/- mice. Flow cytometry and RNA-sequencing analysis revealed that sg*Lgals1* Tex cells conserved effector functions as evidenced by elevated expression of Granzyme B, IFN-γ and TNF-α ($p<0.01$) compared to control T cells. Similarly, in vitro-generated Tex (iTex) lacking Gal1 (*Lgals1*-/-) displayed elevated expression of IFN-γ and TNF-α ($p<0.01$) and reduced levels of the exhaustion marker TOX ($p<0.05$) compared to wild-type iTex. Mechanistically, we evaluated whether soluble Gal1 may contribute to T cell exhaustion by interacting with immune checkpoint receptors. Using solid-phase binding assays and isothermal titration calorimetry, we demonstrated that Gal1 differentially binds to a set of immune checkpoint receptors (including VISTA, PD-1, CTLA-4, TIM3, LAG3, and TIGIT) in a glycan-dependent manner (since binding could be prevented by lactose), suggesting possible autocrine/paracrine effects of the secreted galectin. Our results suggest that endogenous Gal1 expression in CD8+ Tex cells may contribute to their dysfunctional state, possibly through immune checkpoint engagement. Thus, silencing Gal1 may have therapeutic implications in cancer immunotherapeutic regimens including immune checkpoint blockade and adoptive cell transfer.

OI-086

Inhibition of sphingosine kinase 2 (SPHK2) reduces venetoclax resistance in a murine xenograft model of Chronic Lymphocytic Leukemia (CLL)

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Introduction:
Treatment of CLL patients with the BCL-2 inhibitor venetoclax (VEN) has shown efficacy, but the emergence of resistant cells is a current complication. We reported that co-culture of CLL cells with activated autologous T cells (aaT) promotes leukemic clone activation and VEN resistance (Elías et al. 2018). VEN-resistant cells overexpress SPHK2, whose inhibition reduces aaT-induced CLL activation, proliferation and VEN resistance, and resensitizes already resistant CLL cells in vitro (Sarapura Martínez et al. 2023). Objective: To evaluate in vivo treatment with VEN in combination with an SPHK2 inhibitor (opaganib) in a murine xenograft model of primary cells from CLL patients. Methods: 95 NSG mice were injected with peripheral blood mononuclear cells from 6 CLL patients (3 with mutated IGHV (M-IGHV), good prognosis, and 3 with unmutated IGHV (U-IGHV), poor prognosis) along with aaT cells (Patten et al. 2021). After 2 weeks, human $CD45^+$ cell detection confirmed engraftment, enabling 3-week treatment of 4 groups: control, VEN, opaganib, and VEN+opaganib. We euthanized the mice and assessed spleens, bone marrow, and peripheral blood by flow cytometry. Statistical analyses were made using Kruskal-Wallis followed by Dunn test. Results: In this murine model, CLL cells (CD19+CD5+) were resistant to VEN. Opaganib alone did not diminish disease burden, but the combination VEN+opaganib, reduced splenic $CD19^+CD5^+$ cell counts by 50% compared to controls ($p<0.05$; Kruskal-Wallis followed by Dunn test; n=95). We found a trend toward a greater effect in mice with U-IGVH cells, without reaching statistical significance. Few CLL cells were found in bone marrow and blood, with no significative differences among groups. No significant variations in BCL-2 expression were found in any of the 4 treatment groups. VEN+opaganib reduced the number of T cells (CD3+) compared to controls in the spleens of mice injected with cells from U-IGHV patients ($p<0.05$; Kruskal-Wallis followed by Dunn test; n=42). $CD4^+$ and $CD8^+$ T-cell counts exhibited a downward trend in the VEN+opaganib and opaganib groups, reaching significance only for $CD8^+$ cells ($p<0.05$; Kruskal-Wallis followed by Dunn test; n=42). CD8+ T cells in the VEN+opaganib group showed lower PD1 expression ($p<0.05$; Kruskal-Wallis followed by Dunn test; n=73).
Conclusion: This in vivo preclinical trial positions the SPHK2 inhibitor opaganib as a potentially useful therapeutic agent to overcome VEN resistance on CLL patients.

OI-196

A topical administration way of repurposing Vismodegib for the treatment of melanoma by using ultradefomable liposomes

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Vismodegib (VDG) is a Hedgehog pathway (HH) inhibitor approved in 2012 for basal cell carcinoma oral chemotherapy that causes severe systemic side effects. Its efficacy against melanoma has not been evaluated. We propose repurposing VDG for melanoma treatment and developing a nanotechnology-based formulation. We aim to evaluate the efficacy of topically-applied VDG-loaded ultradeformable liposomes (UDL) on melanoma growth, compare it with oral administration (OA), and evaluate inflammatory infiltration (II), and protein expression in the tumoral environment. VDG was encapsulated into UDL via lipid film hydration. In vitro assay: 10.000 B16 cells/well were treated with either free VDG, UDL-VDG, or vehicle for 24h. Cell viability was assessed using Alamar Blue. In vivo assay: C57BL/6 mice were i.d. inoculated with 1×10^5 B16 cells. After tumor detection, mice were treated in three groups: OA, topical UDL-VDG; control. Tumor size was measured for two weeks. A delayed-type hypersensitivity (DTH) assay assessed the anti-tumor immune response. At euthanasia, tumor samples were fixed and sectioned. Sections underwent H&E and immunohistochemical (IHC) analysis. Each tumor received a detection score. VDG inhibited melanoma growth in vitro (50 µM, $p<0.0001$ vs. control). In vivo, UDL-VDG and OA slowed down growth (171.810 mm3 and 177.57mm3 respectively vs 275.17 mm3). UDL-VDG also increased

II. DTH showed a non-significant increase in cellular immune response in both treated groups. IHC revealed high SMAD3 expression in control tumors, but none in the treated groups. Using STRING, SMAD3 was linked to HH activation via a non-canonical route, promoting tumor immune evasion, proliferation, and metastatic potential. TGF-β showed faint cytoplasmic staining but was absent in controls. HIF-1α was expressed only in the OA group, localized in cytoplasm of II areas. NRF2 showed cytoplasmic expression in II necrotic regions of treated tumors, suggesting a response to chemotherapy. NRF2 cytoplasmic signal could reflect an attempt at survival in response to the treatment's effects. CD44 was expressed in tumor cytoplasm in all groups, its presence may be driven by the JAK-STAT signaling pathway. Vimentin and p65 tested negative. In conclusion, topical UDL-VDG represents a promising alternative for treating skin malignancies and repurposing VDG for melanoma may represent a novel target for tumor growth inhibition and immune modulation.

Abstracts preselected for the SATZ Award

Effects of IL-10 on Systemic and Renal Immune Responses to Stx2 in IL-10-/- Mice

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Hemolytic Uremic Syndrome (HUS), caused by Shiga toxin–producing E. coli, leads to hemolytic anemia, thrombocytopenia, and renal failure. Our group showed that IL-10$^{-/-}$ mice become more susceptible to Stx when IL-10 is induced. To further analyze the role of IL-10, IL-10$^{-/-}$ mice received an IL-10–expressing plasmid (PL) and Stx after 48 h (groups: Control, PL, Stx, Stx+PL). To assess an early inflammatory response, corticosterone was measured by ELISA in plasma 3 h post-Stx. Corticosterone levels (ng/mL) rose with Stx but not when IL-10 was present, suggesting a suppression of adrenal activity [Median (IQR) = Control:10.44(4.12–22.03); PL:36.26(27.77–47.34); Stx:44.10(342.60–798.40)*; Stx+PL:6.22(4.23–9.66); *p<0.05]. At 48 h, ROS production (AUC) was assessed by luminol assay in blood. The Stx+PL group showed an increase in respiratory burst [Median (IQR) = Control:138(94–360); PL:351(244–460); Stx:151(151–216); Stx+PL:958(841–1155)*; *p<0.05], indicating a higher activation of phagocytes. At 72 h, kidneys were extracted for flow cytometry and ELISA analysis. Based on our prior findings of increased monocyte kidney recruitment and the role of IL-10 in M2 polarization, we assessed M2-like macrophages (CD45^{+}CD11b^{+}F4/80^{+}CD206^{+}) and found a higher percentage in Stx+PL mice, likely driven by Stx-induced inflammation and IL-10 expression [Median (IQR) = Control:20.25(16.93–23.50); PL:18.50(15.35–20.75); Stx:22.00(20.10–31.00); Stx+PL:45.00(40.00–59.00)*; *p<0.05]. The renal immune profile was studied by measuring MCP-1 and TGF-β. MCP-1 levels increased in the Stx+PL group [Median (IQR) = Control:1830(980.20–2730); PL:1681(1189–2217); Stx:2138(1755–2502); Stx+PL:3831(3502–4024)*; *p<0.05], and TGF-β rose after Stx but not with IL-10 [Median (IQR) = Control:55.40(36.54–69.25); PL:29.33(14.13–66.73); Stx:88.64(85.49–139.00)*; Stx+PL:43.85(21.66–58.47); *p<0.05], indicating altered immune modulation. Overall, our data suggest that Stx with IL-10 increased systemic ROS, indicating an elevated immune activation state despite early corticosterone suppression. Renal MCP-1 levels were higher with Stx and IL-10, consistent with enhanced monocyte infiltration. Meanwhile, TGF-β levels fell and M2 macrophages increased with Stx and IL-10, suggesting modulation of inflammation mediated by the cytokine. IL-10 may be driving an atypical M2 phenotype. These findings support a detrimental and dual role of IL-10 in Stx-induced injury and HUS evolution.

Palabras clave: HUS; STEC; IL-10; Inflammation

Pharmacological Enhancement of Cross-Presentation in Dendritic Cells Promotes Robust Antigen-Specific CD8⁺ T Cell Responses In Vivo: A Promising Approach for Next-Generation DC-Based Immunotherapies

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Dendritic cells (DCs) are uniquely capable of activating naïve T lymphocytes through antigen presentation, costimulatory signaling, and cytokine secretion. Cross-presentation (XP) of exogenous antigens on MHC class I molecules is a key pathway for inducing cytotoxic CD8⁺ T lymphocyte (CTL) responses, with important implications for immunotherapy. In previous work, we demonstrated that pharmacological stimulation of DCs with Pimozide (P), a drug previously used as an anti-psychotic treatment, enhances XP, leading to increased expression and stability of H-2Kᵇ–SIINFEKL complexes in DC surface, endosomal antigen translocation, and the activation and proliferation of naïve CD8⁺ T cells from OT-I mice in vitro. Moreover, DCs treated under these conditions induced potent antigen-specific CTL activity in vivo, as evidenced by specific elimination of SIINFEKL-pulsed target cells. Here, we expanded our studies to evaluate the in vivo immune response induced by DCs loaded with OVA in the presence or absence of P as an adjuvant. FMS-like tyrosine kinase 3 Ligand Bone marrow–derived DCs (Flt3-L BMDCs) were incubated for 2 h with OVA ± P, washed, and intravenously injected into C57BL/6 mice. Seven days post-immunization, splenocytes were isolated and analyzed. Following in vitro re-stimulation with SIINFEKL, mice immunized with DCs + P showed a higher percentage of splenic CD8⁺ IFNγ⁺CD107a⁺ T cells compared to controls. Analysis with H-2Kᵇ–SIINFEKL tetramers confirmed an increased frequency of antigen-specific CD8⁺ T cells in the spleen. Additionally, re-stimulation with the MHCII-restricted $OVA_{323–339}$ peptide showed no significant differences in OVA-specific CD4⁺ T cells between groups.These results demonstrate that enhancing XP in DCs can promote a robust and functional antigen-specific CD8⁺ T cell response in vivo. The strong induction of effector CTL activity highlights the translational potential of this strategy. Future studies will focus on testing this approach using antigens derived from tumor and infectious models, aiming to establish its applicability in both cancer and pathogen-targeted immunotherapies.
Palabras clave: Dendritic cells; Cross Presentation; New generation Immunotherapies

Extracellular acidosis promotes resident memory T cell differentiation

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Tissue-resident memory T cells (TRM) provide frontline defense against infectious diseases and contribute to antitumor immunity. However, despite extensive research, key aspects of TRM differentiation in humans remain unclear. pH is a hallmark of a variety of inflammatory processes in peripheral tissues where TRM are present. Our aim was to evaluate if low pH induces the differentiation of TCD8+ cells in a TRM phenotype, expressing CD69+ CD103+ and enrichment for endogenous TRM gene signatures.PBMC were purified from healthy donors and CD8+ T cells were obtained using magnetic microbeads. CD8⁺ T cells were activated with CD3/CD28 beads for 48 hours and then cultured for 3 more days at either neutral (pH 7.3) or acidic (pH 6.5) conditions. The expression of CD69 and CD103 were determined by flow cytometry. Transcriptome profiling was performed by RNA-Sequencing. Exposure to low pH significantly increased the percentage of CD8+CD69+CD103+ T cells compared to neutral pH (24.8 ± 2.4 vs 4.0 ± 0.7, n=24 $p<0.0001$). These cells showed upregulation of genes

identified as the human TRM core signature, such as CD69, ITGAE, CD101, CXCR3. We did not observe any differences in ITGA1 and PD-1 transcript levels. Transcripts of KLF2, an important gene involved in T cell recirculation was downregulated, further suggesting a residency program. CD8+ T cells exposed to low pH upregulated the expression of TGF-β (n=6, $p<0.05$) and the protein production (n=6, $p<0.05$). We also observed that when CD8+ T cells were treated with ALK5 inhibitor (kinase associated with TGF-β receptor I) the ability of acidic pH to induce the CD69+CD103+ phenotype was suppressed (n=3 $p<0.05$), suggesting an action mediated by TGF-β. Our findings suggest a likely cue for the TRM differentiation process and may enable the efficient generation of human TRM-like cells in vitro for basic studies and translational applications such as adoptive cellular therapy.

Palabras clave: TRM; acidosis; TGF-beta

Abstracts preselected for the Clinical Immunology Award

Expanding Genomic Diagnosis in Inborn Errors of Immunity: From National Cohort to a Dominant-Negative IL10RA Variant

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Inborn Errors of Immunity (IEI) are a heterogeneous group of genetic disorders presenting with recurrent infections, autoimmunity, autoinflammation, allergy, bone marrow failure, and malignancies. Despite advances in massive sequencing technologies (MST), over 550 genetic defects have been associated with IEI, yet Latin American populations remain underrepresented in genomic databases, hampering diagnosis and equity in care.

To address these challenges, since 2023 we established a federal collaborative network in Argentina linking more than 15 hospitals. Between 2023–2025, 250 patients with suspected IEI underwent genomic analysis, representing the largest national cohort to date. Whole-exome sequencing achieved ~50% diagnostic yield, revealing known pathogenic variants and novel candidates requiring functional validation. To overcome the bottleneck of Variants of Uncertain Significance (VUS), we developed bioinformatic pipelines integrating AI–based algorithms. This strategy accelerates variant prioritization and facilitates the discovery of novel disease-related genes.

Functional genomics proved essential to refine diagnoses and uncover novel disease mechanisms. We identified nine unreported STAT3 variants, confirmed compound heterozygous PTPN2 variants, and dissected CARD11 alterations in 15 patients, demonstrating both dominant-negative and gain-of-function effects. Additional variants in RHOG and IL2RG are under validation, underscoring the value of our pipeline in resolving atypical genetic models.Among the most striking findings, we identified a multi-generational Argentinean family carrying a heterozygous nonsense IL10Rα variant (c.787C>T; p.Arg263*). Clinical manifestations ranged from very early onset IBD to CVID with autoimmunity, while some carriers remained asymptomatic. Functional studies demonstrated truncated IL10Rα expression and impaired STAT3 phosphorylation upon IL-10 stimulation in patient-derived PBMCs, supporting a dose-dependent dominant-negative effect. Reporter assays confirmed that the mutant receptor interferes with wild-type function, expanding the spectrum of IL-10R–associated immune dysregulation beyond recessive inheritance.

These results highlight the translational impact of integrating national genomic networks, advanced bioinformatics, and functional validation. Our initiative improves diagnostic equity in Argentina while revealing novel mechanisms of immune disease with direct applications in clinical immunology.

Palabras clave: Inborn Errors of Immunity; Genomics; Functional Validation; WES

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Repositioning Indomethacin as a therapeutic agent for bone Langerhans Cell Histiocytosis: effects on homing and bone homeostasis

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Langerhans cell histiocytosis (LCH) is a rare inflammatory myeloproliferative disorder with cells expressing CD207/CD1a likely derived from monocytes (Mo), dendritic cells (DC), or bone marrow $CD34^+$ progenitors. LCH presents variably, with bone being the most common site. Indomethacin (Indo), a non-selective COX inhibitor, has shown to improve bone healing, though its mechanism remains unclear. The TAM receptors (TYRO3, MERTK, AXL) are crucial negative regulators in immune responses and tissue homeostasis, including bone. We aim to evaluate how Indo modulates the homing of LCH precursors and the differentiation of Mo into DC, Langerhans-like cells (LC), and osteoclasts (OCL). Blood samples from 35 LCH patients (54% male, median age 11, (1-50 years)) undergoing or not Indo treatment were analyzed, alongside 20 healthy controls (HC) for in vitro assays. RANK-L was measured by ELISA and $CD14^+$ Mo were isolated from peripheral blood mononuclear cells (PBMCs). Mo were analyzed directly or differentiated into DC, LC and OCL. The effects of Indo on homing and differentiation were assessed by flow cytometry and qPCR. Untreated LCH patients showed higher mRNA levels of CCR1, RANK, OPG, AXL, TYRO3, and MERTK on $CD14^+$ Mo compared to HC. After Indo treatment, there was a reduction in the expression of CXCR4 (P=0.04), CCR1, CCR2, RANK, OPG, AXL and TYRO3 mRNA. Indo also shifted the Mo populations, decreasing classical CD14++CD16− and increasing non-classical CD14−CD16++ Mo (N=12, P=0.03), with both subsets showing higher levels of TYRO3 (P=0.007 for classical, P=0.01 for non-classical). Plasma RANK-L levels were elevated in untreated patients (P=0.005). In vitro, Indo (100 μM) treatment decreased OPG mRNA (P=0.02) and the % of AXL-expressing (P=0.004) Mo. Mo-derived DCs treated with Indo exhibited lower mRNA levels of CCR1, CCR2, RANK, OPG, and AXL (P<0.02). Similar reductions were observed in LCs, particularly in CCR2, OPG, and AXL (P<0.02). Indo impaired OCL differentiation, shown by lower TRAP staining (P=0.03) and reduced expression of CD51+/CD61+ (P=0.002), and decreased bone resorption and adherence (P=0.03). Indo increased AXL and GAS6 expression in OCLs (P=0.03). The clinical benefits of Indo in bone LCH may be linked to its impact on the homing and differentiation of pathological LC. Indo modifies the expression of key molecules involved in Mo homing and differentiation into DC, LC, and OCL, providing a strong rationale for its use in treating bone LCH.

Palabras clave: LCH; BONE; INDOMETHACIN; RANKL

ABSTRACTS
Posters Session

Allergy and Hypersensibility

AH-019

Design of new immunotherapies for asthma treatment through CAR-T cells

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RATIONALE: Atopic asthma is a chronic inflammatory disease with rising global prevalence and no curative therapy. CAR-T cells are T lymphocytes genetically engineered to express synthetic receptors that redirect their activity toward specific targets, independently of MHC. Following their success in oncology, researchers are working to translate this approach to other immune-mediated conditions, from systemic lupus erythematosus to asthma. Recent preclinical studies targeting IL-5R demonstrate selective immune modulation, supporting CAR-T cells as a promising new therapy for severe asthma. METHODS: Lentiviral vector was produced by co-transfection of 293FT cells with packaging plasmids. Titration of lentiviral particles was performed in HEK293T cells. PBMCs from buffy coat were obtained and activated for 2 days with activation beads and human IL-2 followed by transduction via spin-inoculation with lentivirus particles. Specific reactivity of OVA CAR was measured by co-culture effector cell (OVA CAR-T) and target cell (Ramos-OVA) at different effector-to-target (E:T) ratios in flat-bottom 96-well plates. RESULT: We successfully designed an allergen-specific CAR by cloning a single-chain variable fragment (scFv) against ovalbumin (OVA)— an asthma model antigen—into a validated third-generation CAR backbone vector (OVA CAR). This vector contains a fluorescent marker (mTagBFP) and antibiotic resistance gene (puromycin) to identify transduced cells. Lentiviral particles were produced and concentrated to a final titer of 5×10^8 TU/mL. They were used to efficiently transduce T cells purified from human buffy coats. The functional activity of these OVA-CAR T cells was confirmed in co-culture assays with OVA-expressing Ramos B cells (Ramos-wOVA), demonstrating over 85% specific lysis at a 5:1 effector-to-target (E:T) ratio, with significant cytotoxicity maintained even at a 0.5:1 ratios. Control experiments using non-OVA-expressing Ramos cells and CAR-T cells targeting unrelated antigens showed no cytotoxicity, confirming that activation was strictly antigen-specific. CONCLUSIONS: These results validate the specificity and potency of the OVA-CAR system, which now serves as the foundational platform for the current study.
Key words: Cell therapy; Chimeric Antigen Receptor T cells; Severe allergic asthma

AH-111

B3-TUBULIN EXPRESSION PATTERN REVEALS POOR INNERVATION AND SUGGESTS EPITHELIAL DEDIFFERENTIATION IN COLORECTAL POLYPS FROM PATIENTS SENSITIZED TO FOOD ALLERGENS

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The intestinal mucosa is densely innervated by neurons that maintain close communication with immune cells, thereby contributing to the regulation of inflammatory processes. B3-tubulin (TUBB3) is a neuron-specific cytoskeletal isoform widely used as a pan-neuronal marker. Notably, TUBB3 expression can also be induced in epithelial cells in the context of dedifferentiation and neoplastic transformation. This study aimed to characterize the expression pattern of TUBB3 in the mucosa of colorectal polyps from patients sensitized to food allergens. To this end, we performed immunofluorescence and confocal microscopy on histological sections of colorectal polyps from sensitized patients (n=5) and compared them with healthy colon biopsies (n=15), other non-neoplastic polyps (hyperplastic and inflammatory; n=9), and neoplastic lesions (adenomas, intramucosal carcinoma, and invasive adenocarcinoma; n=25). For transcriptomic analysis, the epithelial compartment of 5 polyps from sensitized patients and adjacent normal tissue were isolated. Total RNA was extracted, and gene expression profiling was performed using Illumina RNA sequencing (RNA-seq). Immunofluorescence analysis revealed that histological sections from sensitized patients exhibited minimal or absent stromal TUBB3 staining. Quantification of the TUBB3-positive tissue area confirmed a significant reduction in innervation compared with the other groups. Interestingly, most allergic samples showed TUBB3 expression within epithelial cells, a pattern that was also observed in intramucosal carcinoma and adenocarcinoma samples. Aligned with this observation, RNA-seq analysis of the epithelial compartment of these polyps revealed differential expression of over 1700 genes when polyp samples were compared with adjacent normal tissue. Subsequently, a comparison with public databases containing transcriptomic profiles of colorectal cancer and healthy colonic tissue enabled the identification of more than 800 genes that were either overexpressed or underexpressed in both, colorectal cancer and sensitized polyps. In conclusion, our findings showed a loss of innervation in polyps from sensitized patients, suggesting that neuronal mediators are involved in this inflammatory process. Furthermore, the epithelial expression of TUBB3 in these samples, along with the differential expression of other genes associated with tumorigenic transformation could reflect an early neoplastic transformation process in these patients.

Key words: B3-Tubulin; Colorectal Polyps; Innervation; Epithelial Dedifferentiation

AH-137

Microbiota from feces and juvenile colorectal polyps of food-sensitized children exhibit an allergic and inflammatory signature

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We previously found that 90% of children with juvenile colorectal polyps (P) from La Plata Children's Hospital are sensitized to food allergens. P showed type 2 inflammation and is a site of active IgE production.Low gut microbiota diversity, with Firmicutes predominating over Bacteroidetes, has been reported in food-allergic children. However, microbiota composition linked to P has not been described. This study aimed to characterize bacterial populations in feces and colonic tissue of food-sensitized children with JCP compared to healthy controls. Stool samples from food-sensitized children (FSC) with P (n=11) and healthy control children (CS) (n=22) were collected. P (n=14) and biopsies (B) (n=5) were obtained by colonoscopy. Microbial DNA

was extracted using commercial kits. 16S rRNA V3-V4 hypervariable regions were amplified and Illumina sequencing was performed. Sequences were analyzed through Qiime2 software. Amplicon Sequence Variants (ASV) abundance and representative sequences were generated. Alpha diversity was evaluated by Shannon and Faith_pd while beta diversity by UniFrac, and visualized by PCoA. Taxonomic analysis was applied where the representative sequences had been assigned taxonomic labels. Alpha diversity showed no significant differences ($p>0.1$), while beta diversity differed significantly between FSC and CS ($p=0.001$). P and B showed no significant differences ($p=0.658$). PCoA differentiated tissue from fecal samples and additionally separated FSC from CS. Taxonomic analysis of the bacterial communities in samples from FSS and CS exhibited similar profiles to those reported, with a reduction of Bacteroidetes and an increase in Firmicutes in FSS. Bacterial microbiota composition differed significantly, at the genus level, between tissue and feces samples. P samples were enriched with Escherichia relative to FSS. The comparison between P and CS showed 28 differences, including an increased abundance of the Fusobacterium A and C clusters. Bacteria of the genus Roseburia were more abundant in FSS, whereas Anaerobutyricum was higher in CS, suggesting a possible role in intestinal inflammation. Overall, we characterized fecal and tissue microbiota in atopic children with P and non-allergic controls. Our results show that sensitized patients harbor a unique microbial signature linked to food allergy and inflammation. These findings suggest a potential causal relationship between microbiota, colorectal polyp development and food-antigen sensitization.

Key words: Allergy; Microbiota; Inflammation; Polyps; Feces

Autoimmunity

AI-022

A new bioartificial platform to study immune response

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Peripheral blood and 2D cell culture models are commonly used to study immune cell parameters, yet they fail to accurately represent tissue-resident cells involved in autoimmune diseases or organ transplantation. The aim of the present study was to generate and validate 3D fibrin hydrogels (FH) as a model to study immune cell parameters inside the tissue. For this purpose, peripheral blood mononuclear cells (PBMCs) were isolated by a Ficoll Hypaque gradient from healthy subjects (HS) and kidney transplant immunosuppressed patients (KTIP). After FH was constructed (RPMI 1640, 40% plasma, and 0.1% CaCl2), PBMCs were seeded on top of it. On the 2nd day, the non- and gel-migrated cells were recovered from the supernatant and the gel, respectively. Cell immunophenotyping was evaluated by flow cytometry. The mean percentages of positive cells for each marker present in supernatants and in the gels were compared, between HS and KTIP (Mann Whitney test) and among themselves (Wilcoxon matched-pairs signed rank test). In HS, the CD4+ and CD8+ cells are distributed equally in the supernatant and the FH. The same was observed for CD8+ cells derived from KTIP. However, there were fewer CD4+ cells in the FH than in the supernatants when cells were derived from KTIP ($p<0.01$). Thus, the CD4/CD8 index was lower for KTP than HS. No differences were found between the levels of CD40L expression in T cells present in supernatant or gel, but a positive correlation was found between these and the percentage of CD19+ CD40+ lymphocytes present in PBMC from KTIP (Spearman non-parametric correlation, $r=0.9$, $p= 0.0417$). We conclude that the culture of PBMCs in 3D FH is a good model to evaluate immune cell parameters in KTIP; perhaps reflecting the immune status of immunosuppression of these patients.

Key words: CD4; CD8; hidrogel

AI-040

Autoantibodies in Focus: Evolution of Diagnostic Methods in Autoimmune Diabetes and Celiac Disease

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The detection of autoantibodies is essential for the diagnosis and follow-up of autoimmune diseases such as Type 1 Diabetes (T1D) and Celiac Diseas (CD). Our group, a reference laboratory for T1D markers by radioligandbinding assay (RBA), has also developed alternative approaches such as ELISA and Flow Cytometry (FC), applicable to medium-complexity laboratories. In T1D, RBA was the first diagnostic method, offering high sensitivity but requiring radioisotopes. Over time, ELISA has replaced RBA in many settings, while FC has emerged as a promising tool for simultaneous detection of different autoantobodies. Characterizatión by Surface Plasmon Resonance (SPR) provides additional information on concentration (q) and affinity (Ka), helping to asses the risk of progression in asymptomatic antibody-positive individuals. CD has a strong immunogenetic link with T1D, supporting its early detection. Anti-tissue transglutaminase autoantibodies (tTgA), usually measured by ELISA, are highly sensitive and specific markers for CD. Combining T1D and CD assays allows for a more complete autoimmune profile and timely intervention. Our laboratory has achieved recombinant expression of the main antigens of T1D (GAD65, IA-2, ZnT8, PI) and CD (tTg) in E.coli or baculovirus-insect cell systems. Following purification and validation, these antigens were used in "double-paratope" immunoassays, where the antibody binds both an immovilized antigen (plate or microsphere) and a biotin-labeled soluble antigen, with detection via streptavidin. This format works for ELISA and FC, the latter enabling combined and discriminative detection of major autoantibodies. Overall, RBA results (validated though external quality controls) showed 98.9-100% specificity and 52-66% prevalence, depending on the marker. ELISA specificity was 90-98%, with sensitivity relative to RBA of 65-75%. For FC, specificity was 90-93% with relative sensitivity to RBA of 73-93%. Agreement between RBA and FC reached 85-92%, supporting FC as a good alternative to the reference method. We have also used SPR to analyze autoantibodies q and Ka in juvenile and adult diabetic patients, revealing distinct autoimmunity profiles with implications for pathogenesis and early prediction. These advances strengthen the diagnostic and monitoring tools available for T1D and CD, facilitating their implementation in diverse laboratory settings and contributing to earlier and more precise patient management.

Key words: Autoantibodies; Immunoassay; Type 1 Diabetes Mellitus; Celiac Disease

AI-066

EXPLORING THE PROINFLAMMATORY ROLE OF SEROTONYLATION IN A SPONTANEOUS MURINE MODEL OF LUPUS

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by inflammation and immune dysregulation, but the most severe consequence is nephritis. Although the mechanisms involved are not fully understood, reduced serotonin (5-HT) levels in sera and increased transglutaminase (TG) activity suggest that serotonylation (a post-translational modification mediated by TGs) may contribute to the pathogenesis. Therefore, we aimed to investigate serotonylation in immune cells from MRL/MpJ-Faslpr/J mice, a spontaneous model of lupus. Using click chemistry and flow cytometry (FC), we assessed serotonylation in bone marrow-derived dendritic cells (BMDCs) from mice before and after disease onset. Additionally, we increased systemic 5-HT levels by administering the precursor 5-HTP and evaluated disease severity, immune infiltration in kidneys and lymph nodes (LN). Besides, we evaluated BMDC differentiation under 5-HT exposure, with or without TG inhibition. Statistical analyses were performed using one-way ANOVA and student t-tests. Our results show that BMDCs from SLE mice exhibit a trend to higher serotonylation capacity than those from pre-SLE mice (p=0.0659). Increased 5-HT levels tend to increase clinical scores and were associated with higher kidney infiltration by monocytes/macrophages (p=0.006), and a trend toward more DCs in LN (p=0.063). BMDCs exposed to 5-HT showed reduced differentiation efficiency (p=0.0014) but higher basal CD86 expression (p=0.0019), and increased CD80/CD86 upon LPS stimulation (p=0.0026), effects reversed by TG inhibition. In conclusion, our findings suggested that 5-HT promotes a proinflammatory environment in SLE through serotonylation, potentially affecting immune cell activation and differentiation. Serotonylation could be proposed as a novel mechanism that contributes to SLE progression and, therefore, a promising therapeutic target.
Key words: Systemic lupus erythematosus; Serotonylation; Serotonin

AI-069

Profiling helper and follicular T cells in the immunopathogenesis of T1D and LADA

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Autoimmune diabetes (AD) is an autoimmune disorder mediated by T lymphocytes (LT) that destroy pancreatic β-islets, leading to reduced or absent insulin production. Within AD, type 1 diabetes (T1D) presents as an early-onset disease, while latent autoimmune diabetes in adults (LADA) is a more slowly progressive form. Although both share pathophysiological mechanisms, their specific immune responses have not yet been fully characterised. Autoantibody production, relevant for diagnosis, results from hyperactivation of B cells in germinal centres, a process regulated by follicular T helper cells (Tfh), cytotoxic follicular T cells (Tfc), and follicular regulatory T cells (Tfr). Additionally, different CD4+ helper T (Th) cell subsets, such as Th17, Th1, Th17.1, and regulatory T cells (Treg), also contribute to this immune imbalance, fostering an inflammatory environment that damages pancreatic β cells. This study aimed to characterise components of cellular and humoral immunity involved in autoreactivity in patients with T1D and LADA. Peripheral blood mononuclear cells were obtained from healthy volunteers (Co; n=25), T1D patients (n=17), and LADA patients (n=8), and T-cell populations were analysed by flow cytometry. Patients with T1D and LADA showed a significant increase in CD25 fluorescence intensity (MFI) on total CD4+ T cells compared to Co (T1D vs. Co, LADA vs. Co; $p<0.05$), indicating a higher degree of activation. Also, frequencies of Th17 and Treg subsets were significantly elevated in both T1D and LADA patient groups compared with Co (T1D vs. Co, LADA vs. Co; $p<0.05$), indicating an active inflamma-

tory response alongside regulatory mechanisms attempting to counterbalance it. Additionally, in T1D, MFI of CD25 was significantly increased in Th17 and Th17.1 cells, suggesting a sustained proinflammatory microenvironment (T1D vs. Co; $p<0.05$). Moreover, both pathologies showed increased frequencies of Tfh and Tfc cells compared with Co (T1D vs. Co, LADA vs. Co; $p<0.05$), suggesting enhanced T–B cell cooperation and potential autoantibody production. In conclusion, T1D and LADA share alterations in adaptive immune responses, characterised by increased activation of autoreactive T-cell subsets, with no significant immunological differences observed between them to date.

Key words: Autoimmunity; Type 1 Diabetes; LADA; Flow cytometry; T cells

AI-101

From biopsy to clinic: Immunological parameters in ocular mucous membrane pemphigoid biopsies according to early and late stages of the disease.

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Ocular mucous membrane pemphigoid (OMMP) is a chronic, immune-mediated, fibrosing disease. Diagnosis relies on clinical findings and/or conjunctival biopsy. Patients are classified according to Foster's clinical stages, based primarily on conjunctival findings, scarring, and adhesions. However, immunological changes associated with disease stages remain poorly characterised, which limits our understanding of disease progression.

This study aimed to investigate immunological parameters in conjunctival biopsies from OMMP patients and assess their relationship with clinical severity according to Foster's stages.

A retrospective observational study was conducted in patients with OMMP. Demographic, clinical, and histological data were collected. Patients were divided into early stages (ES) (Foster 0–1) and advanced stages (AS) (Foster 2–4). Immunological parameters evaluated in biopsies included polymorphonuclear cells, mast cells, eosinophils, and linear deposits of immunoglobulins and complement, which were quantified on a 0–4 scale. All evaluations were performed prior to immunomodulatory treatment.

A total of 210 patients were analyzed according to Foster's stages: 147 (70%) were in ES and 63 (30%) in AS, mostly females (72.2%). The age at diagnosis was 63.3 (SD 13.6) for ES and 64.9 (SD 15) for AS. No significant differences were found in sex or age at diagnosis ($p=0.422$ and $p=0.413$ respectively). Patients in AS had a longer time from symptom onset to diagnosis (median 48.5 vs. 34 months, $p=0.006$) and longer follow-up (median 31 vs. 21 months, $p=0.005$). ES showed a higher presence of polymorphonuclear cells ($p=0.003$), total mast cells ($p=0.004$), and degranulated mast cells ($p=0.037$). Eosinophils were more frequent in ES but not significantly ($p=0.128$). In contrast, lineal deposits of C3 ($p=0.284$), IgG ($p=0.100$), IgM ($p=0.507$), and IgA ($p=0.983$) did not differ significantly between groups.

Despite the progressive nature of OMMP, the distribution of immune reactants such as immunoglobulins and complement remains similar across disease stages. These findings suggest that lineal deposition may represent an early and stable pathogenic event rather than a marker of progression. Importantly, all histological and immunological analyses were performed before immunomodulatory treatment, highlighting their relevance in untreated disease. Further studies are needed to clarify the role of these immune components in disease evolution and fibrosis.

Key words: Immunology; Biopsy; Ocular Cicatricial Pemphigoid

AI-110

Murine immunopeptidome analysis reveals potential cross-reactivity between peptides presented by MHC I under physiological conditions and peptides that may be sourced from Trypanosoma cruzi antigens, evaluated as vaccine targets

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Trypanosoma cruzi is the etiological agent of Chagas disease (CD). Although the persistence of *T. cruzi* in specific host niches remains the primary mechanism implicated in disease development, the possible involvement of autoimmune mechanisms mediated by molecular mimicry is also recognized. This study aims to explore the role of autoreactive CD8+ T lymphocytes in CD through the identification of potential peptides presented in the context of Major Histocompatibility Complex class I (MHC I) molecules that could be involved in cross-reactivity. To this end, raw MHC I immunopeptidomic data from 19 organs of C57BL/6 mice, available in the PRIDE repository (PXD008733), were analyzed using FragPipe version 22 (MSFragger version 4.1), following the strategy of comparing acquired fragmentation spectra against theoretical fragmentation spectra. A FASTA database was built including the reference proteome of *Mus musculus* downloaded from UniProt, common contaminants, and random decoy sequences, and the "HLA nonspecific" workflow was employed to identify peptides of 8–15 amino acids in length with a 1% false discovery rate. After removing contaminants, peptides presented by the different organs were queried using pBLAST against protein sequences from various *T. cruzi* strains with 100% sequence identity along their entire length, also including searches in the IEDB database. Peptides presented under physiological conditions in the context of MHC I molecules were identified across the analyzed organs and may be involved in cross-reactivity, likely due to sequence conservation between related *T. cruzi* and *M. musculus* proteins. Peptides presented by MHC I in the bladder, intestine, stomach, testes, thymus and heart mapped to parasitic surface proteins previously characterized as parasite antigens and vaccine targets against CD, as well as murine autoantigens. Moreover, some sequences contained repetitive motifs characteristic of immunogenic peptides, and IEDB searches indicated that two peptides —one of which was even presented by MHC I in murine heart— could also be presented under physiological conditions in human tissues. While experimental validation is required, these results suggest a probable contribution of molecular mimicry–mediated autoimmune mechanisms to the pathophysiology of CD.
Key words: Chagas disease; Trypanosoma cruzi; Molecular mimicry

AI-119

AUTOANTIBODY SPECIFICITY IN WARM AUTOIMMUNE HEMOLYTIC ANEMIA: BEYOND THE PANREACTIVE PATTERN

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Autoimmune hemolytic anemia (AIHA) is characterized by increased red blood cell (RBC) de-

struction due to autoantibodies, with or without complement activation. According to autoantibody isotype and thermal properties, two main AIHA types are recognized: warm AIHA (wAIHA), caused by IgG with weak complement activation, and cold AIHA (cAIHA), mediated by IgM binding RBCs below 20°C with strong complement activation. Warm autoantibodies usually display broad specificity, reacting with all human RBCs without preferential affinity for a blood group antigen. In rare cases, however, autoantibodies may be specifically directed against Rh antigens, showing stronger reactivity with RBCs expressing particular Rh determinants. Defining such specificities is clinically relevant for understanding disease mechanisms and optimizing transfusion management. The aim of this study was to investigate relative autoantibody specificities in wAIHA. A total of 27 samples from patients with confirmed AIHA were analyzed. Standard immunohematologic procedures were performed to classify AIHA types. Patient sera underwent red cell antibody screening and identification at 4°C, 20°C, and 37°C using commercial 2 cell and 11 cell panels (ID-Diacell and ID-DiaPanel, Biorad, Switzerland). Antibodies were eluted from patients' RBCs using an acid elution kit (Diacidel, Biorad). Specificity was confirmed with antigen-positive and negative reagent RBCs. Autoadsorption was performed with patient RBCs treated with 1% cysteine-activated papain to exclude alloantibodies. Among the 27 cases, 11 were classified as wAIHA (sex: 7F/4M), 10 as cAIHA (5F/5M), and 6 as mixed-type (2F/4M). All samples showed panreactivity with commercial panels. Relative specificity was identified in 4 of 11 wAIHA patients, all showing anti-e reactivity. No alloantibodies were detected. Given the low frequency of such cases in the literature, the proportion found in this study is clinically significant. Identifying autoantibodies with well-defined specificity may help transfusion services select compatible units in otherwise challenging situations. These findings highlight the need for further research into the frequency, mechanisms, and clinical implications of Rh-directed autoantibodies in AIHA, which could contribute to improving diagnostic and therapeutic strategies.

Key words: Autoimmune hemolytic anemia; autoantibodies; relative autoantibody specificities

AI-130

Phenotypic and functional diversification of age-associated B cells reveals distinct trajectories of immune aging

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Aging is associated with profound remodeling of the immune system, characterized by chronic inflammation, reduced immune tolerance, and increased susceptibility to infections and autoimmunity. Among B cell subsets, age-associated B cells (ABCs), defined by CD11c and T-bet expression, expand with age and in autoimmune conditions, yet the influence of sex on their accumulation and function remains incompletely understood. Understanding how ABCs evolve with age is essential to delineate mechanisms of inflammaging and to explain the higher prevalence of autoimmune diseases in females.

We characterized immune populations in peripheral blood and spleen of C57BL/6 male and female mice at 3, 6, and 12 months of age. Flow cytometry revealed age-related reductions in total lymphocytes and increased frequencies of activated $CD4^+$ and $B220^+$ cells, which were more pronounced in females. Serum cytokine profiling showed elevated IFN-γ, IL-6, IL-17, and IL-23 in aged females, consistent with a systemic pro-inflammatory state.

Analysis of B cell subsets demonstrated a marked age-dependent expansion of $CD11c^+$ ABCs in both sexes, with significantly greater accumulation in females. ABCs from aged mice expressed high levels of T-bet, TLR7, CD44, FAS, MHC-II, CD80, and immune regulatory molecules (PDL1, PDL2, CD73, CD39). Chemokine receptors (CCR3, CCR5, CCR6, CXCR3, CXCR4) were also upregulated, indicating broad phenotypic remodeling. Heatmap and PCA analyses confirmed a greater phenotypic diversification of female ABCs across age groups, with MHC-II, T-bet, CD80, FAS, and IgD/IgM-defined subsets as major contributors to heterogeneity.

Functionally, ABCs from aged females displayed heightened spontaneous and TLR7-induced responsiveness, including differentiation into $CD138^+$ plasmablast-like cells, secretion of IFN-γ, IL-

6, IL-17, and production of autoreactive antibodies. Importantly, aged females exhibited elevated levels of anti-dsDNA and anti-histone IgG2b/IgG2c autoantibodies. Sorted ABCs, but not follicular B cells, were identified as the main source of these autoreactive responses.

Our findings reveal that aging drives a sex-biased expansion and activation of ABCs, with females showing greater accumulation, phenotypic remodeling, and autoreactive potential. These results suggest a mechanistic link between female immune aging and increased autoimmune risk mediated by hyperresponsive, autoantibody-producing ABCs.

Key words: Age-associated B cells; aging; inflammaging; sex differences; autoimmunity.

AI-135

Imbalance of effector, regulatory, and follicular T cells in Autoimmune Hepatitis: links to disease activity and therapeutic response

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Autoimmune hepatitis (AIH) is a chronic immune-mediated liver disease characterized by the breakdown of self-tolerance and persistent hepatocellular inflammation. $CD4^+$ T lymphocytes play a pivotal role in disease pathogenesis, yet the contribution of their subsets under treatment remains insufficiently understood. We conducted a detailed immunophenotypic analysis of peripheral $CD4^+$ T cell subsets (including Th1, Th2, Th17, Treg, follicular helper (Tfh), follicular regulatory (Tfr), and memory populations) in untreated AIH patients (AIHwT, n:5), patients under glucocorticoid/azathioprine therapy (AIHT, n:20), and healthy controls. (Co, n:27).*, #p<0.05. Untreated patients displayed a skewed proinflammatory profile with increased Th17 and effector memory $CD4^+$ T cells (EM), along with an expansion of Treg, possibly reflecting a compensatory mechanism (Th17=Co:10.1±0.6, AIHwT:15.5±2.3*, AIHT: 10.6±1; EM= Co:11±1, AIHwT:21±3*, AIHT: 5±2; Treg= Co:6.4±0.3, AIHwT:9.3±1.3#, AIHT: 5.9±0.6, # AIHwT vs Co, *AIHwT vs Co and AIHT). On the other hand, treated patients showed reduced Th1 levels, with polarization toward Th2 and Tfh2 subsets compared with Co, and a lower Th1/Th2 ratio compared with Co, consistent with the immunomodulatory effects of corticosteroids (Th1=Co:18±1, AIHwT:13±2, AIHT: 12±2; Th2= Co:59±2, AIHwT:63±5, AIHT: 71±3*, Tfh2= Co:44±3, AIHwT:78±5, AIHT: 72±4*,*AIHT vs Co). Both AIH groups showed a significant imbalance in Tfh dynamics, characterized by reduced Tfr cells and enrichment of Tfh2 over Tfh1/Tfh17 subtypes, potentially driving autoantibody production and perpetuating disease activity. Moreover, untreated patients presented an increase in central and effector memory subsets, whereas therapy partially normalized these alterations (AIHwT vs Co, p<0.05). This integrated analysis demonstrates that AIH pathogenesis involves profound dysregulation of effector, regulatory, and memory $CD4^+$ T cell compartments. The persistence of Tfh2 and central memory populations under therapy may contribute to chronicity and relapse risk. These findings highlight the importance of monitoring T cell dynamics and provide a rationale for developing targeted immunotherapies aimed at restoring immune tolerance in AIH.

Key words: Autoimmune Hepatitis; Th17; Treg; LTfollicular; Memory T cells

AI-147

Circulating miRNAs and Cytokine Profiles as Biomarkers in Celiac Disease

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Although knowledge about celiac disease (CeD) has expanded in recent decades, its pathogenesis remains partially understood. HLA genes explain 30–40% of disease susceptibility, while non-HLA variants account <10%. MicroRNAs (miRNAs), small non-coding RNAs that regulate gene expression, have been implicated in immune-mediated disorders such as CeD. The aim of this study was to evaluate the expression of inflammatory miRNAs (miRNA-146a, miRNA-155, and miRNA-223) in peripheral blood mononuclear cells (PBMCs) and serum of adult patients with CeD and to correlate them with circulating cytokine and nitrite levels. To achieve this, twenty-nine volunteers were enrolled at the National University of Luján and Posadas National Hospital (Argentina). CeD was diagnosed according to international criteria, requiring positive serology and Marsh histology ≥3a. Participants provided informed consent and completed a structured interview addressing health status, gastrointestinal symptoms, comorbidities, family history, and dietary habits. Peripheral blood was collected for serum and PBMC isolation. Expression levels of the three miRNAs were quantified by qPCR. For absolute quantification, curves constructed with plasmids containing the sequence of each amplicon were used. In addition, serum levels of IL-1b, IL-8, IL-10 (ELISA), and nitrites (Griess method) were determined. Statistical analyses included Kruskal-Wallis and Dunn's post hoc tests. Results: Expression of miRNA-146a, miRNA-155, and miRNA-223 was significantly higher in both active and inactive CeD groups compared with non-CeD controls ($p<0.05$). No significant differences were observed between active and inactive patients. A moderate positive correlation (Pearson's $r = 0.6–0.8$) was identified between the symptoms reported in the previous 15 days and expression of miRNA-146 and miRNA-155 and IL-8 in serum. In conclusion, upregulation of immune related miRNAs in the peripheral blood of CeD patients suggests their involvement in disease related immune pathways. Comparable expression in active and inactive CeD underscores their potential as noninvasive diagnostic biomarkers, even in patients on GFD. On the other hand, IL-8 and nitrites can be proposed as biomarkers for active CeD.
Key words: miRNAs; cytokines; Biomarkers; Celiac Disease; diagnosis

AI-150

"Impact of Hyperthyroidism on the Development of Autoimmunity in Type 1 Diabetes"

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Type 1 diabetes mellitus (T1D) is characterized by autoimmune processes directed against pancreatic β-cells. However, the underlying pathogenic mechanisms remain incompletely understood. Our group investigates how endocrine factors impact the immune system and the role of hyperthyroidism (HyperT) in the development of autoimmunity. We used female control mice (NOD n = 5; BALB/c n = 5), as well as female HyperT mice (NOD n = 5; BALB/c n = 5), and male NOD mice (control n = 9; HyperT n = 9). HyperT was induced by administering T4 (12 mg/L) in drinking water for 28 days. We recorded body weight, food and water intake, basal glycemia, and glycemia after an intraperitoneal glucose tolerance test (IPGTT). Clinical wellbeing was evaluated using the Mouse Grimace Scale and Assessment of Wellbeing Score Sheet, measured the effects of T4 on

overall wellbeing. After treatment, animals were euthanized, and samples were collected. Serum was used for enzymatic glucose testing, and spleens and pancreas were prepared for immunophenotyping by flow cytometry. Control NOD females exhibited significantly higher water intake compared BALB/c controls ($p<0.0001$), consistent with polydipsia. T4 treatment reduced water intake in NOD mice, reaching levels similar to BALB/c control. Treated animals of both strains gained more weight and showed improved wellbeing. Control NOD mice developed pronounced hyperglycemia, showing typical diabetes onset. In contrast, T4-treated NOD mice had significantly lower blood glucose concentrations, comparable to BALB/c controls ($p < 0.0001$). T4 treatment increased spleen weight and spleen index in both strains (BALB/c $p<0.01$; NOD $p<0.001$). In the pancreas of treated NOD mice, both the percentage and absolute number of CD45+/CD4+ cells per milligram of tissue decreased significantly ($p < 0.01$). Specifically, T4-treated groups had fewer CD4+ cells compared to controls ($p<0.05$). In the spleens of treated NOD males, the percentage of CD4+ cells decreased ($p < 0.001$), while the percentage of CD19+ cells increased ($p < 0.05$), indicating distinct effects on B and T lymphocytes in this lymphoid organ. In conclusion, HyperT favorably modulates clinical and immunometabolic parameters in experimental T1D, suggesting a regulatory role in the progression of autoimmunity.

Key words: Diabetes; autoimmunity; hyperthyroidism

AI-170

TOLEROGENIC DENDRITIC CELLS MODULATE GLUTEN-SPECIFIC T CELL RESPONSES IN A HUMANIZED MOUSE MODEL: TOWARD A CELL-BASED IMMUNOTHERAPY FOR CELIAC DISEASE

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Celiac disease (CD) is an autoimmune disorder triggered by gluten ingestion in genetically predisposed individuals, leading to chronic inflammation and intestinal damage. Loss of immune tolerance to gluten is central to CD, and the presence of HLA-DQ2 or HLA-DQ8 molecules increases disease risk by presenting gluten peptides to $CD4^+$ T cells. This study aimed to evaluate whether tolerogenic dendritic cells (tolDCs) can modulate immune responses in vitro by the induction of gluten-specific regulatory T cells (Tregs), to ameliorate gastrointestinal effects associated with CD.

CD103+ DCs were generated from bone marrow of NOD-DQ8 transgenic mice and tolerized with retinoic acid. They were stimulated with gliadin or left unstimulated. Activation markers were analyzed by flow cytometry and cytokines released by ELISA. These gliadin stimulated-DCs (or not stimulated) were co-cultured with naïve $CD4^+$ T cells (isolated from NOD-DQ8 mice) to assess specific proliferation and their phenotype by flow cytometry, and cytokines released by ELISA.

TolDCs upon gliadin stimulation showed reduced activation compared to non-tolDCs, evidenced by fewer $CD86^+HLA\text{-}DQ^+$ cells (40%±0.1 vs 75%±1.3; ****$p<0.0001$, n=2), higher TGF-β (395±67 vs 38±33 pg/ml; ****$p<0.0001$, n=4), and lower IL-12p40 concentration (202±9.2 vs 815±17 pg/ml; ****$p<0.0001$, n=4). In co-cultures, gliadin-stimulated non-tolDCs induced proliferation of $CD4^+CD25^+$ Th1 cells and a small $Foxp3^+$ Tregs subset. TolDCs reduced $CD4^+CD25^+$ proliferation and gluten-specific $Foxp3^+$ Tregs, compared to non-tolDCs (1.4%±0.4 vs 6.4%±0.08; ***$p<0.001$, n=4). Cytokine levels were also lower in co-cultures with tolDCs vs non-tolDCs: IFN-γ (non-detectable [ND] vs 1411±55 pg/ml; ****$p<0.0001$, n=4), IL-2 (43±13.5 vs 628±115 pg/ml; ****$p<0.0001$, n=4), and IL-10 (ND vs 140±62 pg/ml; *$p<0.05$, n=4). No proliferation was observed in unstimulated DCs co-cultured with naïve CD4+ T cells, suggesting that the proliferation observed in the

co-cultures with gliadin is specific.

Our results demonstrate that tolDCs exerted a strong immunomodulatory effect by suppressing T cell activation, including a significant reduction in gluten-specific Th1 cell proliferation (a key driver of inflammation in CD); but not enhancing the induction of gluten-specific Tregs. Together, these findings support the potential of tolDCs-based therapies to restore gluten tolerance by broadly dampening pathogenic immune responses, even in the absence of increased Tregs induction.

Key words: celiac disease; tolerogenic dendritic cells; regulatory T cells; gliadin; immune modulation

AI-212

Pelvic chronic inflammatory pain induces depression- and anxiety-like behaviors in mice and aligns with hippocampal oxidative stress and antigen-specific immune activation

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Chronic pain is a debilitating condition that frequently leads to mood disorders like depression and anxiety. These disorders are refractory to commonly used antidepressants, likely due to neuroinflammatory and oxidative stress processes, with no available pharmacological therapies. This study aimed to assess depression and anxiety-related behaviors, as well as oxidative stress indicators and the activity of antioxidant enzymes in brain regions associated with nociceptive and emotional processes, in animals experiencing chronic pelvic pain (CPP) resulting from the development of Experimental Autoimmune Prostatitis (EAP), a widely used animal model for human Chronic Pelvic Pain Syndrome. EAP was induced in C57BL/6 male mice by immunization with PA emulsified in Complete Freund's Adjuvant (CFA), whereas control groups received CFA alone or saline. At days 24-26, EAP and pelvic pain development were evaluated. Between days 38 and 43, emotional behaviors were analyzed using the open field test (OFT, anxiety-like), sucrose preference test (SPT, anhedonia), and tail suspension test (TST, helplessness). Oxidative stress was analyzed in hippocampus and medial prefrontal cortex by quantifying malondialdehyde (MDA) levels and catalase activity. As expected, PA-immunized mice showed the induction of PA-specific mixed Th1/Th17 immune responses, along with marked prostate tissue inflammation, and the development of chronic pelvic pain. None of these changes were observed in control animals. Interestingly, behavioral data showed that, 35 days post-disease induction, EAP mice subjected to OFT preferred the peripheral area, suggesting an anxious phenotype in contrast to control animals. In the SPT, diseased mice lacked the control group's marked preference, with reduced performance in the TST suggesting anhedonia and depressive-like behavior. Besides, increased levels of MDA and catalase activity were detected in brains from EAP mice with respect to controls.

Our data indicate that EAP induce CPP as well as anxiety and depressive-like behavior associated to hippocampal oxidative stress. These findings provide promising insights for further characterizing neuroinflammation and associated behaviors in EAP, and for assessing the potential of brain-targeted anti-neuroinflammatory agents as a therapeutic strategy for treating CPPS.

Key words: Chronic pelvic pain; chronic prostatitis; depression; anxiety; oxidative stress.

Inborn Errors of Immunity

EI-006

Challenges in caring for adults with common variable immunodeficiency: a case report.

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Common variable immunodeficiency is one of the most prevalent inborn errors of immunity worldwide. Despite this, in many countries, the diagnosis and treatment of patients with this condition are delayed until adulthood. This case report presents the case of an adult woman who suffered from recurrent infections, autoimmune disorders, and even Hodgkin's lymphoma without ever having been evaluated by an immunologist. After years of multiple consultations with specialists, she was diagnosed with common variable immunodeficiency, and treatment with intravenous immunoglobulin was difficult to obtain because the Paraguayan health system does not consider that primary immunodeficiencies can also occur in adults.

This case report, beyond the clinical aspect, also seeks to present how the health system still has many deficiencies in the diagnosis and treatment of patients with inborn errors of immunity in adulthood.

Key words: Common Variable Immunodeficiency; Adult; Paraguay; Public Health

EI-068

Inborn Errors of Immunity in Children: A Chilean Single-Center Experience

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Introduction: Inborn errors of immunity (IEI) encompass a heterogeneous group of primarily genetic disorders that affect the immune system. Since June 2024, eleven patients with IEI have been registered at a single center in the Santiago Metropolitan Region, Chile.

Objective: This retrospective report analyzes the demographic, clinical and genetic characteristics of a cohort of 11 patients with IEI in Chile.

Results: Eleven patients, aged between 7 months and 17 years, were analyzed. Seven had a positive family history, five had relatives who died from suspected or confirmed IEI, and seven were male. No consanguinity was reported. The mean age at diagnosis was 6.48 years (range: 0.19–15.44 years), and the median diagnostic delay was 3.69 years (range: 0.03–12.48 years). According to the IUIS classification: one patient belonged to group I (CD40L deficiency), one to group II (AD-HIES due to STAT3 deficiency), three to group III (X-linked agammaglobulinemia, XLA), one to group IV (perforin deficiency, FHL2), and four to group VIII (hereditary angioedema, HAE). One patient has not yet been classified; she presents with specific antibody deficiency and systemic lupus erythematosus, and whole-exome sequencing is in progress. Initial manifestation was infection in 5 patients, except in the four HAE patients, the FHL2 patient (who presented with hemophagocytic lymphohistiocytosis), and one XLA patient diagnosed through early screening. Regarding treatment, four patients are receiving immunoglobulin replacement therapy, five require antibiotic prophylaxis, and the FHL2 patient is awaiting hematopoietic stem cell transplantation. HAE patients are receiving on-demand treatment. At the time of this report, all patients are alive.

Conclusion: This is the first report from this tertiary-care hospital in Chile. IEIs are rare but still underdiagnosed disorders. Despite advances in the field of primary immunodeficiencies, there is a pressing need to improve awareness to shorten the time to diagnosis and offer appropriate treatment.

Key words: inborn errors immunity; primary immunodeficiency; diagnosis; rare diseases

EI-178

Novel RHOG mutation (p.G12R) identified in a patient with CVID and combined T- and B-cell dysfunction

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RhoG is a member of the Rho family of small GTPases that function as molecular switches cycling between inactive GDP-bound and active GTP-bound states. It regulates cytoskeletal remodeling, immune synapse formation, and T cell activation, mainly through signaling to Rac/WASP pathways and induction of lamellipodia and membrane ruffles. RHOG has recently been included in the IUIS classification of inborn errors of immunity under disorders of immune dysregulation, but clinical reports remain very limited. Glycine at position 12 is highly conserved and considered essential for GTP binding and hydrolysis; variants at this site have been shown to severely impair GTPase function.

We describe an adult patient with a childhood-onset clinical diagnosis of common variable immunodeficiency (CVID), characterized by recurrent respiratory infections, hypogammaglobulinemia, poor vaccine responses, and extensive HPV-induced cutaneous warts. Immunological workup revealed combined T and B cell dysfunction. Whole exome sequencing prioritized a heterozygous de novo missense variant in RHOG (c.34G>A; p.Gly12Arg), absent from population databases (gnomAD) and predicted to be deleterious (REVEL 0.910, AlphaMissense: 0.997). Familial segregation confirmed the de novo status. No other candidate variants explaining the phenotype were identified.

This variant affects the critical glycine residue within the conserved GTP-binding domain, previously recognized as indispensable for proper Rho GTPase activity. Structural considerations predict altered nucleotide binding and defective GTP hydrolysis. Functional consequences may include dysregulated actin remodeling, impaired immunological synapse formation, and defective T cell activation. Clinically, the combination of antibody deficiency, impaired cellular immunity, and viral susceptibility aligns with this mechanism. Although classified as a variant of uncertain significance by ACMG criteria (PM2, PM6, PP3), the strong genotype–phenotype correlation and de novo occurrence support pathogenicity.

We report the first description of a de novo heterozygous RHOG p.G12R variant in a patient with CVID and combined immunodeficiency. This case highlights RHOG as a novel candidate gene for immune dysregulation with viral susceptibility. Ongoing studies, including GTPase activity assays, cytoskeletal reorganization and T cell activation assays, are required to define its role in human immunopathology.

Key words: Inborn Errors of Immunity; Novel variant; Exome sequencing

Adaptive Immunity

IA-053
Adenosine impairs germinal center response and antibody production

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Previously, we observed that antibody-secreting cells (ASC) express high levels of the ectoenzyme CD39. While CD39 is not required for ASC differentiation, it is enzymatically active and

able to generate adenosine (ADO). ADO is an immunomodulatory molecule with predominantly anti-inflammatory functions, and its effects depend on the specific ADO receptors (ADOR) expressed by immune cells. For instance, activation of ADORA_2A has been reported to influence antibody isotype production in bacterial infections and, in T cells to reduce germinal center (GC) response by altering the Tfh/Tfr balance in an immunization model. However, the direct effects of ADO on ASC themselves or on B lineage cells remain unknown. To evaluate the influence of ADO on different B cell subsets, we took advantage of C57BL/6 mice infected with T. *cruzi* intraperitoneally-ip-(5,000 trypomastigotes, Tulahuén strain) or with influenza virus intranasally (5 PFU, H1N1 strain), as both experimental models generate GC B cells and ASC. We found that GC B cells and ASC from T. cruzi-infected mice express ADOR-A_2B and ADOR-A_3, while ASC additionally express ADOR-A_2A, suggesting that ADO can directly act on both cell types. Ip administration of ADO to mice infected with either *T. cruzi* or influenza virus resulted in a reduced frequency of GC B cells compared with PBS-treated infected animals ($p \leq 0.05$), while ASC responses remained unaffected. In contrast, in vitro experiments with ASC purified from the spleen of *T. cruzi*-infected mice showed a significant reduction ($p \leq 0.05$) in antibody secretion in the presence of ADO, without affecting cell viability, compared to ASC cultured without ADO. Furthermore, when B cells from naïve mice were activated in vitro with CpG and anti-CD40 to evaluate the effect of ADO during differentiation, we found that ADO impaired ASC generation, as evidenced by a lower frequency of ASC in activated B cell-cultures containing ADO. Finally, ASC differentiated in vitro in the presence of ADO displayed distinct phenotypic and metabolic profiles compared with those generated in its absence ($p \leq 0.05$).

Our results demonstrate that ADO affects B cell subsets modulating GC B cell frequencies in vivo and impairing ASC generation and function in vitro, while also altering the phenotypic and metabolic profiles of in vitro differentiated ASC. These findings highlight a previously unrecognized role of ADO in regulating B cell responses and suggest potential plasticity of plasmablasts.

Key words: antibody-secreting cells; B cells; Adenosine; Antibody.

IA-062

Metabolic comorbidities exacerbate T-cell immunosenescence in patients with chronic Chagas disease

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Chronic low-grade inflammation drives cellular ageing and contributes to cardiomyopathy development. Metabolic disorders, notably type 2 diabetes mellitus (D2), may exacerbate this process. Chronic Chagas disease (Ch), characterised by persistent inflammation, provides a model to explore how, when combined with D2 influences immunosenescence. We evaluated T lymphocyte (TL) alterations associated with ageing in individuals with Chagas disease (Ch), with or without chronic Chagas cardiomyopathy, considering D2 comorbidity. A cross-sectional study with convenience sampling included indeterminate Ch (iCh), cardiac Ch (CCC), D2, Ch with D2 and healthy controls (Co), matched for age and sex (30–70 years; n = 25–35/group). Naïve and memory TL (CD45RA/CCR7), activation (HLA-DR+), cytotoxicity (CD107a+/IFNγ+), and senescence (CD28-/CD57+) were assessed by flow cytometry. Thymic output was estimated by sjTREC quanti-

fication via qPCR. Data were analysed using ANOVA/Kruskal–Wallis with post-hoc tests. Results showed reduced naïve CD4+ TL in CCC ($p<0.05$ vs. Co), more pronounced in iCh+D2 ($p<0.05$ vs. all groups). CD8+ TL decreased in D2 and Ch+D2, independent of disease severity ($p<0.05$ vs. Co and Ch). SjTREC levels declined in CCC and D2 ($p<0.05$ vs. Co and iCh), with the greatest reduction in Ch+D2 ($p<0.05$ vs. Co, Ch, and D2). Senescent CD4+CD28- cells increased in Ch, particularly CCC+D2 ($p<0.01$), while CD8+CD57+ expanded in D2 and iCh+D2 ($p<0.05$). CD4+IFNγ+ TL were elevated in Ch+D2 ($p<0.05$ vs. Co), and CD107a+ TL were higher in CCC, D2, and iCh+D2 ($p<0.05$). CD4+HLA-DR+ activation rose in Ch, especially iCh and iCh+D2 ($p<0.05$), and CD8+HLA-DR+ activation increased across all groups, peaking in CCC-associated cases ($p<0.01$). Overall, Ch patients exhibit immunosenescence features regardless of clinical severity, which are exacerbated by D2. Chronic inflammation, combined with metabolic dysregulation, fosters a proinflammatory, aged TL profile, potentially accelerating CCC progression. Managing metabolic comorbidities may mitigate immune ageing and inflammatory complications in Chagas disease.

Key words: Immunosenescence; Chagas; Diabetes; T-cell

IA-087

Unraveling the Role of Sorting Nexins in Antigen Cross-Presentation by Dendritic Cells.

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Sorting nexins (SNXs) constitute a family of endosomal proteins that regulate key functions of the endocytic pathway, including endocytosis, membrane remodeling, cargo sorting, and signaling. In dendritic cells (DCs), the endocytic network is tightly regulated to enable cross-presentation, the process by which exogenous antigens are loaded onto MHC-I molecules to activate $CD8^+$ T lymphocytes. The efficiency of this pathway depends on accurate endosomal trafficking and sorting that ensure proper antigen uptake, processing, and delivery of peptide–MHC-I complexes to the cell surface. However, the molecular players controlling these events remain poorly defined. In a previous study, we demonstrated that SNX17 is essential for exogenous antigen internalization, integrin recycling, actin cytoskeleton organization, and phagosomal maturation. Here, we identify another family member, SNX27, as a novel regulator of cross-presentation. Silencing SNX27 expression in DCs selectively impairs the presentation of OVA immune complexes and OVA-coated beads, but not soluble OVA. Furthermore, SNX27 controls early MHC-I recycling and is recruited at late stages to phagosomes, modulating the amount of peptide–MHC-I complexes present in phagosomal membranes, and thereby ensuring efficient MHC-I loading with antigenic peptides. Taken together, our findings identify SNX17 and SNX27 as critical regulators of endocytic trafficking and antigen cross-presentation by DCs.

Key words: dendritic cells; sorting nexins; cross-presentation; intracellular trafficking

IA-092

Metabolic and Mitochondrial Programs in Trypanosoma cruzi-Specific CD8 T Cells

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Chagas disease, caused by Trypanosoma cruzi, remains a neglected tropical disease of growing global relevance. Control of infection requires both CD4⁺ and CD8⁺ T cells, with CD8⁺ T cells playing a dominant role against the intracellular parasite. It has been shown that mitochondrial membrane potential (MMP) and mitochondrial ROS (mROS) might regulate T cells effector function. However, prolonged elevated levels of mROS are detrimental towards effector T cell function. We have demonstrated that, during the acute phase (AP) of T. cruzi infection, the peak of parasitemia coincides with an expansion of total effector (E) CD8 T cells and T. cruzi-specific CD8⁺ T cells (Tc-CD8). Both cell populations exhibited elevated mROS production, glucose uptake, and CD98 expression, consistent with an activated phenotype. However, a substantial fraction of these cells showed loss of MMP along with reduced glucose uptake and CD98 expression, suggesting a dysfunctional subset that may compromise the overall T cell response. To examine phenomena associated with MMP lost, C57BL/6 mice were infected with 5000 Tulahuen trypomastigotes, and spleens were collected at AP. Mitochondrial parameters were analyzed by FACS using MMP-dependent and MMP-independent dyes in combination with Fas-L and Annexin V staining. Mitochondria-depolarized cells displayed a higher frequency of Fas-L⁺ cells ($p<0.05$) and a trend toward increased Annexin V expression ($p=0.07$) compared to cells with preserved MMP. Additionally, to explore the link between mROS production and metabolic capacity, effector (E) CD8 T cells were stratified by mROS levels using MitoSOX dye. During the AP, cells with high mROS (mROShi) exhibited reduced glucose and amino acid uptake, as assessed by 2-NBDG and CD98 staining by FACS, compared to mROSlo ($p<0.05$, $p<0.005$) or mROSdim ($p<0.05$, $p<0.005$) counterparts. Finally, to further characterize the metabolism of these cells, we applied the SCENITH methodology. We found that Tc-CD8 cells displayed lower mitochondrial dependency and respiratory capacity relative to their glycolytic dependency and capacity at this stage ($p < 0.05$ for both). Overall, these findings indicate that E and Tc-CD8 T cells adopt distinct metabolic and mitochondrial programs during T. cruzi infection, with subsets displaying dysfunctional profiles associated with mitochondrial alterations and apoptosis. Further studies are required to clarify the metabolic pathways regulating T cell responses in this context.
Key words: Chagas Disease; CD8+ T cells; mitochondrial metabolism; mROS.

IA-097

Leukocyte-Specific Protein 1 Regulates Adaptive Immune Responses

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The Leukocyte-Specific Protein 1 (LSP1) is a 52 kDa cytoplasmic phosphoprotein expressed in leukocytes and endothelial cells, highly conserved across species. By binding F-actin, LSP1 regulates cytoskeletal dynamics, thereby influencing cell motility and immune interactions. Previous studies showed that Lsp1⁻/⁻ mice display impaired cytotoxic T lymphocyte (CTL) responses, largely due to defective antigen cross-presentation by dendritic cells (DCs). In vitro, LSP1-deficient DCs exhibited reduced capacity to activate CD4⁺ T cells, linked to impaired antigen uptake, processing, and delayed actin polymerization.

To explore the role of LSP1 in vivo, Lsp1⁻/⁻ and wild-type (WT) mice were immunized with OVA/CpG-ODN1826/Coa-ASC16 (OCC) and later challenged by OVA footpad injection to test delayed-type hypersensitivity. Lsp1⁻/⁻ mice showed reduced inflammation, less edema, and failed to generate strong OVA-specific CD8⁺ T-cell memory. B-cell responses were also compromised, with fewer OVA-specific IgM- and IgG-producing cells.

Humoral responses were analyzed after a single OCC dose. Both groups showed comparable antibody titers and/or kinetics of total IgG, IgG1, IgG2c and IgM. An antigen boost induced similar secondary antibody responses. Nevertheless, Lsp1⁻/⁻ mice had significantly fewer CD138⁺ antibody-secreting cells in the spleen.

For cellular immunity, lymph node and spleen cells cultured with OVA peptides exhibited reduced frequencies of OVA-specific CD4⁺ and CD8⁺ T cells in Lsp1⁻/⁻ mice, accompanied by lower

IFN-γ and TNF-α secretion. Finally, naïve Lsp1$^{-/-}$ CD4^{+} T cells stimulated with a polyclonal signal displayed impaired activation, proliferation, and altered cytokine profiles, with increased IL-10 and IL-27 but reduced IFN-γ, IL-2 and IL-17A. Lsp1$^{-/-}$ CD8^{+} T cells also showed diminished activation, proliferation, and TNF-α production.

Together, these findings demonstrate that LSP1 is essential for the development of robust T- and B-cell responses, underscoring its critical role in regulating both cellular and humoral immunity.

Key words: LSP1; T-cell; B-cell; immune-response

IA-102

Single Cell Proteomic and transcriptomic analysis of the tonsillar B cells from pediatric Down syndrome donors reveal enhanced antiviral transcriptomic programs.

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Down syndrome (DS), caused by trisomy 21 (T21), profoundly impacts the immune system. In childhood, this leads to recurrent infections and poor vaccine responses due to combined innate and adaptive immune dysregulation. In adulthood, DS is linked to chronic inflammation, autoantibody production, and increased prevalence of autoimmune disorders. Most knowledge derives from blood studies reporting altered T and B cell compartments, including expansion of Th1/Th17 effector/memory T cells, resistance to Treg suppression, and accumulation of CD11c^{+} T-bet^{+} memory B cells—features often associated with autoimmunity. We hypothesized that this circulating immune dysregulation originates within secondary lymphoid organs (SLOs), where germinal center T–B interactions are likely impaired. Our previous work revealed dysfunction in tonsillar follicular helper T cells from pediatric DS, but multiparametric flow cytometry of B cells (n=14 T21, n=9 controls) showed no major shifts in canonical populations, except for increased CXCR3 expression across subsets.

Here, we investigated the B cell compartment at transcriptomic and proteomic levels using CITE-seq. Palatine tonsil mononuclear cells from six pediatric donors (3 controls, 3 T21) were sorted into B (CD19^{+}) and non-B (CD19^{-}) fractions, stained with 137 oligonucleotide-conjugated antibodies (ADT) and subjected to integrated RNA/protein profiling. After quality control, unsupervised clustering and UMAP visualization were performed using Seurat, cell types were annotated using established tonsillar B cell marker genes. To compare population abundance, we applied MILO neighborhood analysis, which confirmed no significant shifts in canonical B cell frequencies, corroborating flow cytometry.

In contrast, transcriptomic profiling revealed striking differences. Differential expression analysis of total B cells identified 1053 upregulated and 2191 downregulated genes in T21 vs controls. Enrichment analyses (GO, KEGG, etc) showed upregulation of antiviral defense programs and activation of innate immune receptors (TNF, RIG-I, NOD-like, helicase, etc), accompanied by downregulation of mitotic checkpoint regulators, p53 signaling, and cell cycle pathways.

In summary, although canonical B cell distribution was preserved, B cells in T21 tonsils displayed a distinct transcriptional signature, characterized by heightened proinflammatory and antiviral programs that potentially contributes to the autoimmune predisposition observed in DS.

Key words: Down syndrome; trisomy 21; autoimmunity; tonsil

IA-107

Melanoma HLA I immunopeptidome uncovers gut bacterial peptides mimicking tumor antigens with potential to enhance tumor immune recognition

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The gut microbiome has been recognized as a potential novel player in the pathogenesis and treatment of malignant melanoma. This study aims to evaluate through bioinformatic analysis whether bacterial antigens from the gut microbiome exhibit molecular mimicry with peptides presented by human melanoma in the context of HLA I molecules and whether bacterial peptides are also presented in the immunopeptidome of these cells, thereby facilitating tumor recognition and killing by tumor-infiltrating lymphocytes. Immunopeptidome data from melanoma samples of 25 patients available in the PRIDE repository (PXD004894) were analyzed using FragPipe version 22 (MSFragger version 4.1). To compare the acquired fragmentation spectra with the theoretical spectra of peptides potentially presented in these tumors, a FASTA database was built including the predicted neoepitopes, proteomes of various bacterial species constituting the gut microbiota as defined in the Human Gut Microbiome Atlas (UniProt), and the reference proteome of *Homo sapiens* (UniProt), along with decoy sequences and common contaminants. The peptides presented by these tumors were searched with 100% sequence identity in the proteomes of bacteria constituting the gut microbiota, in order to identify taxonomic conservation and potential cross-reactivity with human proteins. Bacterial peptides were identified as being presented, likely derived from the processing of antigens from the genera *Eubacterium*, *Mycoplasma*, *Firmicutes*, *Megasphaera* and *Clostridium*. In most cases, these sequences were conserved among different species within the same genera, and occasionally among different genera. Also, potential antigenic mimicry was observed between peptides derived from human protein processing and bacterial antigens from various genera, with notable taxonomic conservation. In these cases, the most represented genera were *Blastocystis*, *Bacteroides*, *Prevotella*, *Klebsiella*, *Alistipes* and *Fusobacterium*, in addition to those previously mentioned. The analysis suggests that bacterial peptides from gut microbiota can be presented in the immunopeptidome of human melanoma cells and exhibit potential antigenic mimicry with human proteins. Importantly, all the bacterial genera identified in this study have been previously associated with tumors, including melanoma, highlighting a potential link between gut microbiota composition and tumor immune landscapes.
Key words: Gut microbiome; Melanoma; Molecular mimicry; T cell cross-reactivity

IA-143

Immune dysregulation in children with Down Syndrome: Single-cell multi-omics reveal Th1-Skewed CD4 differentiation, reduced mature Tfh, and altered tonsillar architecture

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Introduction
Children with Trisomy 21 (T21) exhibit early-onset immune dysregulation, leading to increased susceptibility to infections, impaired vaccine responses, and autoimmunity. Tonsils, as secondary lymphoid organs, provide a unique opportunity to study T-B cell interactions and differentiation of CD4+ T cells into follicular helper T cells (Tfh).

Methods
Multiparametric flow cytometry, multiplex immunofluorescence and single-cell multi-omics (CITE-seq + TCR-seq) characterized hypertrophied, non-infected tonsils in T21 children compared to age-matched controls. Using Seurat, we identified CD4 clusters. Neighborhood-level abundance shifts were tested with Milo; differential expression and TCR repertoire metrics (Shannon and Gini index) were computed.

Results: Flow cytometry revealed a marked remodeling of T–B compartments in T21 with decreased naïve CD4 T cells and increased memory CXCR3$^+$ CD4$^+$ T cells in detriment of conventional Tfh ($p<0.05$). Multiplex immunofluorescence showed smaller follicles in T21 and a negative correlation between follicle size and PD1- IFNγ$^+$ T-cell frequency ($p<0.05$). Suggesting heightened extrafollicular responses in T21. We also found higher frequencies of IFNγ-producing Tfh. CITE-seq confirmed profound remodeling within CD4$^+$ cells. In T21 tonsils, MiloR detected significantly decreased neighborhoods ($p<0.05$) among differentiated SAP$^+$ Tfh, and increased neighborhoods in central memory (CM), CD4 cytotoxic T lymphocytes (CTL), and less-differentiated Tfh states (CM-PreTfh and Tfh in the T–B zone), suggesting differentiation arrest. Significantly expanded CTL neighborhoods in T21 showed higher expression of effector genes (IFNG, GZMB, GZMH, XCL2, PRF1), while expanded CM neighborhoods expressed genes such as RORA, SMAD7, SLCO3A1, GAB3 and FNDC3B.

Protein-level data showed higher CXCR3 across T21 clusters ($p<0.001$), except CTL. CXCR3+ Tfh upregulated interferon-γ and allograft-rejection pathways. The expression of some genes such as IFNG, F2R, TEX13SD, NUGGC and CXCR3 were particularly increased in T21 CXCR3+ Tfh. In T21 tonsils, TCR-seq revealed higher clonal diversity (Shannon) in CM, lower diversity in differentiated Tfh ($p<0.1$), and higher Gini indices in T helper cells ($p<0.1$) suggesting stronger clonal expansions.

Conclusions: T21 tonsils display reduced maturation of Tfh, Th1-skewed and cytotoxic CD4$^+$ programs, and altered clonal dynamics, providing a mechanistic basis for impaired GC responses and dysregulated antibody production.

Key words: Trisomy21; Tonsils; Tfh

IA-191

Mild hypothermia enhances memory cell generation in CD8+ T cell differentiation.

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T cells are a fundamental component of the adaptive immune system, particularly CD8+ T cells, which specialize in eliminating pathogen-infected and tumor cells. Owing to this cytotoxic capacity, CD8+ T cells have emerged as promising therapeutic tools in the context of cell- and immunotherapies. In adoptive cell therapies, CD8+ T cells are expanded *ex vivo* and reinfused into the patient with the aim of eliciting an antitumor response. During *ex vivo* expansion, CD8+ T cells differentiate into effector and memory cells, the latter being crucial to the success of these therapies due to their long-term persistence, providing prolonged protection over time and generating an effective and rapid response to re-exposure to the antigen. To enhance the generation of memory populations, this study evaluated the effect of mild hypothermia on CD8+ T cell cultures. These cells were obtained from PBMCs of three healthy donors, CD8+ T cells were isolated using the EasySep Human CD8+ T cell isolation Kit (Stemcell) and activated with Immunocult Human CD3/CD28 T Cell Activator (Stemcell). Following activation, cultures were maintained either at control temperature or mild hypothermia. Differentiation status was assessed on days 5 and 10 of culture. The main results obtained to date, through flow cytometry analysis of five differentiation markers that allow distinguishing Tn, Teff, Tem, Tcm, and Tscm populations, indicate that on both day 5 and day 10 there is a significant increase in the central memory T cell (Tcm) population compared to the control. This

change in cell differentiation would indicate that the effect of temperature occurs at the level of cellular metabolism, where mild hypothermia would be affecting glycolysis enzymes, decreasing glycolytic metabolism and promoting oxidative metabolism.

Key words: Mild hypothermia; memory; CD8+ T; cell; Tcm

Immunity to Infection

IAI-009

18-HEPE Impairs Dendritic Cell Activation in Response to Mycobacterium tuberculosis

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), evades host immunity by disrupting dendritic cell (DC) functions. Our previous work identified elevated levels of pro-resolving lipids, including 18-HEPE, in TB pleural effusions. We further demonstrated that Mtb-infected macrophages produce 18-HEPE, which subsequently suppresses their microbicidal activity. Here, we investigated whether 18-HEPE modulates monocyte-derived DC activation during Mtb infection. Human monocytes were differentiated into DCs with IL-4 and GM-CSF in the presence of physiologically relevant doses of 18-HEPE and then exposed to live or irradiated Mtb. Although 18-HEPE did not alter CD1a or DC-SIGN expression, it significantly inhibited the upregulation of activation markers (CD83, CD86, HLA-DR) following Mtb infection or stimulation with irradiated Mtb ($p < 0.05$). Moreover, 18-HEPE-treated DCs exhibited reduced TNF-α and IL-10 production in response to live Mtb ($p < 0.05$). To assess whether these effects were mediated by GPR120, a known receptor for 18-HEPE, we treated DCs with the GPR120 agonist TUG891. GPR120 activation did not impair DC responses to live Mtb, suggesting a GPR120-independent mechanism. Since 18-HEPE serves as a precursor for E-series resolvins, we next evaluated whether its downstream metabolite, RvE1, could mimic the inhibitory effects. However, RvE1 treatment failed to suppress DC activation, indicating that 18-HEPE acts through pathways distinct from its conversion to RvE1.

In summary, 18-HEPE suppresses DC activation during Mtb infection, likely through mechanisms independent of GPR120 and RvE1 production. These findings reveal a novel immunomodulatory role for 18-HEPE in TB pathogenesis, warranting further investigation into the underlying molecular pathways.

Key words: Dendritic Cells; Immune Evasion; Lipids; Tuberculosis

IAI-015

Cooperative role of CR3 and CD206 in determining Bordetella pertussis intracellular fate in human macrophages

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During microbial infections, macrophages (MØ) display remarkable plasticity, transitioning between classically activated (M1) and alternatively activated (M2) phenotypes according to environmental cues. Our group has previously shown that Bordetella pertussis modulates macrophage polarization to favor intracellular persistence.

The aim of this study was to evaluate the specific contribution of complement receptor 3 (CR3; CD11b/CD18) and the mannose receptor (CD206) to the adhesion, internalization, and intracellular trafficking of Bordetella pertussis in human monocyte-derived M0 macrophages in a non-polarized state.

We also compared these processes with M2 macrophages to identify receptor-specific differences. In M0 macrophages under non-blocking conditions, 15 ± 2 bact/cell were associated, with ~50% internalized. Blocking CR3 significantly reduced adhesion to 6 ± 2 bact/cell ($p<0.01$) and phagocytosis to 25% ($p<0.01$), while CD206 blockade decreased adhesion to 4.5 ± 2 bact/cell ($p<0.01$) and internalization to 23% ($p<0.01$). Dual blockade nearly abolished adhesion (1 ± 0.2 bact/cell, $p<0.01$) and markedly reduced uptake ($p<0.01$). In M2 macrophages, higher basal interaction was observed (28 ± 3 bact/cell adhered, 80% internalized). Blocking CR3 significantly reduced adhesion to 18 ± 2 bact/cell ($p<0.01$) and internalization to 50% ($p<0.01$), whereas CD206 blockade decreased adhesion to 15 ± 2 bact/cell ($p<0.01$) and almost eliminated uptake (2%, $p<0.01$). Combined blockade abrogated both adhesion and phagocytosis (<1%, $p<0.01$).

Confocal imaging at 30 min post-infection revealed CR3 and CD206 co-localizing at phagocytic cups, confirming their cooperative role in bacterial capture. In M0, colocalization with the lysosomal marker LAMP-1 showed that CR3-mediated entry resulted in higher lysosomal targeting (~82%) than CD206-mediated entry (~58%, $p<0.01$), indicating receptor-dependent intracellular routing. Together, these results demonstrate that B. pertussis exploits CD206-dependent uptake to evade lysosomal degradation, whereas CR3 engagement favors trafficking to bactericidal compartments, revealing a mechanism by which the pathogen modulates macrophage handling to enhance survival.

Key words: Macrophage polarization; Bordetella pertussis; Docking receptor

IAI-018

ATP metabolism modulates cytotoxic CD4 T-cell function in human and experimental Trypanosoma cruzi infection

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Background: During infection, immune cells release ATP, a molecule with antimicrobial activity that is metabolized by the ectonucleotidases CD39 and CD73 into adenosine (ADO), a potent immunoregulatory mediator. Cytotoxic CD4 T-cells (CD4 CTLs) have recently emerged as relevant effectors in *Trypanosoma cruzi* infection, the causative agent of Chagas disease. However, the factors regulating the differentiation and function of this novel population remain incompletely defined. Objective: To determine the role of extracellular ATP metabolism in regulating CD4 CTLs during murine *T. cruzi* infection, and to analyze the expression of components of the CD73/ADO axis in human infection. Methods and results: Experimental *T. cruzi* infection induced a marked expansion of splenic CD4 CTLs (GranzymeB+perforin+) at 14 days post-infection. Functional assays revealed that perforin was essential for controlling T. cruzi-infected macrophages (% specific lysis: medium, 30.3 ± 2.0 vs +perforin inhibitor, 9.4 ± 1.6). CD73- CD4 T-cells expressed higher levels of granzyme B compared to CD73+ cells ($p < 0.05$). Consistently, cytotoxic capacity was enhanced under ATP-enriched conditions (% GranzymeB+Perforin+CD4+: medium, 6.04 ± 0.6 vs +ATP, 10.0 ± 1.4). Infected CD73-deficient (CD73KO) mice exhibited increased expression of granzyme B, perforin, IFN-γ, and TNF-α, and the degranulation marker CD107a in splenic and cardiac CD4 T-cells, along with higher cytotoxic activity (% specific lysis: WT, 7.4 ± 3.0 vs CD73KO, 18.8 ± 2.4) and reduced parasite burden in both spleen and heart tissues ($p < 0.05$). In humans, asymptomatic chronic Chagas patients displayed elevated plasma ATP levels and a higher frequency of circulating GranzymeB+ CD4 T-cells (% GranzymeB+CD4+: control, 4.0 ± 1.4 vs Chagas, 20.6 ± 7.3). RNA-seq analysis of cardiac tissue from end-stage Chagas disease patients revealed enrichment of transcripts associated with ATP metabolism (CD73 and ADO receptor activity).

Conclusion: These findings suggest that CD73-mediated ATP metabolism critically regulates CD4 CTL function, dampening the immune response and parasite control during T. *cruzi* infection.

Key words: ATP; CD73; Adenosine; Immunoregulation; CD4 CTL

IAI-021

18-HEPE rewires M1 macrophages metabolism impairing control of M.tuberculosis infection

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a major global health challenge. We previously demonstrated that proinflammatory (M1) macrophages exposed to the acellular fraction of pleural effusions from TB patients (TB-PE) exhibit reduced glycolysis via suppression of hypoxia-inducible factor (HIF)-1α, resulting in impaired antibacterial activity. Elevated levels of the pro-resolving lipid mediators Resolvin D5 (RvD5) and 18-HEPE were detected in TB-PE, both capable of limiting glycolysis and macrophage microbicidal function.

Here, we investigated the role of 18-HEPE in modulating M1 macrophage metabolism. Human monocyte-derived macrophages were polarized with LPS/IFN-γ (M1 profile) in the presence or absence of 18-HEPE at physiological concentrations found in TB-PE. Metabolic activity was assessed using SCENITH and lactate assays; cytokines (IL-1β, IL-10) by ELISA; and HIF-1α and MerTK expression by flow cytometry. Efferocytosis was evaluated with CFSE-labeled apoptotic neutrophils, and Mtb clearance was quantified by colony-forming units at day 3 post-infection. Given that 18-HEPE is described as a GPR120 ligand, we tested its mechanism using TUG-891 (agonist) and AH-7614 (antagonist). We also used a ChemR23 inhibitor to assess the role of RvEs (18-HEPE–derived metabolites).

Results showed that 18-HEPE significantly reduced lactate production, glycolytic capacity, and HIF-1α expression ($p<0.05$), while decreasing IL-1β, impairing Mtb killing, and increasing IL-10, MerTK expression, and efferocytosis ($p<0.05$). Stabilizing HIF-1α with dimethyloxalylglycine restored glycolysis, bactericidal activity, and normalized efferocytosis. While TUG-891 suppressed lactate release via GPR120, an effect reversed by the antagonist AH, 18-HEPE-mediated inhibition persisted despite AH treatment, suggesting a GPR120-independent mechanism. Notably, neither ChemR23 inhibition nor RvE1 exposure induced changes in macrophage metabolism.

Key words: macrophages; metabolism; 18-HEPE; proresolution

IAI-025

Role of CD8⁺ T Lymphocytes in IL-33-Mediated Protection During Acute Trypanosoma cruzi Infection

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IL-33, a member of the IL-1 family, is constitutively expressed in the nuclei of epithelial and stromal cells and is released upon tissue damage, acting as an alarmin through its ST2 receptor. Depending on the context, IL-33 can exert either beneficial or detrimental effects during infections triggering diverse immune mechanisms, both effector and regulatory or regenerative. Acute Trypanosoma cruzi infection triggers intense immune activation aimed at limiting parasitism, often at the cost of inducing tissue damage. In previous work, we demonstrated that IL-33 treatment in infected wild-type (WT) mice expanded regulatory tissue-repairing T cells (rtTreg), type 2 innate lymphoid cells (ILC2), and parasite-specific $CD8^+$ T lymphocytes, resulting in reduced tissue damage and parasite burden, and improved survival. Identifying the cellular mediators responsible for IL-33's effects is essential to harness its therapeutic potential in this infection.

To evaluate the role of $CD8^+$ T lymphocytes in IL-33-induced effects, we used CD8 knockout (CD8 KO) mice infected with 1000 trypomastigotes (Tulahuén strain) and treated with recombinant IL-33 or PBS on days 0, 3, and 6 post-infection. Unlike WT mice, IL-33-treated CD8 KO animals displayed higher plasma levels of tissue damage markers (GOT, GPT, LDH, CPK; $p<0.05$) and increased parasite loads in target tissues compared with PBS controls ($p<0.01$). Immunological profiling revealed that, in the absence of $CD8^+$ T cells, IL-33 still promoted the expansion of effector $CD4^+$ T cells, rtTreg, and ILC2 in spleen and liver ($p<0.01$), as well as Treg in skeletal muscle ($p<0.05$). However, this enhancement of immunoregulatory and tissue-repairing populations alone was not sufficient to prevent tissue injury and, critically, without $CD8^+$ T cells as the main cytotoxic subset, IL-33 administration was even associated with exacerbated parasitism in host tissues.

Taken together, our results demonstrate that $CD8^+$ T cells are indispensable for IL-33 treatment to achieve full protective effects during acute T. cruzi infection.

Key words: IL33; cruzi; CD8; repair, Treg

IAI-055

Development and validation of an in vitro diagnostic method for tuberculosis: a locally focused and nationally applicable strategy

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Tuberculosis (TB) remains one of the leading causes of mortality from infectious diseases worldwide. In Argentina, the rising incidence and the diagnostic challenges—especially in underserved populations—underscore the urgent need for accessible and regionally adapted tools. Interferon-gamma release assays (IGRAs) offer sensitive and specific alternatives for TB diagnosis; however, commercial tests are often costly, technically demanding, and not tailored to the local immunogenetic landscape. In this context, we developed IGRA-X, a modular whole-blood-based immunodiagnostic platform that eliminates the need for PBMC isolation. The current version uses synthetic peptides from Mycobacterium tuberculosis (Mtb) antigens ESAT-6 and CFP-10, absent in the BCG vaccine and environmental mycobacteria, ensuring high specificity. While these peptides enable initial validation, IGRA-X is designed to incorporate epitope pools selected through in silico predictions based on the most frequent HLA-DR alleles in the local population, enhancing population-specific immune detection. The experimental protocol involves collection of 2 mL of peripheral blood and stimulation for 16–24 hours with ESAT-6/CFP-10 peptides (BEI Resources). Co-stimulation with anti-CD28 and anti-CD49d monoclonal antibodies is used to ensure optimal T cell activation and cytokine production. IFN-γ is quantified by ELISA from culture supernatants, using a workflow compatible with basic laboratory infrastructure. Computationally guided selection of immunodominant peptides from ESAT-6 and CFP-10 TB proteins was performed using bioinformatic tools (IEDB.org), adjusted to the most prevalent Class I and Class II HLA alleles in the Argentinean population. This strategy aims to maximize antigen presentation and enhance the sensitivity of the assay. Peptide synthesis is currently underway.

Preliminary results from 8 active TB patients and 9 healthy donors revealed significantly elevated IFN-γ levels in TB samples ($p < 0,0001$), with clear group discrimination. Moreover, IGRA-X achieved 100% concordance with the T-SPOT.TB® assay (Oxford Immunotec), reinforcing its technical robustness and diagnostic reliability. Together, these results support the feasibility of IGRA-X as a cost-effective, accessible, and regionally adaptable tool for TB diagnosis, with potential to expand to other emerging pathogens relevant to both human and veterinary health.

Key words: Tuberculosis; immunodiagnosis; IGRA assay; interferon-gamma; HLA-DR.

IAI-071

Study of the immune response to viral respiratory infections in children with post-infectious bronchiolitis obliterans and frequent hospital consultations

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Post-adenovirus infection bronchiolitis obliterans (BOPI) is characterized by airways fibrosis. PIBO patients present the frequent respiratory tract infections. Its development or its long-term consequences have not been studied in depth. This work aimed to evaluate the immune response in children with PIBO. Five patients with PIBO were selected for the study. Blood samples were taken at time of consultation (day 0, t1) and after 21-23 days (t2) for molecular biology studies. Total blood leucocytes were isolated at t1 and t2. The expressions of IFN-β, IFN-γ, Mx1, OASL, IFIT2, STAT1, TNF-α, IL-1β, IL-6, IL-10, IL-15, and IL-17 were evaluated in blood immune cells by RT-qPCR. When the expression of IFNs and antiviral factors were analyzed, all the patients had values that were higher than non-infected controls ($p<0.01$). Four of the patients showed a reduction in the expression of IFN-β, IFN-γ, Mx1 and STAT1 when time 1 and 2 were compared ($p<0.05$), while patient 1 showed an increment of these factors. In all cases the expression of OASL and IFIT2 were reduced from time 1 to time 2 ($p<0.05$). The expression levels of the TNF-α, IL-1β, IL-6, IL-15, and IL-17 in all the PIBO patients were higher than non-infected controls ($p<0.01$). In all cases the expressions of IL-1β and IL-6 were reduced from time 1 to time 2 ($p<0.05$). Four of the patients showed a reduction in the expression of TNF-α and IL-15, while patient 1 showed an increment of these factors ($p<0.05$). Interestingly, IL-17 expression was increased from time 1 to time 2 in all the patients except for patient 4 which showed opposite behavior. We also detected enhanced levels of the regulatory cytokine IL-10 in all the PIBO patients compared to non-infected controls ($p<0.05$). However, the expression of IL-10 at time 1 was higher and lower than time 2 for 3 and 2 patients, respectively. The results suggest that children with PIBO have a predisposition to the development of exacerbated inflammatory responses against respiratory viruses characterized by the increases in IFNs, antiviral factors and inflammatory cytokines. Although the increase of inflammatory mediators was intense and sustained in all patients, the changes in the expression of the regulatory cytokine IL-10 were variable, indicating individual variations in the ability to regulate the inflammatory response. In virus-infected PIBO patients, clinical improvement would relate to the ability to regulate the exacerbated inflammatory response.

Key words: Bronquiolitis Obliterante Post-Infecciosa (BOPI); Molecular Biology; Pediatric patient

IAI-079

Disruption of infection-induced hypoglycemia impairs host defense against Yersinia enterocolitica via IL-17

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Yersiniaenterocolitica (Ye) is a Gram-negative bacteria that causes Yersiniosis, a gastrointestinal infection in humans. While its virulence mechanisms have been extensively studied, the metabolic changes induced during infection and their influence on the host immune response remain poorly understood. In previous studies, we observed that Ye infection in mice induces a persistent hypoglycemic state, along with increased serum IL-17 levels during the course of infection. Building on these findings, we aimed to investigate whether Ye-induced hypoglycemia and IL-17 production contribute to host protection or disease severity. C57BL/6 mice were orally infected with Ye serotype O:8 and randomly assigned to three experimental groups: (1) oral glucose supplementation twice daily, (2) glucose supplementation plus intraperitoneal anti-IL-17 antibody (secukinumab) administered biweekly, and (3) saline-treated infected controls. Non-infected mice served as baseline controls. Body weight, food intake, serum IL-17 levels, and fecal bacterial load were monitored throughout the infection. Survival was recorded over a 45-day post-infection (p.i.) period. Glucose supplementation led to increased weight loss ($p<0.05$) and reduced food intake ($p<0.01$) compared to non-supplemented infected mice. Serum IL-17 levels were elevated in both infected and glucose-supplemented groups as early as day 1 p.i. ($p<0.01$ vs. control), with peak levels on day 3 p.i. in the glucose group ($p<0.0001$ vs. control). Secukinumab treatment delayed IL-17 elevation until day 3 p.i. ($p<0.05$ vs. control). Glucose had no impact on fecal bacterial load, but secukinumab significantly reduced bacterial counts on days 7 and 21 p.i. ($p<0.05$ vs. untreated infected group). Survival was lowest in the glucose-supplemented group (40%) compared to non-supplemented infected mice (80%), while the secukinumab-treated group showed an intermediate survival rate (71.4%). Our data suggest that the hypoglycemic state induced by Ye infection contributes to host protection, whereas glucose supplementation exacerbates disease severity, potentially through early and excessive IL-17 production. IL-17 neutralization improved survival and reduced bacterial burden, underscoring a possible detrimental role for IL-17 in Ye infection. These findings reveal a critical metabolic-immune axis in the host response to Y. enterocolitica, warranting further investigation.
Key words: Yersinia enterocolitica; glucose; secukinumab

IAI-081

Plasma Peptidome Analysis Using Machine Learning and Immune Alterations in Sepsis for Diagnosis and Stratification: A Pilot Study

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Introduction: Sepsis is a leading cause of morbidity and mortality in intensive care units (ICUs), characterized by a dynamic and dysfunctional immune response. Early diagnosis and patient stratification remain major challenges. Emerging technologies such as proteomics and artificial intelligence represent innovative approaches in biomedical research. *Objective*: To evaluate the potential of plasma peptidome fingerprints for sepsis diagnosis and stratification using MAL-

DI-TOF MS technology, complemented by assessment of immune status throughout the disease course. *Materials and Methods*: In this pilot cohort study, 18 ICU patients from Sanatorio Otamendi were classified as sepsis (S, n=10) or non-sepsis (NS, n=8). Peripheral blood samples were collected on days 1, 3, 5, and 7 for MALDI-TOF MS peptidome analysis, generating 576 spectra from S, NS, and healthy controls (Ctl, n=8). Unsupervised clustering (PAM) and supervised methods (Random Forest [RF] and Logistic Regression [LR]) were used to evaluate classification performance. Leukocyte immunophenotyping was performed by flow cytometry on days 1 and 7. *Results*: In S a decreased in T and B lymphocytes levels (% (median (RIQ)), T:CD4: S = 24,4 (13,2-31,2); Ctl = 39,5 (26,2-51,9), p< 0,05; B:CD19: S = 10,5 (5,2-14,7); Ctl = 21,4 (14,7-28,9), p< 0,05) was observed, in conjunction with an PD-1 increase on T lymphocytes (%(median (RIQ) TCD4+/PD-1+): S = 43,5 (23,3-67,8); NS = 15 (11,9-20,8); Ctl = 13,5 (9,4-15,4), p< 0,05). Monocytes showed increased PD-L1 (%CD14+/PD-L1+ (median (RIQ)): S = 46 (34,4-74,6); Ctl = 3,9 (1,3-4,6), p< 0,05) and decreased HLA-DR (MIF (RIQ)): S = 344 (167-8036); Ctl = 23109 (17794-27604), p< 0,05). An increase in immature neutrophils (% CD11b+/CD10- (median (RIQ)): S = 30,2 (6,3-40,4); Ctl = 2,6 (1,1-4,4), p< 0.05) was observed, with elevated PD-L1 levels (% CD11b+/CD10-/PD-L1+ (median (RIQ)): S = 29,1 (7,3-95,8); Ctl = 1,4 (0,4-4,4), p< 0.05). Machine learning models effectively discriminated between sepsis and non-sepsis showing high performance in accuracy (A), sensitivity (SE), specificity (SP), and ROC curve (AUC). RF predictive values were: A = 86%, SE = 88%, SP = 82%, AUC = 0.88. *Conclusion*: Immune alterations were similar in septic and non-septic patients, but peptidome analysis distinguished sepsis fingerprints, enabling predictive modeling, supporting its potential for early diagnosis and patient stratification.

Key words: sepsis; MALDI-TOF MS; peptidoma; magine learning

IAI-082

Phenotypic and functional characterization of CD4+LAG-3+ T lymphocytes in patients with chronic Chagas Disease

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, remains a major public health challenge in Latin America and an emerging concern worldwide due to migration. While many chronically infected individuals remain without clinical symptoms, approximately 30% develop cardiac manifestations. Since it is known that immune regulatory pathways play a key role in chronic disease, we decided to study Lymphocyte Activation Gene-3 (LAG-3) which shares homology with CD4 and binds MHC class II with high affinity. We previously reported differential LAG-3 expression in $CD4^+$ T cells from chronic Chagas Disease patients (CCD) without detectable symptoms (A) and with cardiac manifestations (C). Here, we extended our study by analyzing both, the memory/Th phenotype and functional profile of total and *T. cruzi*-specific $CD4^+LAG\text{-}3^+$ T lymphocytes in CCD patients and non-infected (NI) donors. Peripheral blood mononuclear cells (PBMCs) were cultured for 24h with medium or *T. cruzi* lysate (Dm28c). For phenotypic analysis, cells were stained with antibodies against CCR7/CD45RA (naïve/memory), CCR4/CCR6/CXCR3 (Th), and OX40/CD25 (antigen-specific cells) and analyzed by multiparameter flow cytometry. For functional assessment, PBMCs were incubated under the same conditions in the presence of co-stimulatory antibodies (CD28/CD49d), with brefeldin A and monensin added during the last 4h, followed by intracellular cytokine staining (ICS) for IFN-γ, TNF-α, and IL-17A. $CD4^+LAG\text{-}3^+$ T cells displayed predominantly Th1 and central memory profiles across groups. Upon *T. cruzi* stimulation, CCD patients showed a decrease in the frequency of naïve $CD4^+LAG\text{-}3^+$ T cells, accompanied by an increase in effector memory cells compared with NI donors. As for the Th profile, $CD4^+LAG\text{-}3^+$ T cells from CCD patients displayed a shift toward a Th17 phenotype, with a slight decrease in Th1 cells. *T. cruzi*-specific ($OX40^+CD25^+$) $CD4^+LAG\text{-}3^+$ T cells predominantly exhibit-

ed a Th17 profile compared with non-activated cells in both groups of CCD patients. Functional analysis supported this observation, revealing a higher frequency of IL-17A$^+$ cells within the LAG-3$^+$CD4$^+$ subset in cardiac patients compared with the LAG-3$^-$ population. These findings highlight the potential relevance of this subset in modulating immune responses during chronic *T. cruzi* infection and contribute to understanding the complex dynamics of T cell responses in chronic Chagas disease.

Key words: Chagas disease; Adaptive immunity; CD4$^+$ T cells; LAG-3

IAI-093

T. cruzi Modulates Macrophage Mitochondrial Dynamics to Enhance Parasite Proliferation

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Macrophages are the first line of defense against *Trypanosoma cruzi*, the etiological agent of Chagas disease, but the parasite can exploit them for survival and replication. Mitochondria, key regulators of metabolism and immune responses, undergo dynamic fusion mediated by Mitofusins (MFN) and OPA1, while DRP1 phosphorylation drives fission. In macrophages, fission is linked to M1-like activation, whereas fusion correlates with M2-like features. We hypothesized that *T. cruzi* modulates mitochondrial dynamics to favor proliferation. We previously showed that infection promotes a transient fusion-like state, with mitochondrial elongation and increased MFN1/OPA1 expression peaking at 30-48 h post-infection (p.i.). To test whether a fusion- or fission-biased environment influences infection, resident peritoneal macrophages (PEMs) were treated with optimize doses of Mdivi-1 (fission inhibitor; 15µM) + M1 (fusion promoter; 30µM) overnight, and CCCP (positive control for fission; 0.5µM for 2h) selected for their ability to alter mitochondrial morphology (immunofluorescence) without affecting viability (LDH). Treated PEMs were washed and infected with *T. cruzi* Tulahuen strain at a 1:5 cell-to-parasite ratio; non-infected PEMs were used as controls. At 48 h p.i., mitochondrial morphology, parasite replication, nitric oxide (NO), and IL-1β were assessed using MitoSpyOrange, immunofluorescence, Griess and ELISA. Data were analyzed by one-way ANOVA. Infection rates were similar across groups, but both Mdivi-1+M1 and CCCP significantly increased amastigote burden ($p \leq 0.05$). MitoSpyOrange staining showed that infection enhanced mitochondrial footprint and branch length in DMSO and Mdivi-1+M1 treated cells ($p \leq 0.0001$). In CCCP-treated cells, infection counteracted CCCP-induced fission, restoring a more connected network with increased footprint, length, and branching ($p \leq 0.0001$), correlating with higher parasite replication. IL-1β and NO significantly increased upon infection compared to non-infected controls ($p \leq 0.01$) but showed no treatment-dependent differences. Arginase activity was also unaffected by infection under most conditions, however, in CCCP-treated PEMs infection reversed the reduction seen in non-infected PEMs ($p \leq 0.01$). These findings suggest that *T. cruzi* induces mitochondrial fusion in macrophages and that a fusion-like profile associates with parasite replication, uncovering a link between mitochondrial dynamics, macrophage function, and infection outcome.

Key words: Trypanosoma cruzi; macrophages; mitochondria; Immunometabolism.

IAI-095

Enhancing CD8$^+$ T Cell Metabolic Fitness and Effector Function with Nicotinamide Riboside During Trypanosoma cruzi Infection

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Trypanosoma cruzi, the causative agent of Chagas disease, is a neglected tropical disease endemic to Central and South America and a growing global health concern. Both $CD4^+$ and $CD8^+$ T cells are essential for infection control, with $CD8^+$ T cells playing a central role. Our previous studies showed that during the acute phase (AP) of infection, $CD4^+$ T cells accumulate mitochondrial reactive oxygen species (mROS), lose mitochondrial membrane potential (MMP), and express PD-1—changes linked to reduced functionality and apoptosis. Similar mitochondrial alterations were observed in $CD8^+$ T cells, including Tc-$CD8^+$ populations. Because immune cell function is tightly linked to metabolism, we investigated whether supplementation with Nicotinamide Riboside (NR), a NAD^+ precursor, could modulate $CD8^+$ T cell differentiation and function in vitro. NR-differenciated $CD8^+$ T cells showed enhanced mitochondrial fitness, with increased respiration, MMP, biogenesis, reduced mROS leading to lower apoptosis, higher proliferation and increased IFN-γ and Granzyme B compared to vehicle-differentiated cells. To validate in vivo, male C57BL/6 mice were injected with 5000 Tulahuen trypomastigotes. Non-infected (NI) animals served as controls. Mice received NR (500 mg/kg/day by oral gavage) or PBS from 5–20 days post-infection (dpi) (I-NR or I-PBS). Blood was collected at 10, 14, 16, 18, and 21 dpi, and spleen cells at 21 dpi. We found that NR treatment had a better control of parasitemia during AP, without affecting body weight. While the frequency of effector $CD8^+$ T cells remained unchanged, $CD8^+$ T cells from I-NR animals showed increased pEBP41 expression ($p<0.05$), while PD-1 expression was reduced within effector T cells compared to I-PBS ($p<0.05$), with a decreased frequency of $mROS^+$ cells in this population ($p<0.05$). Although no differences were observed in the proportion of depolarized/preserved MMP cells, mROS production was reduced in I-NR animals among cells with preserved MMP ($p=0.0538$). Moreover, NR treatment enhanced the expression of IFN-γ, TNF-α, and Granzyme B in effector $CD8^+$ T cells compared to PBS treatment ($p<0.05$, $p<0.05$, $p<0.005$, respectively). Altogether, these results show that NR supplementation not only improves metabolic and mitochondrial parameters but also enhances the cytotoxic potential of $CD8^+$ T cells. Our findings highlight NR as a promising immunometabolic modulator with potential applications in strengthening T cell responses during infections.
Key words: Chagas Disease; Immunometabolism; CD8+ T cells; Nicotinamide Riboside.

IAI-106

The impact of Clostridioides difficile on Caco-2 model of the human intestinal barrier.

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Clostridioides difficile (*C. difficile*) is an anaerobic, gram-positive and spore-forming bacterium, that infects the human intestine under conditions of microbiota dysbiosis and it can cause symptoms ranging from mild/moderate diarrhea to severe cases and even death. The human epithelial cell line Caco-2 is widely used as a model of the intestinal barrier due to its ability to differentiate and acquire characteristics typical of enterocytes. As such, it pro-

vides a valuable tool for evaluating the effects of C. *difficile* on intestinal epithelial cells. Our objective was to determine the effect of C. *difficile* on differentiated Caco-2 cells, as an *in vitro* infection model. Caco-2 cells were differentiated for 15 days and then, treated with a non-toxigenic strain (CD160), a toxigenic strain (NAP1) and/or the secretome of C. *difficile* (NAP1), which contains the toxins among other secreted factors. Cells were stimulated in a ratio of 1:1 (cell:bacteria) and with 450 ng/µl of the secretome, quantified by total protein concentration. By hematoxylin and eosin staining we observed that toxigenic C. *difficile* strain and/or its secretome causes loss of monolayer integrity with a significant increase in the cell free area ($p<0.05$). Fluorescence microscopy revealed altered Claudin-1 expression and Actin-F formation ($p<0.01$), as well as increased MUC1 expression ($p<0.05$). By flow cytometry, we determined that this treatment affects the expression of the cytokines IL-10 and TNF-α. Finally, we also observed that C. *difficile* treatment increased Defensin 2 secretion as measured by ELISA ($p<0.05$).

Our results establish and characterize an *in vitro* model to study for C. *difficile* infection on the human intestinal context. This model reveals how C. *difficile* disrupts epithelial integrity by altering tight junctions, the cytoskesletal organization, and mucin expression, while also reshaping cytokines and antimicrobial peptide responses.

Key words: Clostridioides difficile; Intestinal epithelium; Caco-2

IAI-112

Brucella abortus RNA interferes with T cell functionality

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Brucellosis is a zoonotic disease caused by Brucella spp. These pathogens can survive inside macrophages, persisting inside the host. In order to survive, *Brucella abortus* (*Ba*) must trigger different strategies to evade the adaptive T cell response it elicits. We have recently demonstrated that *Ba* RNA (a PAMP related to pathogens' viability or *vita*-PAMP) activates lymphocytes at early time points but it may later contribute to circumventing this activation. More specifically, *Ba* RNA increases the expression of the early activation marker CD69 on peripheral blood mononuclear cells (PBMCs) at 24 h. In contrast, *Ba* RNA decreases the expression of the senescence marker CD28 only in anti-CD3/CD28 pre-stimulated PBMCs at 96 h. However, these phenomena on PBMCs were not observed in purified CD3+ T cells. These results suggest that the modulation of T cells by *Ba* RNA may require the presence of monocytes. So, first we aimed to elucidate the role of monocytes in these effects. For this, unstimulated or anti-CD3/CD28 pre-stimulated CD3+ T cells were co-cultured or not with monocytes and treated with *Ba* RNA. At 24 h, CD69 expression was then measured by flow cytometry. *Ba* RNA was only able to increase CD69 expression when T cells were co-cultured with monocytes. Next, we wanted to evaluate the effect of *Ba* RNA on other functional aspects of T cells, such as cytokine secretion and proliferation. First, we evaluated the effect of *Ba* RNA on IL-2 and IFN-gamma secretion. For this, unstimulated or anti-CD3/CD28 pre-stimulated PBMCs were treated with *Ba* RNA for 24 to 96. Supernatants were collected, and cytokine secretion was quantified by sandwich ELISA. *Ba* RNA significantly reduced IFN-gamma secretion at 72 h ($p<0.05$). For IL-2, a non-significant decrease was observed at 24 h. Next, we evaluated the effect of *Ba* RNA on the lymphocyte proliferation. For this, we performed a CFSE assay by flow cytometry. *Ba* RNA did not affect proliferation in unstimulated PBMCs at any of the evaluated time points. However, it significantly reduced the proliferation in anti-CD3/CD28 pre-stimulated PBMCs at 96 h ($p<0.05$). Overall, our results show that *Ba* RNA interferes with T cell functionality and this seems to require the presence of monocytes. This impairment of T cells may favor the establishment of a chronic infection.

Key words: Brucella abortus; PBMCs; T cells; RNA

IAI-114

Role of the response regulators RisR and RisA in the intracellular survival of Bordetella pertussis within human macrophages

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B. pertussis, the etiologic agent of whooping cough, can establish intracellular infections in human cells, including macrophages, potentially promoting persistence in the host. The establishment of intracellular infection involves remodeling of the B. pertussis gene expression profile. Transcriptomic analyses of B. pertussis during macrophage infection suggest that the bacteria adopt an avirulent phenotype intracellularly, whereas proteomic data reveal the sustained expression of adenylate cyclase and pertussis toxin, two toxins characteristic of the virulent phase. Our group has shown that both toxins contribute to bacterial survival within macrophages. The two-component systems reported to regulate B. pertussis virulence are BvgAS and RisAK, which control the expression of vags (virulence activated genes) and vrgs (virulence repressed genes). Omics analyses suggest that these systems may contribute to intracellular adaptation, but additional regulatory proteins are likely required to fully trigger this process. To investigate how B. pertussis adapts to the intracellular environment, we studied another response regulator, RisR, which was identified as differentially expressed in intracellular bacteria by a proteomic analysis of macrophage infection. By constructing a risR mutant and performing comparative proteomics with wild-type bacteria in both virulent and avirulent phases, we identified RisR as a novel regulator of both vags and vrgs. Intracellular infection assays in THP-1 macrophages showed that RisR is required for bacterial survival at 24 and 48 h post-infection. Because the RisR regulon partially overlaps with that of RisA, we next assessed whether these regulators cooperate in intracellular adaptation. Construction of a risA mutant and a risR/risA double mutant followed by macrophage infection assays revealed that the risA mutant displayed reduced survival similar to that of the risR mutant, whereas the double mutant was severely impaired, indicating a synergistic action. In conclusion, our findings suggest that RisR and RisA act coordinately to promote B. pertussis adaptation to the macrophage intracellular environment, supporting bacterial survival and potentially contributing to persistence.
Key words: Bordetella pertussis; macrophage; two-component system

IAI-117

Human neutrophils represent a potential source of Bordetella pertussis for respiratory epithelial colonization

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Bordetella pertussis is the etiologic agent of whooping cough, a reemerging respiratory disease. Previous studies have shown that this pathogen, historically considered extracellular, can survive in intracellular location within both immune and non-immune human cells. Our group recently showed that B. pertussis is internalized by polarized respiratory epithelial cells and might establish a long-term persistence niche inside these cells. B. pertussis is also able to survive inside neutrophils and macrophages which, considering their high mobility and relatively short lifespan, may constitute a transient niche with the potential to transport viable bacteria and disseminate the infection. In order to explore this possibility, we evaluated whether bacteria phagocy-

tosed by neutrophils were able to infect respiratory epithelial cells. To this end, primary human neutrophils previously incubated with B. pertussis were co-cultured with non-infected polarized epithelial cells (16HBE14O-) for 24 h. We used two-colour fluorescence microscopy to evaluate bacterial attachment and internalization, confocal microscopy to evaluate bacterial intracellular trafficking, and polymyxin B protection assays to determine intracellular survival. Our results showed that after 24 h of coincubation, there were bacteria attached to and internalized by epithelial cells. Forty-eight hours after co-incubation, most intracellular bacteria were found in phagosomes with late endosomal characteristics, previously described as persistence phagosomes in this cell type. Accordingly, a high number of intracellular colony-forming units were recovered at this time point. Microscopy analysis combined with confocal studies using occludin as a marker of tight junctions showed an intact epithelial monolayer without neutrophils associated with them, suggesting that bacteria likely reach epithelial cells after being released from neutrophils. Taken together, these results indicate that B. pertussis can establish intracellular infections in epithelial cells even after being phagocytosed by neutrophils and that this secondary infection is similar to that developed by free-living bacteria. Moreover, they suggest that neutrophils, one of the immune cells expected to eliminate the pathogen, may instead act as carriers, delivering viable bacteria to uninfected sites where new persistence niches could be established.

Key words: Bordetella pertussis; neutrophil; epithelium

IAI-124

Immunomodulatory and endocrine effects of Rifampicin: a study on human macrophages and adrenal cells

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Tuberculosis (TB) remains a major global health challenge. The etiologic agent, *Mycobacterium tuberculosis* (Mtb) is transmitted by air and captured by lung macrophages (Mf). Effective Mf activation along with an efficient cellular immune response (IR) are essential for Mtb elimination. However, these activated cells can also contribute to tissue damage. We early demonstrated that newly diagnosed TB patients exhibit an immune-endocrine imbalance characterized by high plasma levels of pro- and anti-inflammatory mediators and cortisol (Gc), as well as markedly reduced levels of Dehydroepiandrosterone (DHEA). During specific anti-TB treatment, proinflammatory mediators and DHEA levels reached values like those found in healthy controls. Rifampicin (R), a key component of TB treatment, is a potent antimicrobial agent. There is evidence that R can also modulate host IR, influencing lymphocyte migration, cytokine production, phagocytosis; and it is a known activator of P450 (CYP) family enzymes in the liver, with a probable effect on the adrenal glands by activating P450 enzymes, where the synthesis of both cortisol and DHEA takes place. Accordingly, we assessed the effect of R (30 and 45µg/ml) on the proinflammatory response of Mf derived from monocytes from healthy volunteers (MDMh, n=10) stimulated with irradiated Mtb (Mtbi), added or not with DHEA (10-7M) and/or cortisol (10-6M), and on adrenal human cell line (n=3). All cultures were stimulated and/or treated for 24 hrs. Culture supernatants of Mtbi-stimulated MDMh had increased levels of IL-1β and IL-6 compared to non-stimulated Mf ($p<0.05$). R treatment reduced both cytokines production in stimulated MDMh cultures ($p<0.05$ vs. those only stimulated) and in a dose-dependent way. Similar results were achieved in Mf+DHEA+GC+Mtbi cultures, with the lower amount of the pro-inflammatory mediators found in cultures of Mf+DHEA+GC+Mtbi+R45. As it was expected, cultures of activated adrenal cells produced DHEA, DHEA-S and cortisol in the supernantant; treatment with R decreased the former one levels in a dose-dependent way ($p<0.05$), without changing the other hormones quantities. These findings suggest that R not only modulates the pro-inflammatory response of macrophages, even under stress-associated hormonal conditions, but also alters adrenal steroidogenesis by selectively reducing DHEA production. This dual action of R may contribute to the immune-endocrine changes observed in TB patients under treatment.

Key words: Tuberculosis; Rifampicin; human macrophages; DHEA; Cortisol

IAI-125

Cutaneous antifungal immunity: Nannizzia gypsea induces IL-6 in HaCaT keratinocytes

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Skin infections caused by dermatophytes are among the most common human diseases, yet the mechanisms that recognize fungi and control infection remain poorly understood. Previously, we demonstrated that IL-17A mediates antifungal defenses independently of neutrophil recruitment in the skin. Here, we further investigated early host–pathogen interactions by analyzing cytokine and chemokine production in HaCaT keratinocytes (an immortalized human keratinocyte cell line) following exposure to the human pathogen Nannizzia gypsea. A suspension of N. gypsea (Ng01UNC strain), containing hyphae and conidia from 10-day cultures, was prepared in PBS. Heat-killed fungus (HKNg) was obtained by incubation at 60 °C for 1 h, while viable fungus (Ng) was maintained at room temperature. HaCaT cells (1×10^5) were incubated in DMEM 5% FCS in the absence or presence of Ng or HKNg at conidia-to-cell ratios of 0.5:1, 1:1, and 2:1. After 3, 12, and 24 h, cell viability assessed by MTT was not decreased in presence of fungus. Supernatants were analyzed for cytokines/chemokines using LEGENDplex™ (BioLegend) and flow cytometry. Statistical significance was determined by two-way ANOVA ($p < 0.05$). HaCaT cells produced elevated levels of IL-6 in response to both Ng and HKNg, compared to unstimulated controls. Viable fungus (Ng) induced ~6-fold and ~25-fold increases in IL-6 production (2:1 ratio) at 12 and 24 h, respectively ($p < 0.01$ vs. cells in medium alone), showing a clear dose- and time-dependent response. HKNg induced IL-6 at lower conidia-to-cell ratios (0.5:1), though without a dose-dependent effect. At 24 h, both Ng and HKNg stimulated IL-8 (CXCL8) and TNF but suppressed MCP-1 release. At early time points (3 h), Ng transiently induced IL-1β and IL-2, while HKNg stimulated IL-12p70 ($p < 0.01$). No IL-17A, IFN-γ, or CXCL10 production was detected. These findings indicate that keratinocytes rapidly sense the dermatophyte N. gypsea and respond primarily by producing IL-6 and inflammatory chemokines and cytokines, highlighting their key role in initiating antifungal immunity.
Key words: Fungi; Skin; innate immunity

IAI-127

Modulation of Macrophage Apoptosis by Yersinia enterocolitica YopP and IgG: crosstalk at the FcγR Interface

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Yersinia enterocolitica employs the effector protein YopP to manipulate host immune responses, particularly by inducing apoptosis in macrophages. Its interaction with Fcγ receptors has not been previously described, and it remains unclear how the adaptive immune response, especially IgG, may modulate its activity. Structural modeling (AlphaFold2, RoseTTAFold2) and comparative anal-

ysis with PopP2 (a YopJ family member secreted by the plant pathogen *Ralstonia solanacearum*) were used to identify conserved residues, predict disordered domains, and perform molecular docking with FcγRI, FcγRIIa, FcγRIIb, FcγRIII, and IgG. In vitro studies evaluated apoptosis and YopP–IgG interactions in mouse peritoneal macrophages infected with wild-type Y. *enterocolitica* (Ye wt) or a ΔyopP mutant (Ye Δ*yopP*). Binding was validated by immunofluorescence, flow cytometry, and western blot. In vivo analyses employed orogastric infection models with or without FcγR blockade, followed by DNA laddering and TUNEL assays. Structural modeling revealed homology between YopP and PopP2 (RMSD = 1.85) and a disordered N-terminal domain. Docking suggested weak interactions between YopP and FcγRs but strong binding with IgG, supported by favorable ΔG values and larger contact areas. Experimental data confirmed the conformational dependent YopP–IgG binding. Ye wt infection induced macrophage apoptosis ($p \leq 0.05$), while Ye Δ*yopP* infection decreased cell death ($p \leq 0.05$). Notably, IgG alone induced apoptosis ($p \leq 0.01$) but also attenuated YopP-mediated effects ($p \leq 0.05$), probably via FcγRIII competition or direct interaction. In vivo, wild-type infection triggered DNA fragmentation at early and late stages, while genomic DNA integrity was preserved at day 7 post-infection, partially influenced by FcγR blockade. Thus, YopP exerts a dual regulatory role during infection: it promotes apoptosis to facilitate bacterial dissemination, yet its activity is later modulated by IgG. IgG, through both receptor-dependent and direct YopP interactions, may act as an adaptive mechanism to limit Y. enterocolitica virulence.

Key words: Innate Immunity; Cell Death; Yersinia enterocolitica; Fcγ Receptors

IAI-128

Co-infection of C57BL/6 mice with enteroaggregative Escherichia coli (EAEC) and Enterohemorrhagic Escherichia coli (EHEC) increases EHEC intestinal colonization and elicits a specific immune response both locally and systemically.

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Hemolytic Uremic Syndrome (HUS) is a life-threatening condition caused by Shiga toxin-producing Enterohemorrhagic E. coli strains (EHEC). Recently, the isolation of Enteroaggregative E. coli strains (EAEC) in children with HUS was reported by the National HUS Reference Centre in Argentina. Our previous results demonstrated that the preinfection of Caco-2 cells with EAEC increases EHEC adherence, as well as, *il6*, *il1β* and *ccl20* transcription levels compared to EHEC monoinfection. We aimed to evaluate whether the preinfection of mice with an EAEC strain favors HUS outcome after EHEC infection. To do this, a model of coinfection in C57BL/6 mice at weaning was set up. Mice were intragastrically infected with 1010CFU EAEC and coinfected with 1011CFU EHEC 24 h later. We compared weight, food intake, bacterial shedding, intestinal permeability, and differential leukocyte counts, anti-EHEC and -EAEC IgG antibodies, and urea levels in plasma from EAEC:EHEC (coinfected) mice with mice infected separately with EAEC or EHEC (monoinfected) by ANOVA or Kruskal-Wallis. The presence of IgA-coated bacteria was also evaluated in feces from infected mice by flow cytometry. EAEC:EHEC infected mice showed a decreased weight from 48 h post-EHEC infection ($p<0.05$), which correlated with a decreased food intake ($p<0.05$) and a trend to increase plasmatic urea levels at 72 h compared to monoinfected mice. An increased intestinal permeability was observed from 120 h ($p<0.05$), and EAEC:EHEC infected mice shed higher CFU numbers of EHEC in feces than monoinfected animals at day 8 ($p<0.0001$). Besides, EAEC:EHEC mice showed higher plasmatic levels of anti-EHEC and -EAEC IgG antibodies than monoinfected mice by day 8 ($p<0.05$). Finally, an increase in anti-IgA coated bacteria was observed in EHEC:EAEC mice compared to EAEC monoinfected mice ($p<0.05$). These results indicate that EAEC:EHEC mice show increased damage and colonization in the intestine, which could probably facilitate Shiga toxin access to the blood, and lead to a worse outcome than that observed in monoinfected mice. Nevertheless, the coinfection was able to induce an increased systemic and specific immune response, which could contribute to mice survival. However, more studies are needed in order to confirm this hypothesis.

Key words: Co-infection; enteroaggregative Escherichia coli; Enterohemorrhagic Escherichia coli; HUS

IAI-132

Effect of antimicrobial peptides on Shiga toxin-producing bacteria and their impact on neutrophil activation.

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Hemolytic uremic syndrome (HUS) develops secondary to infection with Shiga toxin (Stx) producing Escherichia coli (STEC) strains and is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal dysfunction. In Argentina, STEC O157:H7 is the serotype most associated with HUS. There are no specific treatment or vaccine. Antibiotics are not recommended since their capacity to induce Stx production. Today, progress is being made in the study of the role of antimicrobial peptides from the skin of amphibians, particularly the mexican frog Pachymedusa dacnicolor, which have been isolated and characterized, demonstrating immunomodulatory and microbicidal functions, among others. The objective was to evaluate the effect of antimicrobial peptides derived from the skin of P. dacnicolor, to modulate the growth and effect of STEC on neutrophils. The effect of Na2 and Pept5 peptides from the epidermis, obtained and characterized by HPLC, was evaluated. Both inhibited the growth of STEC bacteria (105 CFU/ml) in vitro, measuring the optical density at 600nm every 20 min during 18h in a multimode microplate reader (AUC (hxOD 600nm): STEC= 259.0±3.9; STEC+Na2 (5 M, 2 M= 99.0±4.5*; 94.6±0.21*); STEC+ Pept5 (5 M, 2 M = 96.9±2.1*; 97.5±0.6*);* p<0.05, n=4). In parallel, Shiga toxin levels were evaluated by ELISA, observing that the afore mentioned treatments inhibited Stx production: STEC = 43.73±20.9 ng/mL; STEC + Na2 (5 M, 2 M)= ND); STEC + Pept5 (5 M, 2 M) = ND. Since neutrophils are recruited to intestinal tissue after STEC infection, the peptides capacity to activate these leukocytes after 3h was evaluated. The expression of activation marker CD11b and chemokine receptor CXCR2 were assayed on neutrophils by flow cytometry. Preliminary results are expressed as the percentage of expression: %CD11b/CXCR2: Control= 86±3/86±2; STEC= 95±1/67±5; STEC + Na2 (2 M)= 95±1/63±4; STEC+ Pept5 (2 M)= 83±15/59±4, Na2= 69±1/92±1, Pept5: 87±1/82±10 (n=2).
We conclude that these peptides show a notable antimicrobial capacity against STEC by inhibiting the production of Stx toxin. While further studies are needed to confirm their effects on neutrophils, these proteins could be a valuable tool for controlling STEC infections without affecting leukocyte activity.
Key words: STEC; Stx; frog peptides

IAI-133

Tissue-Specific Features of Follicular Cytotoxic CD8⁺ T Cells in Trypanosoma cruzi-infected mice

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During experimental acute T. *cruzi* infection, we identified splenic follicular CD8⁺T cells (CX-

CR5⁺PD1⁺), named Tfc, which peaks at 18 dpi alongside a strong plasmablast (PB) response. Tfc display phenotypic traits of both Tfh cells (ICOS, CD40L, Bcl6), the canonical B-cell helper population, and cytotoxic CD8⁺T cells (granzyme B, perforin, IFNγ). We also demonstrated that, *in vitro*, splenic Tfc cells enhance antibody secretion by naïve B cells and induce PB death via Fas–FasL, performing distinct functions depending on the type of B cell they interact with. We investigated whether the tissue microenvironment shapes the phenotype and function of Tfc cells by comparing those located in inguinal lymph nodes (iLN) with that in the spleen (S). For that, C57BL/6 mice were infected intraperitoneally with 5000 trypomastigotes of *T. cruzi* Tulahuen strain and Tfc characteristic were evaluated in both tissues. Flow cytometry (FC) showed a similar kinetic between iLN-Tfc and splenic Tfc (S-Tfc) cells, peaking at 18 dpi and then declining at the end of acute phase. Bulk RNA-seq of sorted S-Tfc and iLN-Tfc revealed a clear transcriptional segregation (PC1 explaining 77% of the variance) between two populations, with 676 genes upregulated and 612 downregulated in S-Tfc vs iLN-Tfc. Gene set enrichment analysis indicated that S-Tfc were enriched in pathways related to inflammation, glycolysis, and B-cell activation compared to iLN-Tfc. FC revealed higher ICOS expression in iLN-Tfc, while Bcl6 and CD40L levels were similar in both subsets. S-Tfc were predominantly effectors (CD44⁺CD62L⁻), whereas iLN-Tfc comprised effector and central memory (CD44⁺CD62L⁺) cells. iLN-Tfc also exhibited higher expression of T-bet, Eomes, and TCF-1 ($p<0.05$), genes associated with effector/memory programs. The frequency of parasite-specific Tfc was comparable in both tissues ($p>0.05$). iLN-Tfc showed higher phospho-mTOR MFI than S-Tfc ($p<0.05$), indicating greater mTOR pathway activation, with similar glucose uptake. We evaluated B-cell helper capacity and cytotoxic activity toward PB, performing *in vitro* co-cultures. Both subsets enhanced antibody secretion, while simultaneously inducing PB death. In summary, Tfc exhibit tissue-specific transcriptional and phenotypic profiles while maintaining comparable B-cell helper activity and cytotoxicity toward PB. However, how these differences influence their survival, cytokine production, and regulatory functions across tissues remains to be determined.

Key words: Tissue microenviroment; CD8+ Tfc cells; B cells.

IAI-138

Purinergic signalling is involved in sexual dimorphism and the regulatory profile of in vitro activated CD4 T cells

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Background: Several evidence show that male patients exhibited increased severity of Chagas cardiomyopathy (CC), though the reasons for this gender disparity remain unclear. Purinergic signaling shapes the outcome of immune activation through the balance of pro-inflammatory ATP and anti-inflammatory adenosine (ADO). Potential sex-related differences in purinergic components remain largely unexplored. Objectives: This study aimed to evaluate the impact of sex on purinergic system components and the regulatory phenotype of CD4⁺ T cells. Methods: CD4⁺ T cells were isolated from spleens of male and female wild-type (WT) and CD73 knockout mice using a negative selection kit. Cells were activated with anti-CD3/CD28, activation markers and purinergic components were assessed by spectral flow cytometry at 0, 24, 48, and 72 h. In addition, to assess whether ADO promotes differentiation toward a regulatory phenotype, it was added at the onset of activation and evaluated 72 hours later. Results: At baseline, CD4 T cells from female mice showed a higher expression of ATP receptor P2X7 compared to cells from male mice ($p<0.01$). This difference was also observed at 24 h ($p<0.05$). However, the tendency inverted at 72 h ($p<0.001$). CD73 in cells from WT female and male mice showed similar dynamics ($p<0.05$ for all times points). The addition of ADO resulted in a significant higher expression of FoxP3 and IL-10 in cells from male mice compared to females at 72 h ($p<0.01$). Conclusions: Higher baseline P2X7 expression in cells from female mice may enhance ATP sensing, CD73 upregulation, and an an-

ti-inflammatory environment. However, after activation, females showed a more inflammatory profile, with reduced CD73 levels. ADO addition promoted a more regulatory phenotype in cells from male mice. These findings suggest a stronger early immune response in females, potentially affecting long-term outcomes such as CC. Further studies are needed to better understand these mechanisms.

Key words: Adenosine; CD73; IL-10

IAI-144

Shiga toxin reduces repair functions on mature endothelial cells

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Hemolytic Uremic Syndrome, the main cause of pediatric acute renal failure, is caused by E. coli producing Shiga toxins (Stx) and characterized by massive endothelial damage, which is worsened by inflammation, or bacterial lipopolysaccharides (LPS). After endothelial injury, the vascular repair process involves restoration of a functional endothelial monolayer. Endothelial regeneration may involve nearby resident mature EC themselves endogenous) and/or cells other than the resident EC population, such as the circulating stem/progenitor cells (EPC) (exogenous). We have previously demonstrated that Stx decreased repair functions on EPC. Although Stx toxic effects on mature EC have been widely studied, its action on angiogenesis and self-repair abilities remain to be elucidated. We here aim to analyze and compare the effect of Stx alone or in combination with LPS on self-repair abilities on EC.

Human umbilical vein endothelial cells (HUVEC) were treated with LPS (0.3 ug/ml) and/or with Stx (10 ng/ml) for 24 h. Dead detached cells were discarded and the remained adhered cells were use in indifferent repair assays. Typically, when vascular EC are damaged, matrix proteins are exposed. The ability of EC to adhere to these proteins reflects their capacity to initiate reparative processes. Considering this, we performed a matrix protein binding assay. We observed that HUVEC treated with Stx and to a greater extent Stx+LPS had reduced ability to adhere to both collagen and fibronectin compared to untreated HUVEC ($p \leq 0.05$ n=3).Then we performed a functional healing assay. When creating a scar with a tip on a monolayer of treated HUVEC, the ability of HUVEC to migrate and close the scar was reduced when treated with Stx and more markedly when treated with LPS+Stx compared to untreated HUVEC ($p \leq 0.05$ n=3). Finally, we observed that Stx toxin reduced the microtubule-forming capacity of treated HUVEC compared to untreated cells The combination of Stx+LPS increased it back to control levels. This could be explained by the fact that LPS was a strong tubule inducer itself ($p \leq 0.05$ n=5).

Results obtained here show that Stx toxin and to a greater extent combined with LPS significantly affect the intrinsic repair functions of mature EC. We propose that Stx could have implications in the development of the disease by affecting the reparative abilities of EC, affecting the possibility of reducing the endothelial damage that occurs in Hemolytic Uremic Syndrome.

Key words: Shiga toxin; Mature endothelial cells; Endothelial self-repair function

IAI-158

Impact of Trypanosoma cruzi Infection on Gut-Associated B Cell Responses

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The acute phase of T. *cruzi* infection is characterized by splenomegaly, lymph node (LN) enlargement, early extrafollicular B cell response with delayed germinal center reaction and non- and specific-antibodies in the serum. However, an integral analysis of the B cell response has not been conducted in this infection. Here, we investigated the B cell response in the gut in T.*cruzi* infection, given that intestine contains the highest number of B cells. For that, 8–12-week-old C57BL/6 mice were ip injected with 2,500 trypomastigotes of T.*cruzi* (Tulahuén), or with PBS as controls. Peyer's patches (PP), mesenteric LN (MLN), spleen, inguinal LN (iLN) and liver were collected at different days post infection (dpi). Macroscopic evaluation revealed a reduction in the size of PP and MLN at the peak of parasitemia (18 dpi). The decrease in PP size was transient, as they returned to normal by 82 dpi. Immunofluorescence analysis showed a marked reduction in follicular dendritic cells as well as B cells, CD4+ and CD8+T cells in PP at 18dpi. MLN presented a follicular disorganization and reduction in lymphocytes but with an increase in the frequency of antibody-secreting cells (ASC) ($p<0,05$). PP cellularity decrease was not due to apoptosis, as TUNEL assay was negative; instead, apoptotic signals appeared in follicles and medullary zone of MLN from infected mice. To evaluate if cellular diminution in PP is consequence of cellular migration, infected mice were intraperitoneally injected with FTY720 (1mg/kg), which inhibits lymphocyte egress, or saline solution. An increase in IgA+ASC was observed in PP and a decrease in MLN in FTY720-treated infected mice, while an increase in IgA+ASC was noted in MLN of mice treated with saline solution ($p<0.05$). Interestingly, qPCR analysis revealed that PP harbored a parasite load approximately 100-fold lower than that detected in the spleen and iLN. By ELISpot, we determined that ASC from PP were not specific for T.*cruzi* antigens. Supporting the notion that IgA+ASC mobilized beyond the gut, flow cytometry analysis of the liver showed an increased frequency of IgA+CD138+CD98+ASC in infected mice at 18dpi compared with controls ($p<0,05$). The absence of apoptosis in PP, together with the redistribution of IgA+ASC observed upon FTY720 treatment, suggests that these cells abandon the gut to other tissues. Our findings indicate that T.*cruzi* infection impacts the gut immune response, potentially influencing both local and systemic immunity.

Key words: B cell; IgA+ ASC; Peyer´s patches

IAI-161

IL-10 Overexpression Reduces Susceptibility to STEC Infection in a Mouse Model of Hemolytic Uremic Syndrome

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Shiga toxin (Stx)-producing Escherichia coli infections can lead to Hemolytic Uremic Syndrome (HUS), characterized by anemia, thrombocytopenia, and renal failure. Previously, we demonstrated that IL-10-deficient (IL-10-/-) mice are protected against Stx2, suggesting a detrimental role for IL-10 in HUS evolution. Based on this, we aimed to explore the role of IL-10 in HUS using an infectious model. BALB/c mice were inoculated with either an IL-10-producing plasmid (PL) or saline (control). After 48 hours, they received an oral inoculation of STEC (10^9 CFU/mouse) or saline (control, PL, STEC, and STEC+PL groups). At 96 h post-inoculation, the mice were given intragastrical FITC-dextran and bled 3 h later. FITC-dextran levels in plasma, revealed increased gut permeability in the STEC group. This effect was prevented when IL-10 was present [Median (IQR) = Control: 330(250-380); PL: 240(220-321) STEC: 786(668-857); STEC+PL: 443(356-503)*; n=5; *$p<0.05$]. At 120 h post-infection. Plasma urea levels (mg/dL) indicated more severe kidney injury in STEC mice, also prevented by IL-10 (mg/dL) [Median (IQR)= Control: 71.22 (64.88-77.56); PL: 88.29 (69.27-94.15) STEC: 152.20 (129.30-302.90)*; STEC+PL: 114.60(59.02-163.90); n=5; *$p<0.05$]. Circulating Neutrophil percentages, assessed, were higher in STEC mice but reduced in STEC+PL mice [Median(IQR) = Control: 11(10-15); PL: 12(10-15) STEC: 40(38-51)*; STEC+PL: 22(15-31); n=5; *$p<0.05$. Antibody titers against bacteria were higher in STEC+PL mice at 120 h after STEC infection, suggesting an enhanced humoral response [Median (IQR) = Basal: ND; PL: ND STEC: 0.143(0.069-133.30); STEC+PL: 0.238(0.224-0.506)*; n=5; *$p<0.05$]. Given our previous results and the capacity of IL-10 to reduce immune response, we were expecting the opposite outcome. How-

ever, our findings suggest that IL-10 overexpression reduces susceptibility to STEC infection. This discrepancy may be due to the influence of IL-10 in preserving gut barrier integrity and enhancing humoral response. These findings indicate that IL-10 might have different effects in modulating immune response during the development of HUS, being beneficial during the infectious phase but detrimental when Stx2 exerts its effects.

Key words: HUS; STEC; IL-10; Inflammation

IAI-179

Improved Cardiac Protection Using Low-Dose Benznidazole Combined with CD73 Inhibition in Experimental Chagas Disease

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Background: Chagas disease (CD) follows a heterogeneous course, with chagasic cardiomyopathy representing the leading cause of morbidity and mortality. Benznidazole (BZ) is the first-line drug for CD therapy, but exhibits limited bioavailability, high cumulative doses, long treatment, and adverse effects. We evaluated the efficacy of low-dose BZ delivered free or uploaded to a modified drug-delivery system (BZ-MDDS) in combination with α,β-methylene ADP (APCP), an inhibitor of the immunoregulatory ectoenzyme CD73, for murine acute Trypanosoma cruzi infection treatment. Methods: BALB/c mice were infected ip with 1000 trypomastigotes (Tulahuen strain). BZ or BZ-MDDS (50 mg/kg every 48h, days 15–55) was orally administered in combination with APCP (20 mg/kg/day every day ip, days 15–18) or with a saline solution (SS). Cardiac function was assessed by ECG and ECHO at 105 days post-infection (dpi). At this time point, a blood sample was obtained to evaluate the shift to a T. cruzi–specific CD8 T-cell population with central memory phenotype using the TSKD14/kd tetramer, a parameter proposed to measure treatment efficacy. At 120 dpi, blood was collected to measure parasitemia and serum biomarkers of tissular damage after immunosuppression. Results: BZ-based regimens significantly improved survival ($p=0.01$) in comparison to infected non-treated mice. After immunosuppression, parasitemia was detectable in all treated groups, but was lower in BZ-MDDS than in BZ+APCP ($p<0.05$). There were no significant differences in the relative weights of hearts, spleens, adipose tissues, and livers or in serum tissue damage markers among treatments. BZ+APCP was the most effective treatment for preserving cardiac function. The percentage of CD8+ T. cruzi-specific T cells was 0.44±0.66% with very low cell counts, not allowing further analysis. Given this result, the population was further comparatively analyzed in the spleens of BALB/c (3.2±0.8%) and C57BL/6 (7.8±2.7%) mice at 15 dpi.

Conclusion: Together, these results suggest that integrating pharmacokinetic optimization (MDDS) with purinergic modulation (CD73 blockade) supports the use of intermittent low-dose BZ regimens as a potential therapeutic strategy for CD. Furthermore, the data suggest that the transition from an effector to a central memory phenotype in T. cruzi-specific CD8 T-cell population, which serves as a useful marker of treatment efficacy in C57BL/6 mice, may not be reliable in BALB/c mice due to low sensitivity.

Key words: Chagas disease; benznidazole; modified drug-delivery system; APCP; CD73

IAI-203

KUNITZ TYPE PROTEIN FROM FASCIOLA HEPATICA AS A NOVEL COAGULATION INHIBITOR

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Kunitz-type (KT) protease inhibitors are low molecular weight proteins with serine and cysteine protease inhibitory activity and are involved in physiological processes including blood coagulation, fibrinolysis, and inflammation. However, there is no information regarding the role of Fasciola hepatica Kunitz inhibitors in blood coagulation, a process potentially fundamental for parasite feeding.

Among the Kunitz family members in F. hepatica, FhK4 exhibits serine protease inhibitory activity. Since coagulation factors are serine proteases, FhK4 inhibitor could affect coagulation. This study aimed to evaluate the effect of FhKT4, produced as a recombinant fusion protein, on blood coagulation.

Preliminary assays tested whether FhK4 could modify activated partial thromboplastin time (APTT). A pool of normal human plasmas was supplemented with FhK4. The addition of FhK4 prolonged coagulation times in the APTT assay: 67 ± 2.0 seconds for FhK4 versus 31 ± 1.0 seconds for the control (buffer) ($p < 0.00027$, Student's t-test). Although the Kruskal–Wallis test showed no significant difference ($p = 0.21$), the Mann–Whitney U test comparing control and FhKT4 yielded a large effect size ($r = 0.77$), indicating a substantial difference. The fusion protein control (without FhK4) showed no effect on APTT time. These results suggest a possible interaction of FhKT4 with intrinsic pathway factors (VIII, IX, and XI). To evaluate this, plasmas deficient in Factors VIII, IX, and XI and APTT reagent were used. The levels of intrinsic coagulation pathway factors were measured. The results showed significantly decreased concentrations of Factors VIII, IX, and XI in the presence of FhKT4 compared to the control ($p < 0.0008$, $p < 0.001$, and $p < 0.02$, respectively, Student's t-test). Dilution curves for Factors FVIII, FIX, and FXI showed a progressive increase in activity with FhKT4 dilution, validating the methodology.

Our preliminary findings suggest that FhKT4 may modulate the intrinsic coagulation pathway by targeting serine proteases involved. Further studies with larger sample sizes are needed to clarify the biological relevance and potential implications for parasite-host interactions and posible therapeutic development.

Key words: Kunitz-type (KT) protease inhibitor; FhKT4; Cogulation inhibition; Intrinsic coagulation pathway.

IAI-205

Evaluation of the effect of iota-carrageenan on Andes virus infection in human respiratory epithelial cell lines.

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Hantavirus Pulmonary Syndrome (HPS) is a zoonotic disease caused by some viruses of the Orthohantavirus genus, characterized by a high case fatality rate of ≈ 40%. Andes virus (ANDV) is one of the viruses responsible of HPS in Argentina, and infections occur through inhalation of contaminated aerosols by infected rodents, although human-to-human transmission has been documented in our country. Iota-carrageenan is a sulfated polysaccharide that has demonstrated antiviral activity against several respiratory viruses by the interaction between viral particles and cell surfaces, thereby preventing the viruses from entering cells. Our objective was to evaluate the antiviral potential of iota-carrageenan against ANDV in vitro. Cell viability was evaluated with MTS/PMS assays (Promega) and by flow cytometry with BD Horizon™ Fixable Viability Stain 660. Cells were seeded in 96-well plates, incubated overnight (37 °C, 5% CO_2), and then treated for 48 h with iota-carrageenan or vehicle. A549 and Calu-3 cell lines were treated or not with 600, 60 and 6 µg/ml iota-carrageenan or vehicle. These treatments were performed before, during, simultaneously with, and after infection. The cell lines were infected for 1 hour at a MOI of 1 with ANDV/Arg and ARG-Epuyén strains, using viral stocks previously titrated in Vero E6 cells. After 4 days of infection, RNA was extracted from cells using the QIAamp® Viral RNA kit, and viral load was determined by real-time RT-PCR. We evaluated cell markers such as β-integrin (CD51/CD61), HLA-DR, and surfactant proteins, as well as cytokine production, after these treatments by flow cytometry. Cell markers such as β-integrin (CD51/CD61), HLA-DR, and surfactant protein (SP-D), as well as cytokine production, were evaluated by flow cytometry. As a result, we observed no differences in cell viability was observed in iota-carrageenan-treated cells compared to vehicle treated control cells. We observed that concentrations of 600 and 60 µg/ml reduced viral replication in both cell lines, in 2 of 4 treatments evaluated under continuous and simultaneous infection conditions ($p<0.05$). Flow cytometry analysis showed that viability was high in all samples (>99.9%), confirming that the treatments were not cytotoxic and that the exposure to carrageenan induces differential expression for both B-integrin and SP-D. Our preliminary results provide new insights into immunopathogenesis and antiviral response in ANDV.
Key words: Hantavirus; Andes virus; Iota-carrageenan; Respiratory Epithelial cell lines.

IAI-214

Impact of prior SARS-CoV-2 infection on the Mtb-specific adaptive immune response.

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SARS-CoV-2 pandemic has posed new challenges for tuberculosis (TB), including potential effects on diagnosis, treatment, and clinical outcomes. Coinfection is associated with more severe TB, higher mortality, and pulmonary sequelae, highlighting the need to understand how COVID-19 impacts immunity against Mycobacterium tuberculosis (Mtb). In this context, the concept of trained immunity—functional and epigenetic changes in innate cells following a primary stimulus—is relevant for exploring new immunological strategies for post-COVID-19 anti-TB treatment. Our previous results showed that in vitro prior SARS-CoV-2 abortive macrophage infection abrogated Mtb-induced $CD4^+$ T-cell proliferation, CD69 and CD137 upregulation, as well as the activation of Th1 and Th17 subsets and IFN-γ production in culture supernatants.

Here, we studied the effect of SARS-CoV-2 infection on trained immunity and its impact on anti-TB responses. We established a SARS-CoV-2-trained immunity model using macrophages differentiated from peripheral blood monocytes of healthy donor buffy coats. Monocytes were differentiated with GM-CSF for 3–5 days, "trained" with SARS-CoV-2, incubated for 6 days, then infected with Mtb (H37Rv strain) and co-cultured with autologous T lymphocytes.

We characterized $CD4^+$ memory/effector T-cell populations, cytokine functionality (TNF-α,

IFN-γ), and regulatory T cells (Tregs, $CD127^{-}CD25^{+}$). T cells cultured with SARS-CoV-2-trained macrophages infected with Mtb showed lower frequencies of IFN-γ^{+} ($p<0.005$), TNF-α^{+} ($p<0.005$), IFN-γ^{+}TNF-α^{+} ($p<0.05$), and total Mtb-specific T cells (IFN-γ^{+} and/or TNF-α^{+}, $p<0.01$), along with increased $CD4^{+}$PD-1^{+} T cells ($p<0.05$), compared with lymphocytes activated by untrained macrophages. Although overall Treg frequency did not differ, we found a reduced proportion of IFN-γ^{+} and TNF-α^{+} Tregs ($p<0.05$), suggesting impaired protective roles.

Our findings indicate that prior SARS-CoV-2 infection significantly impacts Mtb-specific adaptive immunity. The reduction in T-cell functionality in the presence of SARS-CoV-2-trained macrophages could hinder responses against Mtb. These results underscore the need to consider SARS-CoV-2 history in TB treatment of coinfected patients. A deeper understanding of these mechanisms will be crucial for developing post-COVID-19 therapeutic strategies against tuberculosis.

Key words: Tuberculosis; SARS-CoV-2; Trained immunity; Infectious diseases

IAI-216

IMPACT OF STRESS AND HYPERGLYCEMIA ON CYTOKINE AND GALECTIN PRODUCTION IN PBMC FROM PATIENTS WITH PULMONARY TUBERCULOSIS WITH OR WITHOUT TYPE 2 DIABETES FOLLOWING EXPOSURE TO IRRADIATED MYCOBACTERIUM TUBERCULOSIS

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, remains a global health challenge. Pulmonary tuberculosis (PTB) is the most common clinical form. Type 2 diabetes mellitus (D2) is a significant risk factor for active PTB. The cellular immune response (IR) is central to infection containment, although its dysregulation contributes to parenchymal damage. Elevated plasma cortisol exerts anti-inflammatory effects and suppresses the IR, thereby limiting tissue injury. Other immunomodulators include galectins (Gal), soluble proteins with proinflammatory (Gal-3), immunosuppressive, or macrophage microbicidal properties (Gal-9). Previous studies from our group showed that PTB patients display an immune–endocrine–metabolic (IEM) imbalance, characterized by elevated levels of pro- and anti-inflammatory cytokines, cortisol, and circulating galectins. To further explore these mechanisms, we evaluated the response of PBMCs from PTB patients with or without D2 and control group-HCo, cultured under stress (0.1 μM cortisol) and glycemic conditions (high glucose-HG, 20mM; normal glucose-NG, 5mM), and challenged with irradiated Mtb (Mtbi) for 24h. We assessed the IR by measuring several T helper cytokine profiles (Th1: IL-2 and IFN-γ; Th2: IL-4; Th17: IL-17), other cytokines (IL-1β, TNF-α, IL-6, IL-10), and galectins (Gal-3, Gal-9) in supernatants (SN). Analysis of cytokine production in SN showed that all cytokines except IL-4 and IL-17, which displayed undetermined levels—were increased in Mtbi-stimulated (Mtbi-S) cultures from all groups ($p<0.04$ vs basal cultures-BS), while stress conditions (SC) partially reduced cytokine production ($p<0.03$ vs Mtbi-S). Mtbi-induced IL-2 and IFN-γ production was significantly higher in the TBD2 group ($p<0.04$ vs all groups). No significant differences were observed between NG and HG conditions for any mediator. Gal-3 production was enhanced by Mtbi stimulation in all groups except HCo under both NG and HG conditions ($p<0.05$ vs BS). A similar pattern was observed for Gal-9 ($p<0.02$ vs BS). Stress conditions, unlike their effect on cytokine responses, did not suppress Mtb-induced galectin production. No differences between NG and HG conditions were observed for any mediator. Taken together, our findings suggest that cytokine and galectin and secretion are differentially modulated by Mtbi challenge

and SC, depending on the origin of the PBMCs. However, additional studies are required to further clarify these mechanisms*Key words*: Tuberculosis; Galectins; Immune response; Macrophages

Clinical Immunology

IC-054

IMPACT OF JAK INHIBITORS ON T CELL IMMUNE RESPONSE: PROOF OF CONCEPT IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Janus kinases (JAKs) are tyrosine kinases downstream of multiple cytokine receptors, essential for immune cell development and function. JAK inhibitors (JAKis) are widely used to treat immune-mediated diseases such as rheumatoid arthritis (RA). Despite efficacy, JAKis increase herpes zoster risk, suggesting impaired antiviral immunity. We previously reported that RA patients on JAKi is accumulate senescent T cells and that tofacitinib hampers T cell function. As naïve (N) and memory (Mem) CD8 T cell activation requires metabolic reprogramming, we investigated whether JAKis disrupt this process. Sorted N and Mem CD8 T cells from healthy donors (HD) were policlonally activated 3 days ± JAKis. Activation, glucose uptake (2-NBDG), glycolysis (glucose/lactate), mitochondrial function and ultrastructure (MitoTracker, TEM), lipid content (BODIPY), and metabolic flexibility (SCENITH) were analyzed. Transcriptomic profiling was performed on Mem CD8 T cells. Ex vivo assays used PBMCs from HD and RA patients treated with JAKis or methotrexate (MTX) to assess CD8 T cell activation and metabolic parameters. JAKis significantly impaired activation of both N and Mem CD8 T cells, reducing CD69/CD25 expression, GLUT1 levels, 2-NBDG uptake, and lactate production compared to activated controls. FlowSOM analysis identified a highly activated cluster ($CD69^+$C-$D25^+IRF4^+GLUT1^+HIF\text{-}1\alpha^+$) that was absent in JAKi-treated cultures. Mitochondrial dysfunction was evident, with depolarization and mitochondrial damage. BODIPY493/503 staining showed neutral lipid accumulation, suggesting a metabolic shift toward fatty acid oxidation. Transcriptomics showed downregulation of glycolysis and amino acid metabolism, decreased mTOR/MYC targets, and enrichment of p53/starvation pathways. SCENITH confirmed reduced protein synthesis and glycolytic dependence, with increased mitochondrial capacity. Ex vivo, RA patients on JAKis significantly displayed fewer activated CD8 T cells, lower mTOR/GLUT1, reduced protein synthesis, and a metabolic profile consistent with oxidative reliance, unlike HD or MTX-treated patients.

Altogether, JAKis disrupt the metabolic reprogramming required for CD8 T cell activation, impairing glycolysis, protein synthesis, and effector functions, while promoting mitochondrial reliance, lipid accumulation, and p53-driven senescence. These alterations may underlie viral susceptibility in patients and highlight potential targets to preserve immune competence under JAK inhibition.

Key words: JAKs inhibitors; CD8 T cells; immunometabolism

IC-065

Non-infectious anterior uveitis: When to suspect spondyloarthritis? Review of 128 cases.

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INTRODUCTION: Anterior uveitis is the most common type of uveitis involving the iris and ciliary body. Although it can be infectious or idiopathic, the main rheumatological causes are associated with spondyloarthritis. Rheumatologists and ophthalmologists often lack in-depth knowledge of the associated diseases, which can delay proper investigation and early referral.
OBJECTIVE: To analyze the factors associated with spondyloarthritis in patients who consulted for non-infectious anterior uveitis.
MATERIALS AND METHODS: The medical records of patients with anterior uveitis who consulted at the Juan A. Fernández Hospital between January 2008 and March 2021 were reviewed. Infectious cases were excluded. Patients were divided into two groups: anterior uveitis secondary to spondyloarthritis, and anterior uveitis of idiopathic cause and other autoimmune diseases. A multiple explanatory logistic regression analysis was performed to estimate the association of the different variables studied with anterior uveitis secondary to spondyloarthritis.
RESULTS: A total of 128 patients with non-infectious anterior uveitis were included, 80 (62.5%) of whom were female. The mean age at diagnosis was 36.2 years (SD 19.24). In the logistic regression model, we observed that the presence of HLA B27 (OR 15.2; 95% CI 2.6-90.3; p 0.003) and extraocular involvement at diagnosis (OR 104.3; 95% CI 16.5-657.5; p <0.001)were strongly associated with spondyloarthritis, adjusted for sex, age at diagnosis under 40 years, severe visual acuity loss, acute uveitis, bilateral uveitis, and recurrent uveitis.
CONCLUSIONS: Extraocular involvement and/or the presence of HLA B27 are strongly associated with spondyloarthritis. Screening for both phenomena in individuals consulting for anterior uveitis could allow an early diagnosis.
Key words: non-infectious anterior uveitis; spondyloarthritis; autoinmunity

IC-088

IL-33/ST2 Axis in the Immunopathology of Chronic Liver Allograft Rejection: a potential Pathogenic Pathway and Therapeutic Target

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Introduction: Chronic rejection (CR) is the main cause of graft dysfunction after liver transplantation, characterized by persistent immune activation leading to fibrosis, loss of function, requiring re-transplantation. Although the IL-33/ST2 axis is implicated in hepatic fibrosis, its role in human liver transplantation remains undefined. This study investigated the contribution of IL-33/ST2 to post-transplant fibrosis in CR. Materials and Methods: Liver biopsies and explanted tissue from transplanted patients were analyzed by immunohistochemistry (IHC) [Non-Rejectors (NR), n = 8; Chronic Rejection (CR), n = 10] and by gene expression profiling [NR, n = 12; CR, n = 6; Controls, n =

9]. Control samples were obtained from histologically normal donor liver tissue. Immune cell populations were quantified by IHC, fibrosis was assessed by Masson's trichrome staining and collagen deposition by Sirius Red staining. Gene expression was measured by qRT-PCR. The frequency and distribution of ST2⁺ cells were evaluated by immunofluorescence (IF). Institutional Review Board of HUFF (DDI 2419). Results: Patients with CR exhibited significantly higher frequencies of intrahepatic CD3⁺ and CD4⁺ lymphocytes localized to the periportal regions, with an increased density of CD20⁺ cells, when compared with patients with NR ($p<0.05$). The expansion of these immune cells showed a strong correlation with advanced fibrosis, increased collagen deposition, and biochemical evidence of impaired liver function, elevated AST, ALT, and ALP levels ($p<0.05$). Hepatic expression of IL-33 and its receptor ST2 was markedly elevated in CR compared with controls. ST2 expression demonstrated a positive correlation with alterations in liver function markers (AST, ALT, ALP, and total bilirubin; $p<0.05$), suggesting its upregulation in response to hepatocellular and cholestatic injury. At the molecular level, fibrogenic genes α-SMA and TGF-β were significantly upregulated in the CR group compared with both SR and control cohorts. Expression of α-SMA positively correlated with METAVIR fibrosis stage ($p < 0.05$). Furthermore, immunofluorescence analysis confirmed increased ST2 protein expression in CR liver tissue relative to NR samples ($p<0.05$). Conclusions: The IL-33/ST2 axis is modulated during chronic rejection after liver transplantation. Moreover, inhibition of this pathway could represent a promising therapeutic strategy to mitigate fibrosis progression in transplanted patients with CR.

Key words: Liver; IL-33/ST2; Chronic rejection

IC-131

Sepsis-Induced Immunosuppression: HLA-DR Expression as a Predictor of Subsequent Septic Events and Mortality

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Background: Sepsis is characterized by a profound immune dysregulation, transitioning from a pro-inflammatory response to anti-inflammatory state, resulting in sepsis-induced immunosuppression, leading to reinfections and death. Decreased HLA-DR expression on monocytes appears as a surrogate marker of this suppressive state that correlates with impaired cytokine release in response to PAMPs. This study aims to investigate HLA-DR expression as a predictor of subsequent septic events and mortality, and its relationship with TNF-α levels after LPS stimulation.

Methods: We conducted a multicentric prospective cohort study, including adult patients who met Sepsis-3 criteria requiring assessment at the Intensive Care Unit at Hospital Italiano de Buenos Aires and Hospital Austral. On day 7 after sepsis diagnosis,a blood sample was collected to determine HLA-DR levels on monocytes by flow cytometry and to perform an LPS stimulation assay followed by TNF-α measurement. Results: We analyzed 17 patients with a median age of 69 years (IQR 64-78), predominantly male (71%). Among these, 6 patients experienced reinfections, and 3 patients died following their first septic event.

Patients who experienced reinfections had a significantly lower median percentage of HLA-DR expression compared to those without reinfections (28% vs 58%, $p = 0.002$), lower median number of HLA-DR molecules per cell (9152 vs 18768, $p = 0.01$) and a reduced median fluorescence intensity (MFI) (57920 vs 115935, $p = 0.01$). Similarly, patients who died had a significantly lower median percentage of HLA-DR expression compared to survivors (9% vs 50%, $p = 0.002$), along with a lower median number of molecules per cell (5086 vs 18544, $p = 0.04$) and MFI (32846 vs 115268, $p = 0.03$). Regarding the LPS stimulation test, the median TNF-α concentration was significantly lower in patients who experienced reinfections compared to those without reinfections (50.5 vs 216.1, $p = 0.02$) and those who died compared to survivors (28.3 vs 237.4, $p = 0.01$)

Conclusions: Our preliminary analysis revealed that reduced HLA-DR expression on monocytes and LPS-induced TNF-α production were significantly associated with an increased risk of reinfection and mortality. The next step will be to determine the optimal cut-off value for HLA-DR expression that best predicts reinfection risk, and to further characterize immunosuppression by analyzing plasma cytokine levels and checkpoint molecule expression between the two groups.

Key words: sepsis; severe infections; monocytes; TNF-alfa

IC-173

Early and Sustained Thymic Dysfunction Following Pediatric Liver Transplantation

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The thymus is central to immune regulation, but its contribution to tolerance and the impact of immunosuppression after pediatric liver transplantation are not well defined. This study evaluated the effects of immunosuppression on thymic morphology and function in pediatric recipients. We conducted a prospective study of patients (0–18 years, n=12) undergoing first-time liver transplantation at the Sanatorio de Niños de Rosario (2015–2019). Thymic function was assessed by qPCR quantification of sjTREC and β1TREC, calculating the sj/β1-TREC ratio and the estimated number of intrathymic divisions ($\log_2$[sj/β1]). Recent thymic emigrants (RTE; $CD3^+CD4^+CD45RA^+CD31^+$) were analyzed by flow cytometry. Thymic morphology was measured by ultrasonography at baseline and 3, 6, and 12 months post-transplantation. Induction immunosuppression included methylprednisolone, tacrolimus, and basiliximab. A healthy pediatric cohort served as control. sjTREC levels declined significantly at 1, 3, and 12 months post-transplant ($p<0.05$). Both the sj/β1-TREC ratio and the number of intrathymic divisions decreased over time, indicating impaired thymopoiesis. RTE frequencies also declined, especially at 6 and 12 months, though not reaching statistical significance. Age at transplantation negatively asociated with sjTREC and RTE, while sjTREC, sj/β1 ratio, intrathymic divisions, and RTE showed strong positive correlations. Ultrasonography revealed reduced thymic size, consistent with acute involution. Induction immunosuppression in pediatric liver transplantation is associated with early and sustained thymic impairment, evidenced by reduced sjTREC, decreased sj/β1-TREC ratio and intrathymic divisions, diminished RTE, and smaller thymic size. These findings highlight the need to re-evaluate immunosuppressive protocols to preserve thymic output, maintain immune competence, and potentially enhance long-term graft tolerance.

Key words: thymus; liver transplantation; TREC

IC-199

Prevalence of HLA-B27 and HLA-B51 in individuals with clinical features of autoimmune/autoinflammatory disorders in Argentina

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Background: Certain human leukocyte antigen (HLA) alleles are important in the diagnosis of autoimmune/autoinflammatory disorders. The expression of HLA-B27 has been associated with spondyloarthritis and arthritis, while HLA-B51 has been linked to Behçet syndrome. However, overlapping clinical features between these conditions and population-specific variations in

HLA-disease associations should also be taken into account. This study aimed to describe and analyze HLA-B27 and HLA-B51 allele frequencies in individuals with autoimmune/autoinflammatory disorders and general population. Materials and Methods: In this retrospective cross-sectional study, all individuals with HLA-disease association testing in our center between 2022-2025, were included. A group of healthy unrelated transplant donors was included as control. HLA-B typing was performed by Luminex-based technology SSO assay, and results were analyzed with Fusion Software. Statistical analysis, including *chi-square test*, was performed on GraphPad. Results: The study group included 306 individuals [age:46(3-79)years, gender: 54%F,nationality:70.9%argentinian] with clinical features of spondyloarthritis, arthritis or Behçet syndrome. The control group included 298 individuals [age:38(5-75)years, gender: 54%F, nationality: 72.5% argentinian]. HLA-B27 frequency was significantly higher in the study group compared to control (7.13%vs0.84%; $p<0.0001$), whereas HLA-B51 frequency showed no significant differences (7.30%vs9.56%; $p=0.1576$). Similar results were observed when we considered HLA-B27 allele frequency only in individuals with clinical features of spondyloarthritis or arthritis (8.23%vs0.84%; $p<0.0001$) and HLA-B51 in individuals with clinical features of Behçet syndrome (8.75%vs9.56%, $p=0.7540$). Conclusions: individuals with clinical features of spondyloarthritis or arthritis showed a significantly increased frequency of HLA-B27, consistent with previous reports. However, no significant difference in HLA-B51 frequency was observed in individuals with suspected Behçet syndrome, in contrast with existing literature. This discrepancy may be due to other genetic and environmental factors, especially in the context of Behçet syndrome's non-specific clinical presentation. These results highlight the importance of analyzing HLA allele frequencies in diverse populations, as phenotypic associations may vary globally. Further studies involving larger cohorts are needed to support and validate these findings.

Key words: HLA-B 27; HLA-B 51; spondyloarthritis; Behçet syndrome

IC-204

HLA-DQ genotyping distribution and duodenal biopsy among individuals with clinical features of celiac disease in Argentina.

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Background: Celiac disease (CD) is an autoimmune disorder triggered by the ingestion of gluten in genetically susceptible individuals. It is strongly associated with human leukocyte antigen (HLA) alleles HLA-DQ2, HLA-DQ8, and more recently, with DQ2/DQ7 haplotype. Diagnosis can be challenging due to a highly variable clinical presentation. Although duodenal biopsy remains the gold standard for diagnosis, less invasive biomarkers are needed. This study aims to describe the HLA genotyping and duodenal biopsy findings in individuals with clinical features of CD. Material and Methods: we conducted a retrospective cross-sectional study involving a cohort of individuals with HLA genotyping testing for CD in our center, between 2022-2025. Clinical features were recovered from data bases. HLA-DQ typing was performed using Luminex-based technology SSO assay and results were analyzed with Fusion Software. Statistical analysis and *chi-square test* were performed on GraphPad. Results: A total of 74 individuals [age:34(3-84)years, gender:72.9%F,nationality:81.3% argentinian] were included in the study. All participants presented symptoms compatible with CD, had other autoimmune diseases, or family history of CD. HLA genotyping revealed the following haplotypes associated with CD susceptibility alleles and without CD susceptibility (DQx): DQ2/DQ2 (n=4;5.4%), DQ8/DQ8(n=1;1.4%), DQ2/DQ7(n=10;13.5%), DQ2/DQ8(n=5;6.8%), DQ2/DQx(n=10;13.5%), DQ8/DQx(n=19;25.7%) and DQx/DQx (n= 25; 33.8%). Overall, 66.2% (n = 49) carried at least one allele associated with CD susceptibility. Among the 74 participants, 34 underwent duodenal biopsy. Based on histological findings, in-

dividuals were categorized into two groups: NORMAL(N; n=21, 61.8%) and PATHOLOGICAL (P; n=13, 38.2%). There was no significant difference in HLA-DQ allele frequencies between individuals with N and P biopsy findings (DQ CD-susceptibility alleles= N:57.1%vsP:76.9% ; DQx-=N:42.9%vsP:23.1%; *p*=0.2409).

Conclusions: HLA genotyping could be an useful tool for diagnosis evaluation of CD as the majority of symptomatic individuals in our cohort carried at least one susceptibility allele. However, it cannot currently replace duodenal biopsy, which remains the gold standard for CD diagnosis. Further studies with larger sample sizes are necessary to more accurately determine the positive and negative predictive values of HLA-DQ alleles, particularly in individuals who undergo duodenal biopsy histological evaluation.

Key words: HLA-DQ2; HLA-DQ8; HLA-DQ7; Duodenal Biopsy; Celiac Disease.

Innate Immunity

II-005

Cannabidivarin (CBDV) induces intracellular calcium fluxes and inhibits neutrophil functions

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Cannabinoids are a family of compounds obtained from Cannabis sp. plants that have gained increasing interest as a therapeutic option due to their ability to interact with the CB1R and CB2R receptors of the human endocannabinoid system. Beneficial effects have been reported for CBD and THC—the most abundant cannabinoids—in the treatment of epilepsy, seizures, nausea, pain, and other conditions. While research has focused on the effect of cannabinoids on the nervous system, the expression of the CB2R receptor in immune cells has encouraged the study of cannabinoids as immunomodulators. Given the growing use of cannabis for pain relief in autoimmune diseases such as arthritis, we wondered about the role of cannabinoids on neutrophils, the main mediators of immunopathology. To this end, we purified neutrophils from the blood of healthy donors and studied respiratory burst by examining dihydrorhodamine (DHR) oxidation by flow cytometry. By exposing neutrophils for one hour to a broad panel of cannabinoids, we found that cannabidivarin (CBDV) consistently inhibits N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced respiratory burst (Mean 390,288 vs 248,751; n=16; p<0.0001****). This effect is dose-dependent, occurring between 5-50 µM of CBDV, with significant differences from the control group between 30 minutes and 6 hours after exposure. We ruled out that CBDV affects neutrophil viability by microscopy and by Annexin V and propidium iodide staining by cytometry (Mean 2.88 vs 2.93 (M); n=3). In turn, CBDV decreased neutrophil IL-8 production measured by ELISA at 18 hours post lipopolysaccharide (LPS) stimulation (Mean 7313 vs 2522; n=4; p=0.0009***). Upon investigating the mechanism, we discovered that exposure to CBDV induces an instantaneous increase in intracellular Ca+2 levels measured with the Fluo-2 AM probe by flow cytometry (Mean 60,506 vs 95,161; n=11; p=0.0008***). We ruled out the entry of extracellular Ca+2 using Ca+2-free media and found that, after 1 hour of exposure to CBDV, neutrophils have elevated intracellular Ca+2 levels, which explain a suboptimal respiratory burst upon stimulation by fMLP but not by Phorbol 12-myristate 13-acetate (PMA). Our results demonstrate that CBDV induces intracellular Ca+2 fluxes that affect the functionality of human neutrophils.

Key words: Cannabidivarin; Neutrophils; Calcium flux

II-012

Combined exposure to glyphosate- and chlorpyrifos-based pesticides impair NK cell function in vitro

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Growing evidence links pesticide exposure to cancer development, thus little is known about the impact pesticides can have on human immunity. Since immunosurveillance plays a major role in the development of neoplasms, pesticides should be evaluated for immunotoxicity. Also, most studies focused on the effects of individual pesticides, while the effect of pesticide mixtures remains poorly explored. We assessed the impact of environmentally relevant doses of Round-up® (R), a glyphosate (G)-based herbicide, Clorpi48® (C48), a chlorpyrifos (C)-based insecticide, and their combination (R+C48) on NK cell functions. We also evaluated the effect of the active ingredients G, C and their combination (G+C). Methods: Peripheral blood mononuclear cells (PBMCs) and NK cells from healthy donors were exposed in vitro to environmentally relevant doses of either R, C48 and R+C48, or G, C and G+C. T-bet expression and IFN-γ production on NK cells were analyzed by flow cytometry (FC). NK cell cytotoxicity against the tumor cell line K562 and NK cell:K562 cells conjugates were also assessed by FC, while cellular localization of LFA-1 and perforin polarization were analyzed by confocal microscopy. Oxidative stress was evaluated by assessment of superoxide (O2-) production using DHE staining at different time points. Results: Exposure of NK cells to the combination of R+C48, but not to the individual formulations, reduced T-bet expression and IFN-γ production ($p<0,01$). Moreover, cytotoxicity was also significantly reduced after R+C48 exposure, with a reduced frequency of NK cell: K562 cell conjugate formation. This effect was accompanied by a decreased presence of LFA-1 at the immune synapse ($p<0,01$) and a defective perforin polarization in pesticide treated NK cells. Notably, the combination of G+C, but not the individual active principles, significantly impaired NK cell cytotoxicity ($p<0,05$). The combination of G+C increased the production of O2- after 1h and 4h of pesticide exposure, but this effect was lost after 18 h ($p<0,01$). The increase of O2- levels at early timepoints showed no correlation with NK cell cytotoxicity impairment. Conclusion: Our findings indicate that combined exposure to G- and C-based pesticides disrupt key NK cell effector functions and immune synapse organization. Therefore, our results underline the need to evaluate pesticide mixtures for potential immunotoxic effects.
Key words: immunotoxicity; pesticides; oncology

II-027

Role of innate receptor NLRX1 on macrophage M1/M2 polarization and susceptibility to modulation by cannabidiol

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NLRX1, an innate immune receptor of the NOD-like receptor family, is distinguished by its mitochondrial localization and anti-inflammatory role. It is involved in mitochondrial functionality and homeostasis with implications for different pathophysiological conditions. However, there are still open questions on its role as a connecting hub for mitochondrial function, metabolism and immune cell fate. Cannabidiol (CBD), a non-psychoactive component of cannabis, has multiple biological effects including anti-inflammatory properties. Evidence indicates that CBD may act through modulation of mitochondrial function but molecular mechanisms are not fully understood and participation of NLRX1 on CBD-mediated effects has not been evaluated. The aim of this study was to analyze the contribution of NLRX1 to macrophage's functional profile and to assess whether CBD's effects on macrophage's response is dependent on NLRX1. Bone marrow derived macrophages (BMDMs) from NLRX1 knockout (KO) and wild type (WT) mice were

stimulated with LPS & IFNg (M1) in presence/absence of CBD or left untreated (M0). Macrophage response was evaluated by measuring lactate, nitrites and IL-6 production and expression of surface markers by flow cytometry. Metabolic profile under basal conditions was evaluated following Mito-Stress assay by SeaHorse. Under basal conditions, no significant differences were found between NLRX1 KO and WT BMDMs regarding CD86, MHC-II and metabolite production. Nevertheless, NLRX1 KO BMDMs presented a higher basal ($p=0.0072$) and maximum respiration ($p<0.0001$). In M1 conditions, NLRX1 KO BMDMs produced less amount of nitrites ($p<0.0001$) and IL-6 ($p=0.0399$) than WT BMDMs. BMDMs in M0 and M1 conditions were treated with CBD (1, 5 and 10 uM). At M0 condition CBD had no effect on analyzed parameters regardless of concentration or NLRX1 functionality. In M1 proinflammatory state, CBD 10 uM decreased IL-6 ($p=0.0285$) and nitrite ($p<0.0001$) production in WT but not in NLRX1 KO BMDMs.

These preliminary results suggest that NLRX1 KO BMDMs exhibit a weaker differentiation towards the M1 profile compared to WT BMDMs. Moreover, CBD's modulatory effects on macrophage pro-inflammatory polarization seem to be partially dependent on NLRX1 function.

Key words: NLRX1; Cannabidiol; macrophage polarization

II-031

Staphylococcal Superantigens Drive Innate Immune Dysregulation. Is it a mechanism for early pathogen spreading?

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Bacterial superantigens (SAgs) are enterotoxins, mainly produced by Staphylococcus aureus. These toxins are well known for simultaneously binding T-cell receptors (TCRs) and MHC-II molecules on Antigen Presenting Cells (APCs), inducing cytokine storms and dysregulation of the adaptive immune response, leading to toxic shock syndrome and food poisoning. The impact of SAgs on cells of the innate system has not been extensibly addressed. This study aims to characterize the interactions of staphylococcal SAgs encoded in the egc operon with human innate immune cell populations. Four SAgs from the egc operon, SEN, SEU, SEI, and SEO, were cloned and expressed in E. coli, and then purified. Human PMNs and macrophages differentiated from PBMC-derived monocytes were incubated with SAgs at different time points. NET release was assessed by confocal microscopy using an antibody panel, cytokine production was quantified via ELISA, and oxidative burst was measured using dihydrorhodamine 123 fluorescence. Cell death was determined by propidium iodide staining, while macrophage STAT-3 activation was evaluated using flow cytometry with anti p-STAT-3 antibody. In addition, intracellular distribution of SAgs was examined using FITC-labeled SAgs. The effects of SAgs on myeloid-derived suppressor cells (MDSCs) were assesed by flow cytometry after staining with an antibody panel. Results showed that SEN potently induced NETosis, increased PMN death, and promoted the release of proinflammatory cytokines, while both SEN and SEU significantly enhanced respiratory burst and matrix metalloproteinase (MMP) activity ($p < 0.05$). In macrophages, SEN and SEU increased apoptosis, cytokine secretion, and oxidative burst ($p < 0.05$). Notably, SEN also induced STAT-3 phosphorylation ($p < 0.05$) and exhibited an unconventional intracellular distribution compared to the standard protein OVA ($p < 0.05$). Furthermore, SEU, SEI, and SEO reduced the overall proportion of MDSC and altered the distribution of their subsets ($p < 0.05$). These results suggest that egc SAgs have an impact on innate immune cells, directly modulating neutrophil, macrophage, and MDSC functions. By disrupting innate immune regulation in addition to their established effects on T cells, SAgs may amplify systemic inflammation and immune dysregulation. These effects could accelerate the progression of sepsis and other severe staphylococcal diseases.

Palabras clave: Superantigens; innate immunity; inflammation

II-098

S-layer proteins from clinical isolates of Clostridioides difficile show different immunostimulatory activity on macrophages

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The bacterium Clostridioides difficile is responsible for 15–30% of cases of diarrhoea associated with antibiotic use. Infection can range from being asymptomatic to causing severe diarrhoea and can even trigger fulminant pseudomembranous colitis. Currently, there are no vaccines available. The underlying pathology is attributable to variants that yield toxins A (TcdA), B (TcdB) and binary (CDT), in addition to their respective combinations. Other non-toxigenic virulence factors are present, including S-layer proteins (SLP) which form a two-dimensional self-assembled coat on the bacterial surface of significant importance in growth and pathogenicity. The SLP is composed of one high molecular weight (HMW-SLP) and one low molecular weight (LMW-SLP) subunits, with a molecular weight ranging from 35 to 55 kD.

In this study, the immunostimulatory capacity and immunoreactivity of SLPs from clinical isolates belonging to different toxinotypes were characterised, with the aim of subsequently evaluating their usefulness as vaccine antigens.

A total of eight clinical isolates (CD1, CD3, CD4, CD16, CD18, CD28, CD117, and ALCD3) and a reference strain (VPI 43255) were evaluated. SLPs were extracted with 0.2 M glycine (pH 2.2), and SDS-PAGE revealed the presence of two main bands in each of the isolates studied, with apparent molecular weights between 45 and 55 kD for HMW-SLP and between 35 and 40 kD for LMW-SLP. Of the all SLPs examined, only those derived from strains CD1, CD3, CD4, and CD16 could activate the RAW264.7 cell line and inducing IL-6 secretion, as determined by capture ELISA. Conversely, Western blot assays revealed with a mouse anti-SLPVPI43255 serum showed that cross-reactivity was strain-dependent. Only SLPs from strains CD18, CD28, CD117 and ALCD3 were recognised.

It can be concluded that SLPs from different C. difficile strains exhibit molecular heterogeneity, which translates into differences in immunoreactivity and immunostimulatory capacity. These characteristics could impact the pathogenicity of C. difficile and its ability to evade the immune system. These results encourage further investigation into the potential of C. difficile SLPs as vaccine antigens.

Key words: Clostridioides difficile; S-layer proteins; immunostimulatory capacity; vaccine antigens

II-109

Providencia stuartii and Providencia rettgeri induce vital NETs but inhibit suicidal NETs formation in human neutrophils

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Antimicrobial resistance is an increasing challenge in the treatment of infections. *Providencia stuartii* (Ps) and *Providencia rettgeri* (Pr) are multidrug-resistant pathogens associated with nosocomial infections, whose interaction with the innate immune response has not been previously described. In previous studies, we observed that upon exposing human neutrophils (PMNs) to clinical isolates of both species, although Pr and Ps were phagocytosed, PMNs failed to effectively eliminate them, suggesting active immune evasion mechanisms. Additionally, PMNs did not release suicidal neutrophil extracellular traps (NETs), and DNases associated with bacteria were detected, which may contribute to the degradation of these structures. In this study, we aimed to elucidate whether Pr or Ps affects a key step in NETs formation, such as the migration of elastase to the nucleus, leading to DNA decondensation, or if these NETs are induced but not detected due to their rapid degradation by bacterial DNases. To evaluate elastase migration to the nucleus and DNA decondensation, we quantified by confocal microscopy the percentage of PMNs with nuclear elastase at 90 min post-incubation with Pr or Ps (MOI 10) using propidium iodide and a specific anti-elastase antibody. The results showed that neither of the two bacteria induced nuclear decondensation or elastase migration, compared to the positive control, PMA (40 nM) ($p<0.05$; $n=6$).

Additionally, vital NETs is an alternative response mechanism, characterized by the rapid release of DNA. Thus, we also evaluated the ability of Pr or Ps to induce the release of this type of NETs. PMNs were incubated with Ps, Pr, or *Escherichia coli* ATCC (Eco) as a positive control for different times (15-180 min), and NETs were determined by measuring the release of extracellular double-stranded DNA, and by observation using confocal microscopy. Both Pr and Ps induced early DNA release (30 min) ($p<0.05$), which then decreased to basal levels by 180 min. For Eco, an opposite pattern was observed, with no early DNA release but a significant increase at 180 min.

In conclusion, we have elucidated two distinct evasion mechanisms by which Pr and Ps affect the formation of NETs: one preventing elastase migration to the nucleus and inhibiting suicidal NETs induction, and the other degrading vital NETs probably through baterial-derived DNases.

Key words: NETs; neutrophil; Providencia spp.

II-155

Effects of air pollution caused by vegetable oil-processing industries located in the industrial zone of Rosario city on the immune-endocrine system.

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Rosario and its surrounding area are characterized by intense agro-industrial activity, which exposes the population to a distinctive pattern of environmental pollutants. In particular, large-scale vegetable oil–processing industries produce edible oils through processes that involve residual pesticides and the solvent n-hexane, leading to potential exposure of both workers and nearby residents. This scenario is further compounded by airborne particulate matter (PM) originating from grain milling, which may exert additional physical toxicity. Such combined exposures raise concern for functional alterations in exposed individuals, with possible implications for respiratory and cardiovascular health. The aims of this study were: (OB1) To evaluate whether occupational exposure leads to immune-endocrine alterations in blood samples from workers in the vegetable oil–processing industry. (OB2) To assess the role of airborne PM, collected monthly throughout 2024 using a portable device developed by our group, on the innate immune response through stimulation of human THP1-derived macrophages. Findings for OB1: Workers in the grain unloading (GU) and hexane extraction (HE) sectors exhibited significantly reduced plasma levels of the hormone DHEAS ($p<0.01$), while maintenance workers (MW) showed lower Vitamin D levels ($p<0.01$) compared with healthy controls (HCo) and other workers. Flow cytometry analysis revealed sector-specific alterations: increased CD4+ TL in HE, elevated CD8+ TL in GU, and higher BL counts in MW (all $p<0.05$ vs

HCo). Findings OB2: Collected PM triggered an early inflammatory response in macrophages, mediated by NF-κB signaling. Moreover, PM physicochemical characteristics and collection timing differentially influenced the inflammatory response. Although PM concentrations surpassed WHO guidelines yet remained within provincial legal limits, underscoring the permissive and outdated nature of local regulations. In conclusion, our results suggest that environmental pollution associated with vegetable oil–processing industries may induce alterations in the immune–endocrine system, potentially increasing susceptibility to disease. This work provides scientific evidence supporting that Rosario and its surrounding area, with its strong agro-industrial base, face the dual challenge of addressing characteristic pollution patterns while advancing toward sustainable development that safeguards both health and the environment.

Key words: air pollution; oil-processing industries; inmmune system; Rosario

II-156

Characterization of virulence factors and antifungal resistance in clinical isolates of Acute and Recurrent Vulvovaginal Candidiasis: modulation of human Beta Defensins response in an in vitro epithelial cell model

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Recurrent mucosal fungal infections are linked to host predisposition, pathogen intrinsic characteristics, and ineffective therapy. Vulvovaginal candidiasis (VVC) is an acute inflammatory disease affecting ~75% of women of reproductive age, with 9% developing recurrent VVC (RVVC; ≥4 episodes/year). *Candida albicans* (*Ca*), the main cause, shifts from yeast to hyphae in the vaginal microbiome to establish infection. This transition is regulated by environmental cues and transcription factors that control hyphal formation, adhesins, hydrolytic enzymes, and biofilm formation—key virulence traits. β-defensins (BDs) are antimicrobial peptides in the female genital tract with microbicidal, chemoattractant and immunomodulatory roles, but their involvement in VVC is unclear. Objectives: To characterize the virulence profile and antifungal resistance in acute(AVVC) and recurrent(RVVC) *Ca* isolates, and assess how these strains and Pattern Recognition Receptors(PRRs) affect β-defensins expression in human epithelial cells (EC). Methods: *Ca* isolates from RVVC(n=33), AVVC(n=25), and reference strain SC5314 were evaluated for morphogenesis, adherence, biofilm(BFC), and hydrolytic enzyme activity (lipases [LIP], aspartyl proteases [SAP]). UMAP analysis was used to define virulence clusters. Antifungal resistance was assessed by VITEK2. HeLa cells were infected at *Ca*:cell ratios (0:1, 0.5:1, 1:1 and 5:1). PAMP agonists (LPS, Pam3CSK4, zymosan, curdlan, DNA-*Ca*, PolyI:C) were used. hBD1 and hBD3 expression (4h) was quantified by immunofluorescence. Results: *Ca*-AVVC and *Ca*-RVVC produced longer hyphae than SC5314($p<0.001$). *Ca*-AVVC had higher adherence($p<0.05$) and BFC($p<0.001$) than *Ca*-RVVC($p<0.001$). *Ca*-RVVC showed higher SAP and lower LIP than *Ca*-AVVC($p<0.05$). Four phenotypic virulence clusters matched acute, recurrent, or mixed profiles. Both groups resisted fluconazole and voriconazole (higher in AVVC). In EC, *Ca*-SC5314 induced hBD1 and hBD3 at 1:1 and 0.5:1, respectively($p<0.001$); interestingly, all vaginal isolates suppressed both peptides' expression at 5:1 ratio($p<0.0001$). hBD1 was induced by LPS; hBD3 by LPS, Pam3CSK4, and nucleic acid PAMP (DNA-*Ca*, Poly I:C;$p<0.0001$). Conclusions: hBD1 and hBD3 regulation varies with strain origin, fungal load, and host immune recognition, highlighting the importance of this balance in the development of VVC. *Ca*-AVVC and *Ca*-RVVC isolates differ in virulence and resistance, and can inhibit peptide expression in EC, favoring fungal persistence.

Key words: Vulvovaginal candidiasis; Candida albicans; Virulence factors; Antifungal resistance; β-defensins

II-157

Systematic Evaluation of the Proliferative Capacity of Human Monocyte-Derived Cells

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Although monocytes (Mo) and macrophages (Macs) were historically considered quiescent, over the past 30 years, subpopulations with proliferative capacity have been identified both in vivo and in vitro. However, most studies on Mo/Mac proliferation have been performed in animal models, making extrapolation to humans difficult. Here, we systematically evaluated the proliferative capacity of human monocyte-derived cells in vitro. Human monocytes were cultured in RPMI + 10% FBS with M-CSF or GM-CSF. Proliferation was assessed by flow cytometry using CFSE. GM-CSF induced significant proliferation across all concentrations (1, 10, 100 ng/mL), whereas M-CSF supported viability at low doses and triggered significant proliferation only at 100 ng/mL. Proliferation began around Day 6, and by Day 13 up to four successive generations were detectable. We also assessed the potential of monocyte-dervied cells to differentiatie into dendritic cells (DCs). Cells, after 7–13 days of culture with M-CSF (100 ng/mL), were incubated for 5 days with either GM-CSF (GM) (50 ng/mL, control), GM-CSF + IL-4 (30 ng/mL), or GM-CSF + Temsirolimus (T) (50 ng/mL) + GW9662 (GW) (10 ng/mL) to induce DC differentiation. We observed that all generations that had proliferated possessed the potential of differentiating into DCs, with no significant difference from that of the non-proliferative population. GM+T+GW yielded the highest % of CD1a+ cells across all generations: non proliferating, 1st gen, 2nd gen, and 3rd gen (73.8 ± 8.5, 70.0 ± 8.3, 65.0 ± 6.8, and 60.0 ± 6.3) in sharp contrast with GM-CSF treated cells, where CD1a expression was uniformly low across groups, with NP showing 12.0 ± 4.3, compared to 1G: 8.0 ± 2.9, 2G: 7.0 ± 2.6, and 3G: 9.0 ± 2.6 (n=4-6). Finally, and to explore mechanisms underpinning monocyte/Mac proliferation, we tested 70 compounds—including metabolic modulators, PAMPs, and cytokines—on monocyte-derived cell proliferation in the presence of M-CSF (100 ng/mL) or GM-CSF (100 ng/mL). Several compounds dramatically reduced proliferation (e.g., LPS, R848, PGE_2, IL-4, IFN-1β, C188-9 [a STAT-3 inhibitor], pimozide [a STAT-5 inhibitor]), whereas others—such as SB 202190 (a p38 inhibitor)—significantly enhanced proliferative capacity (n=3-5) ($p<0.01$). Our work highlights the proliferative capacity of human monocytes, uncovers potential modulatory pathways, and confirms that post-mitotic Mo/Macs can differentiate into dendritic cells in vitro.
Key words: Monocytes; Macrophages; Proliferation; Dendritic Cells

II-166

Cytokine-induced PD-L1 defines an activated NK cell subset with enhanced effector function

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Natural Killer (NK) cells are key effectors against tumors and virus-infected cells. Our previous studies demonstrated that NK cells stimulated with the pro-inflammatory cytokines IL-12 and IL-18 express high levels of PD-L1. The aim of this study was to characterize PD-L1$^+$ NK cells phenotypically and functionally. NK cells were isolated from healthy donors and stimulated with IL-12 and IL-18 (10 ng/ml) for 24 h, after which surface marker expression and functional responses were assessed by flow cytometry. PD-L1$^+$ NK cells exhibited significantly higher expression of the activation markers CD25 and CD69 compared to PD-L1$^-$ NK cells ($p<0.01$), but lower expression of CD57, a marker of terminal differentiation ($p<0.001$). Cytokine-induced activation of CD56 NK cells resulted in downregulation of CD16 and CD62L with PD-L1 expression en-

riched in these subsets ($p<0.01$ and $p<0.05$, respectively). Functionally, upon IL-12+IL-18 stimulation, NK cells initially produced IFN-γ followed by upregulation of PD-L1, with PD-L1$^+$ NK cells producing significantly more IFN-γ than PD-L1$^-$ NK cells ($p<0.001$). To assess cytotoxic capacity, cytokine-stimulated NK cells were exposed to K562 tumor cells for 4 h. PD-L1$^+$ NK cells showed increased degranulation, as reflected by both a higher percentage of CD107a$^+$ cells ($p<0.001$) and greater CD107a mean fluorescence intensity compared to PD-L1$^-$ NK cells, indicating more robust per-cell granule mobilization ($p<0.01$). Notably, PD-L1 blockade did not alter IFN-γ production, suggesting that PD-L1 functions as a marker of activation rather than a functional regulator in NK cells. These results highlight PD-L1 as a dynamic marker induced by pro-inflammatory cytokines that defines a phenotypically and functionally distinct NK cell subset characterized by enhanced activation and cytotoxic capacity. Rather than serving only as an inhibitory ligand, PD-L1 identifies a hyperactivated NK cell population with potential implications for immune regulation and tumor immunity.

Key words: NK cell; PD-L1; Cytokines

II-181

Glucose Transporter type 1 (GLUT1) Independence in Neutrophil Pro-inflammatory Activity Induced by Calcium Pyrophosphate Dihydrate Crystals

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Calcium pyrophosphate dihydrate (CPPD) crystal deposition disease is the third most common cause of inflammatory arthritis. In its acute form, a large number of neutrophils frequently accumulate in affected synovial joints. We have previously observed that in vitro stimulation of neutrophils with calcium pyrophosphate dihydrate (CPP) crystals increases the pro-inflammatory activity of these cells, measured by their ability to generate reactive oxygen species (ROS) and neutrophil extracellular traps (NETs). The aim of this study was to determine if the glucose transporter GLUT1 plays a role in the pro-inflammatory profile of neutrophils activated by the presence of CPP crystals. For this, peripheral blood neutrophils from healthy subjects were stimulated with CPP crystals to evaluate their pro-inflammatory effect through the release of ROS and NETs. Additionally, the drugs SFT-31 and BAY-678 were used as GLUT1 inhibitors. Data were analyzed using two-way ANOVA followed by Bonferroni's posttests; $p < 0.05$ was be considered significant. We observed that inhibition with STF-31 did not affect the ability of neutrophils to release NETs ($p = 0.83$) or ROS ($p = 0.34$) when stimulated with CPP crystals. Likewise, inhibition with STF-31 or BAY-678 had no effect on glucose uptake (measured by 2-NBDG incorporation) by neutrophils when incubated with the crystals ($p = 0.85$ and $p = 0.79$, respectively). Finally, through qRT-PCR assays, we observed that neutrophils expressed GLUT3, suggesting that this receptor might be responsible for glucose uptake when GLUT1 is inhibited. In conclusion, these results may indicate the independence of the GLUT1 receptor in the pro-inflammatory activity of neutrophils in response to stimulation with CPP crystals.

Key words: CPP Crystals; Neutrophils; Glucose Transporters

II-183

The gut-lung conversation: intestinal homeostasis disruption reprograms pulmonary structure and macrophages populations

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Immune axes involve complex bidirectional communication pathways between the immune system and other vital organs, such as the gut. The gut plays a key role in the development and training of the immune system. Both innate and adaptive responses can be disrupted in the local environment by intestinal alterations. However, the impact on distal sites, such as the lung, remains unclear.

We induced alterations in the gut by using a cocktail of antibiotics (the leading cause of intestinal dysbiosis) and inoculating mice with the intestinal pathogen Clostridioides difficile. C57BL/6 mice received colistin, metronidazole, vancomycin, and cephalosporin in the drinking water, followed by an i.p injection of clindamycin. Another group was additionally inoculated with 1×10^5 C. difficile spores to induce C. difficile infection (CDI). Mice were euthanized at different time points to collect gut and lung tissues. Histopathological score was studied using H&E staining and QuPath and Fiji software. Lung macrophage (MΦs) populations (total MΦs: CD64+, alveolar MΦs: SiglecF+, interstitial MΦs: CD11b+) and SLAMF1 (a costimulatory molecule and microbial sensor) expression were analyzed by fluorescence microscopy.

Antibiotic-induced intestinal dysbiosis compromised gut integrity, with mucosal alterations persisting beyond the resolution of overt clinical signs. Interestingly, gut dysbiosis led to structural lung damage, evidenced by changes in lung parenchyma, alveolar septa, and the distribution and number of alveolar spaces. While lung recovery was observed on day 5 after dysbiosis, CDI mice failed to resolve the structural compromise caused by the initial dysbiosis. Moreover, total MΦs number was reduced in CDI animals' lungs. Interstitial MΦs increased at day 2 and reached baseline by day 5, whereas alveolar MΦs showed the opposite trend. SLAMF1 levels raised on day 5 post-dysbiosis and post-infection in the colon. Conversely, pulmonary SLAMF1 expression peaked at day 2 in both groups of treated animals, but was significantly reduced in CDI mice compared with the antibiotic-treated group. We finally re-analyzed published human transcriptomic data, finding that CDI patients had reduced SLAMF1 blood levels and changes in the innate immune system supporting our results.

Overall, this study demonstrates that alterations in intestinal homeostasis compromise the distal lung; pointing the need to clarify the gut-lung axis pathways that could underlie complex immune pathologies.

Key words: gut; lung; macrophages

II-189

Analysis of leukocytes subpopulation recruited after Shiga toxin injury.

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Hemolytic Uremic Syndrome (HUS), primarily caused by Shiga toxin (Stx)-producing E. coli, is a severe condition characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, affecting children. Upon bacteria ingestion, Stx induces apoptosis on endothelial and epithelial cells, and triggers vascular damage. Inflammatory response follows, driven by polymorphonuclear neutrophils (PMNs) releasing damaging factors and by monocytes/macrophages secreting cytokines and chemokines after Stx interaction. The aim of this work was to deeply characterize leukocyte subpopulations during renal damage induced by Stx. For this purpose, mice were intravenously injected with Stx type 2 (Stx2). After 72h, PMN, monocytes and macrophages were analyzed in kidney samples by spectral flow cytometry. There was an increase in PMN (CD45+Ly6G++CD11b+CD43+, %±SE): control= 10.6±1.5; Stx= 39.7± 0.7*, *p<0.05 and in classical monocytes (CD45+Ly6C++CD11b+): control= 10.6±1.1; Stx= 20.8±1.9*,

p<0.05. In parallel, percentage of renal macrophages (Mac) within myeloid subset was decreased in Stx-treated mice, Mac (CD45+F4/80+CD11b+MHCII+, %±SE): control=71.3±5.3; Stx=51.3±1.2, *p<0.05. However, the distribution of renal Mac between major subset (F4/80++CD11b+CCR2+):-control=76.3±1.2;Stx=74±4; and minor (F4/80+CD11b++CCR2++): control= 12.7± 0.3; Stx= 15.3±2.7) was conserved. These results suggest that Stx, besides the specific effect on endothelial cells, triggers inflammatory signals that modify the profile of renal leukocytes. Then functional imaging of myeloid cells by in vivo multiphoton microscopy was set up in kidney from control mice. For this purpose, transgenic mice expressing three fluorescent probes (RGB-Mac mouse) along with second harmonic generation (SHG) to detect collagen and elastin fibers and Coherent anti-Stoke Raman scattering to detect the surrounding architecture of the tissue (epitheliums, vessels, nerves) without the need of an exogenous fluorescent reporter. This technique will let us observe the renal tubules and capillaries, resident macrophages detected by the expression of GFP (green) and circulating monocytes detected by ECFP (Cyan). Our data showed the modulation of renal leukocytes after Stx injury. In this case, the combination of spectral cytometry and optical techniques would help us to better characterize myeloid cell distribution.

Key words: HUS; Stx; leukocytes

II-190

Human neutrophils' extracellular vesicles generated in response to Shiga toxin mediate host kidney damage in vivo

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Infections by Shiga toxin (Stx)-producing Escherichia coli (STEC) can cause from self-limiting diarrhea to Hemolytic Uremic Syndrome (HUS), a condition characterized by non-immune hemolytic anemia, thrombocytopenia, and acute renal failure. Previously, we characterized the extracellular vesicles (EV) released by human neutrophils exposed to Stx (EV-Stx) and determined that they carry functional Stx. These EV were captured by primary renal endothelial cells and renal tubular epithelial cells in vitro, inducing their death. Here, to gain insight into the amount of Stx contained in the EV-Stx, we isolated the EV produced by 107 neutrophils treated with 100 ng/ml Stx and tested the cytotoxic capacity of different fractions of these EV on Vero cells. The EV released spontaneously (EV-C) were evaluated on the cells as a control. As expected, the EV-C did not reduce Vero cell viability. The EV-Stx induced significant cytotoxicity levels at all the evaluated fractions (n=5, p<0.05). By interpolating the viability data in a standard curve generated by treating Vero cells with known Stx concentrations, we determined that the amount of Stx contained in the EV-Stx produced by 107 neutrophils was ~0.119 ng and the estimated concentration was 0.396 ng/ml. Then, we used this concentration to determine whether EV-Stx could transport Stx to the kidneys to induce tissue damage. To this end, we used a mouse model of Stx intoxication as a HUS positive control, and inoculated human EV-C, EV-Stx or vehicle (Sham) in Rag1KO mice. Sham mice underwent ~30% increase in their body weight over the 72-hour experimental period. By contrast, mice inoculated with pure Stx and those inoculated with EV-Stx did not experience significant weight change. Mice inoculated with EV-C experienced a body weight increase comparable to that of sham mice (n=7, p<0.05). Uremia levels were significantly increased compared to those in sham mice in either Stx- and EV-Stx-inoculated mice but not in EV-C-inoculated mice (n=9, p<0.05). Histology examination showed that EV-Stx-inoculated mice exhibited renal tissue damage, similar to those inoculated with Stx.. These findings support that neutrophil-derived EV-Stx can transport Stx to the kidney, inducing tissue injury. Considering that neutrophilia at HUS presentation is a consistently worse-prognosis factor for the disease, our results suggest a critical

role for neutrophil-derived EV in the pathophysiology of HUS.
Key words: Neutrophils; Extracellular vesicles; Hemolytic Uremic Syndrome

II-198

Interaction between glioblastoma-derived extracellular vesicles and gamma delta T cells: the role of MIC in their activation.

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Gammadelta T lymphocytes (GDTL) are innate immune cells that can migrate to tumors and induce malignant cells' apoptosis. Glioblastoma multiforme (GBM) is the most aggressive and common primary brain tumor, with a median survival of less than a year after diagnosis. Due to their anti-tumoral capacity, GDTL-based immunotherapies have been proposed for GBM treatment. Our previous in silico studies showed that higher GDTL presence in GBM correlates with better patient survival. We additionally observed that GBM cells' supernatants enhanced GDTL activation, promoting an anti-tumoral profile (DOI:10.1007/s11060-021-03787-7). Among other factors released by tumoral cells, extracellular vesicles (EVs) play an important role in cell communication, particularly in tumor modulation of its microenvironment. We have already seen that EVs from GBM cell line U251 (U251 EVs) physically interact with GDTL and induce their activation in a dose dependent manner. We also observed that U251 EVs can contain PD-L1 and MIC. In this work, we studied the mechanisms responsible for GDTL activation by GBM-derived EVs, together with the effect of these EVs on GDTL from patients. For that, GDTL were purified from peripheral blood by a microbead isolation kit. Tumoral EVs were obtained from U251 cell supernatants by differential centrifugation. GDTL were incubated with EVs overnight, with or without ligand-blocking antibodies, and GDTL activation was assessed by CD69 immunostaining and flow cytometry. PD-L1 neutralization in U251 EVs didn't affect GDTL activation state, but MIC blockade diminished CD69 expression in GDTL ($p<0,05$). Importantly, CD3+/TCRγδ+ cells were found in a disaggregated biopsy of GBM, which supports our in silico studies. As well as in healthy donors, U251 EVs tended to increase CD69 expression in patients' GDTL. Our findings suggest that GBM EVs induce GDTL activation, and that MIC may mediate GBM EVs effect on GDTL activation. These results provide valuable insights for developing targeted immunotherapies in GBM patients.
Key words: Glioblastoma; Gammadelta T lymphocytes; Extracellular vesicles; MIC

II-207

Autophagy as a key mechanism involved in TLR2-induced microglial neurotoxicity

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Objective: autophagy is a conserved mechanism that engulfs cytoplasmic components and damaged organelles into autophagosomes, which fuse with lysosomes to maintain cellular homeo-

stasis. Its disruption has been linked to disorders including neurodegeneration. Microglia, the resident central nervous system (CNS) phagocytes, express pattern recognition receptors such as Toll-like receptors (TLRs). Previous studies by our group revealed a link between TLR2 and autophagy. However, the transcriptional regulation of microglia pathways upon activation remains poorly explored. Here, we aim to determine whether microglial autophagy is a necessary mediator of TLR2-induced neurotoxicity by in vitro experiments and transcriptomic gene set enrichment analysis (GSEA) assessment. Methods: murine BV2 microglia was stimulated with Pam3CSK4, PGN, or LPS in the presence or absence of 3-MA. Nitric oxide (NO) production was quantified by Griess assay. LC3-I/LC3-II conversion was analyzed by Western blot. Microglia-induced cytotoxicity was studied in cocultures with neuronal N2a cells, and death measured by flow cytometry (PI staining). Public RNA-seq datasets from TLR2-stimulated or ATG5 knockout cells were retrieved from GEO-NIH and analyzed with Python packages and DESeq2 within a workflow developed in our laboratory.

Results: TLR2 stimulation increased NO release in BV2 cells, which was prevented by 3-MA ($p < 0.001$). GSEA revealed modulation of autophagy and mitophagy pathways in TLR2-stimulated microglia, accompanied by mTOR upregulation and TFEB downregulation ($p < 0.001$). Conversely, ATG5 knockout datasets showed opposite trends, suggesting compensatory transcriptomic changes. Autophagy induction and inhibition by 3-MA were confirmed by LC3-I/LC3II conversion ($p < 0.001$). Cocultures showed that neurotoxicity induced by TLR2-activated microglia was prevented by 3-MA or aminoguanidine, an iNOS inhibitor.

Conclusions: our findings suggest that microglial autophagy may be required for TLR2-induced neurotoxicity. Pharmacological inhibition with 3-MA suppressed NO release, impaired LC3 processing, and protected neurons from microglia-derived toxicity. Transcriptomic analyses further indicated that TLR2 signaling modulates autophagy- and mitophagy-related pathways, with TFEB and mTOR as central regulators. Overall, these results support the possibility that autophagy acts as response involved in TLR2-dependent microglial activation to neuronal damage.

Key words: Microglia; Autophagy; TLR2; Neurotoxicity

II-209

TRANSCRIPTOMIC EVALUATION OF MICE BRAINS AFTER SYSTEMIC LIPOPOLYSACCHARIDE CHALLENGE.

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Introduction: Brain-resident microglia and blood-derived monocytes are key regulators of immune responses in the central nervous system. These cells become activated and migrate in response to chemokines, potentially contributing to the progression of neuroinflammation. We previously demonstrated that systemic lipopolysaccharide (LPS) administration induces glial activation and recruits CD45hi leukocytes to brain vasculature, particularly within circumventricular organs. In this study, we analyzed the brain transcriptome of LPS-systemically treated mice to elucidate gene expression patterns associated with leukocyte recruitment and neuroinflammatory processes.

Methods: Wild-type (WT) C57BL/6 and IFN-γ knockout (KO) mice (n=4 per group) were administered with PBS or lipopolysaccharide (LPS; 1.6 mg/kg, intraperitoneally). Following perfusion, brains were harvested and processed to isolate immune cells, which were subsequently stained and analyzed by flow cytometry. Additionally, bulk RNA-seq datasets from brains of LPS-systemically treated mice were retrieved from the NCBI Gene Expression Omnibus (GEO) and analyzed using the integrated Differential Expression and Pathway (iDEP) analysis tool.

Results: Systemic LPS administration significantly increased monocyte recruitment in the brains of wild-type (WT) mice compared to PBS-treated controls ($p < 0.05$). This effect was absent in IFN-γ knockout (KO) mice ($p > 0.05$), suggesting that type II interferon is required for monocyte

recruitment into the brain. RNA-seq analysis revealed upregulation of pro-inflammatory genes following LPS stimulation. Differentially expressed genes (DEGs) were enriched for cytokines, chemokines, and adhesion molecules ($p < 0.01$). KEGG pathway analysis indicated significant modulation of Cell Adhesion Molecules and Leukocyte Transendothelial Migration pathways ($p < 0.001$), highlighting molecular mechanisms underlying neuroinflammatory responses.

Conclusion: These results suggest that systemic inflammation in mice induces leukocyte recruitment to the CNS in a type II interferon-dependent manner. Type II interferon may act as a key regulator of adhesion and chemoattractant gene expression, thereby promoting the recruitment of peripheral myeloid cells to the CNS.

Key words: Neuroinflammation; Transcriptome; Brain; LPS

Mucosal and Reproductive Immunology

MR-003

Saccharomyces Boulardii CNCM I-745 treatment alleviates inflammation in experimental long-term small intestinal enteropathy

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Background: We previously showed that intragastric (IG) administration of the gliadin peptide p31–43 activates innate immunity and induces epithelial stress in the mouse small intestine, mimicking some features of Celiac Disease, such as reduced villus-to-crypt (V/C) ratio, intraepithelial lymphocyte (IEL) infiltration, cell death, inflammasome activation, and increased proinflammatory mediators. We also found that a three-week pre-treatment with *Saccharomyces boulardii* CNCM I-745 (S.b.) prevented both histological and inflammatory responses to a single p31–43 dose. Aims & Methods: This study aimed to evaluate whether S.b. can prevent intestinal damage caused by repeated p31–43 exposure over three weeks. Eight-week-old C57BL/6 mice received IG p31–43 (40 µg/mouse) five times per week, simultaneously withe daily oral gavage of either S.b. (3 g/kg/day) or vehicle. Sixteen hours after the final dose, the small intestine was collected for histological analysis (V/C ratio, IEL and Goblet cell counts) and molecular studies (Western blot and RT-qPCR). Statistical analysis was performed using ANOVA. Results: Co-administration of *Saccharomyces boulardii* (S.b.) prevented mucosal damage induced by repeated p31–43 exposure. The V/C ratio was restored in S.b.-treated mice, with a trend toward reduced IEL infiltration ($p = 0.21$). Goblet cell counts remained unchanged, though *Muc2* mRNA levels modestly increased after p31–43 treatment, regardless of S.b. administration. The *Atoh1/Hes1* ratio suggested enhanced differentiation of secretory epithelial cells in the p31–43 group. *Cxcl10* expression was upregulated by p31–43 and partially reduced by S.b. ($p = 0.126$). Western blot confirmed that S.b. attenuated p31–43–induced caspase-1 activation, indicating suppression of inflammasome. activity. Conclusion: S. *boulardii* CNCM I-745 restores intestinal histology and reduces inflammation caused by repeated p31–43 exposure, supporting its potential to mitigate gliadin-induced and sterile inflammation-driven intestinal pathology.

Key words: small intestine inflammation; probiotic; celiac disease

MR-036

Oral Lactate treatment ameliorates allergic response in a pre-clinical model of food allergy

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Food allergy (FA) is an immune-mediated adverse reaction to food antigens and its prevalence has increased worldwide. Intestinal microbiota and microbial metabolites play a key role in orchestrating immune regulatory circuits and development of oral tolerance providing an avenue for FA prevention. Lactate, an endogenous/microbial metabolite, has multiple biological functions that greatly impact cellular biology and tissue physiology. Its immunoregulatory role has been demonstrated both in homeostasis and pathophysiological conditions. However, its contribution to gut immunobiology, is less explored. In this work, we aimed to analyze the effect of oral lactate supplementation on a food allergy mouse model. BALB/c mice received 6 weekly intragastric administrations of cow's milk protein (CMP) with cholera toxin (CT) (sensitized group, Sen) or without CT (control group). Animals in the Lactate group (Lac) received lactate 200mM in drinking water throughout the sensitization protocol. Mice were orally challenged with CMP, and the immune response was evaluated both in vivo (clinical score and cutaneous tests) and in vitro (serum specific IgE, IgG1 and IgG2a; secretion of IL-5, IFN-γ, and IL-17 by spleen cells and analysis of cytokines and Treg cells in the intestinal mucosa. Oral administration of lactate partially reduced clinical signs after CMP challenge and ameliorated skin test ($p<0.05$). Accordingly, serum-specific IgE levels were significantly decreased (2.5±0.3 Sen vs. 0.82±0.7Lac; $p<0.05$) while specific IgG1 levels showed a non-significant reduction. Lactate also induced increased IFN-γ secretion by CMP-stimulated splenocytes from sensitized mice ($p<0.05$) with no significant change in IL-5 secretion. In the intestinal mucosa, lactate induced a significant decrease in IL-17 levels ($p<0.01$) while a trend toward reduced IL-5 levels was observed. Analysis of CD4+CD25+Foxp3+ Tregs in the small intestine lamina propria showed no significant changes in lactate-treated mice, whereas a significant reduction was observed in Peyer's patches ($p<0.05$). Our preliminary results suggest that oral lactate supplementation may modulate the induction of adaptive immune response in the intestinal mucosa, attenuating allergic sensitization. Further studies are needed to elucidate the underlying mechanisms.

Key words: Lactate; Intestine; Mucosa; Food; Allergy

MR-046

Adenoids and tonsils play a similar immunological role and experience age-related involution since puberty

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The Waldeyer's ring is a circle of lymphoid tissues extending throughout the naso-and oropharynx which includes the palatine, pharyngeal, lingual and tubal tonsils. Whether the different structures of the ring are equivalent in function remains enigmatic. Extrapolation from animal studies is misleading due to extensive anatomical differences between species. Here,we aimed to compare adenoids and palatine tonsils in terms of histology and main immunological subsets from the same patient, analysing both samples from several donors at different ages. 18 paired (adenoids and tonsils) samples from patients aged 2 to 12 years old were analyzed by H&E, immunohistochemistry staining and flow cytometry (FACS). When their respective mononuclear cells were analysed by FACS, no statistical differences were detected between the proportion of the germinal centre and naïve B cells in adenoids and tonsils from the same patient. Histological appearance was also similar in both organs, most patients exhibited follicular hyperplasia driven by intensively active germinal centres that led to the surgery. To investigate the evolution of both organs with ageing, we divided the samples into 3 age groups. Group 1 comprised 2-5 years old-yo-; group 2, 6-9 yo and group 3, 10-13 yo. BGC cells represented well over one third of all the B cells from combined adenoid and tonsillar mononuclear cells (ATMC) within group 1 (38.2 % ± SD 3.3%). ATMC from children in group 2 rendered 24.4% ± SD 2.6% BGC cells and in the third group we obtained 21.1% ± SD 3.3% BGC. Means between groups 1 and group 2 resulted statistically different (**$p<0.01$; Tuckey test post two-factor Anova test) and between groups 1 and group 3 as well (***$p<0.005$; Tuckey test post two-factor Anova test). Finally,by establishing primary cell cultures with adenoid mononuclear cells (AMC), we tested whether specific memory B cells (mBc) generated by parenteral Ag administration in the arm could

be detected in adenoids. To do so, we cultured AMC for 3 days in media supplemented with tetanus toxoid (TT) and tested for anti-TT Igs in the culture supernatant. The presence and functionality of TT+mBC as well as their reactivation capabilities within AMC were demonstrated through this assay. In conclusion, adenoids and tonsils seem to play redundant roles in immunological terms and both appear to undergo involution around puberty. Additionally, they are a distant niche of persistent specific TT+mBC, generated at a distant location.

Key words: Adenoids; Germinal center; Ageing

MR-050

Annexin A1 in Intestinal Inflammation: Comparative Analysis in Murine Models of Enteropathy and potential implications in celiac disease

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Annexin A1 (ANXA1) is involved in the regulation of immune responses, apoptosis, cell differentiation, and inflammation, and has been implicated in intestinal disorders such as inflammatory bowel disease. We aimed to investigate whether ANXA1 plays similar roles in the small intestine, and particularly on celiac disease (CD).

While ANXA1 has been studied in colon using the dextran sodium sulfate (DSS)-induced colitis model, its expression in the small intestine remains unexplored. To address this, we evaluated ANXA1 expression in two mouse models of enteropathy: one involving intragastric administration of the gliadin-derived peptide p31–43 (40 µg/mouse), other using 3% DSS in drinking water. Following treatments, small intestine and colon tissues were collected. In addition, duodenal samples from CD patients and control subjects obtained during the routine diagnostic protocol were also evaluated. To assess ANXA1 expression, samples were analysed by qPCR, confocal immunofluorescence microscopy, and Western blotting.

ANXA1 expression was detected in the small intestine. In p31–43 model, intragastric delivery of p31–43 at different time points revealed a transient increase in ANXA1 expression in proximal small intestine ($p<0,01$), with a peak at 16 hours post-administration. In DSS-treated mice, ANXA1 expression by qPCR was observed in colon ($p<0,05$), as previously reported, but also in small intestine ($p<0,01$). In both models, ANXA1 was mainly expressed in lamina propria cells, with no evidence of epithelial staining.

Co-localization studies in p31–43-treated mice revealed that ANXA1 expression was restricted to neutrophils, as indicated by co-staining with myeloperoxidase (MPO). No co-localization with $CD3^+$ T lymphocytes was observed. In contrast, analysis of duodenal biopsies from CD and health controls showed an increased number of $ANXA1^+$ cells in CD ($p<0,01$). ANXA1 expression co-localized with MPO, CD66b, and CD3, indicating that both neutrophils and T lymphocytes can produce ANXA1 in the small intestine of CD.

These findings demonstrate a context-dependent expression pattern of ANXA1 in intestinal inflammation. ANXA1 expression varies not only between different regions of the intestine but also depending on whether the inflammatory setting is acute or chronic. These observations are relevant for the understanding of the role of ANXA1 in intestinal immunity and particularly in CD.

Key words: ANXA1; intestinal inflammation; celiac disease

MR-077

Endothelial cell function at early pregnancy: Effect of extracellular vesicles from trophoblast cells overexpressing VIPR2

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Vascular remodeling is a critical step in correct placentation. Extravillous trophoblasts (EVT) contribute directly and indirectly to spiral arteries remodeling. Along with this, the expression of vasoactive intestinal peptide receptor 2 (VIPR2) increases in human placental villi and can exert proangiogenic effects on the murine vasculature. During pregnancy, the number of extracellular vesicles (EVs) increases, and those derived from trophoblast cells EVs (Tb-EVs) are known to play a key role in regulating placentation. However, it is unclear whether VIPR2 expression by Tb cells contributes to the pro-angiogenic effects of EVs during placentation. Here, we examined the effect of human trophoblast cells basally or overexpressing VIPR2 (Tb-VIPR2) EVs on endothelial cell function.

Human uterine microvascular endothelial cells (HUtMvEC) were cultured without or with conditioned media from human first trimester EVT(EVT-CM), collected and used for RNAseq analysis. EVs were obtained from the human first trimester trophoblast cell line Swan 71, non-transfected (Non-T) and transfected either with a VIPR2 plasmid (Tb-VIPR2) or an empty vector (control, Tb-C) using differential centrifugation. Tb-EVs were characterized using transmission electron microscopy, nanoparticle tracking analysis and imaging cytometry analysis. Endothelial cells, EA. hy926 and HUVEC, were used for wound healing, reactive oxygen species (ROS) detection and tube formation assays.

HUtMvEC treated with EVT-CM showed a slight increase in total gene expression, and gene enrichment assay revealed metabolic changes in endothelial cells that favor cell proliferation. When we evaluated Tb-Non-T EVs they showed pro-migratory effects. Tb-VIPR2 EVs carry VIPR2, along with the tetraspanins CD9 and CD81 on the membrane. Compared to Tb-C EVs, they exhibited a smaller size range and a slightly lower number of particles/mL. Tb-VPR2 EVs enhanced endothelial cell migration (*P< 0.05) and showed a trend towards reduced ROS production. They also increased the number of meshes, nodes, pieces and segments in tubulogenic assays compared to Tb-C EVs (*P< 0.05).

These findings suggest that extravillous trophoblasts exert a modulatory role on endothelial cells, partially through extracellular vesicles. The impact of EVs produced by human trophoblast cells expressing VIPR2 on endothelial cell function indicates that VIPR2 plays a regulatory role in adaptations associated with human placentation.

Key words: Trophoblast Cells; Endothelial Cells; Extracellular Vesicles

MR-078

Senescence and Inflammaging: links between endometrial aging and impaired functionality

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The decidualization of human endometrial stromal cells (HESC) generates two distinct subpopulations: mature (mDec) and senescent (snDec) decidual cells. Far from being detrimental, physiological senescence contributes to implantation by establishing a senescence-associated secretory phenotype (SASP) that shapes the immune microenvironment, supports tissue remodeling, and allows trophoblast invasion. During aging, however, the endometrium is exposed to inflammaging, a chronic low-grade inflammatory state linked to cellular stress responses and an altered balance of senescent cells, which may compromise receptivity.

Here, we investigated the dysregulation of the balance between mDec and snDec and its implications for endometrial aging, inflammation, and implantation competence. *In silico* analyses revealed specific expression patterns of mDec and snDec markers that were validated in an *in vitro* model of decidualization, where we found upregulation of both mDec and snDec markers, highlighting the controlled induction of senescence and the SASP as integral to endometrial receptivity.

In an *in vitro* model of endometrial aging, based on HESC pretreated with thapsigargin (Tg), an endoplasmic reticulum stress inducer, we observed reduced X-gal staining and a shifted balance between subpopulations. Similarly, endometrial biopsies from older patients (>40 years) showed increased expression of senescence and inflammation markers such as CLU, PTGS1, ATF6, and IL-1β compared to the younger group (<40 years), paralleling the Tg-induced alterations in the *in vitro* model.

Next, Atomic Force Microscopy and force volume quantification were employed to confirm that decidualization decreases HESC stiffness (Young's modulus, a measure of elasticity), rendering them more permissive for embryo implantation. In fact, excessive inflammation induced by Tg increased decidual cell stiffness to levels comparable to those of undifferentiated cells. Finally, the reduction in the elastic properties of decidual cells impacts the adhesion and expansion of blastocyst-like spheroids, impairing implantation in an *in vitro* implantation model.

Together, these findings suggest that alterations in the mDec/snDec balance impair SASP-driven immune modulation and maintain a mechanically rigid, pro-inflammatory environment. By uncovering these mechanisms, we expect to deepen our understanding of the endometrium-immune dialogue and its impact on implantation success and age-associated infertility.

Key words: SASP; ER stress; Endometrial receptivity

MR-085

Impact of ageing in oro-naso-pharynx microbiota

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Human tonsils are considered comparable to the naso-pharynx-associated lymphoid tissue (NALT) of rodents, they are part of the mucosa-associated lymphoid tissue. We use them as a model to study immune-senescence as they experience involution since puberty. We have recently shown an age-dependent accumulation of a B cell subset that co-express the ecto-enzymes CD39 and CD73. These enzymes act in tandem to catabolize adenosine triphosphate (ATP) into adenosine. In turn, adenosine regulates immune function by interaction with several receptors. Our goal was to further characterize CD20+CD39highCD73+ cells and to assess putative effects of their accumulation on the bacteriological landscape of tonsils. To achieve it, we have used a combination of classical biochemical methods and advanced molecular techniques as next generation sequence (NGS). We have previously demonstrated that the majority of tonsillar CD20+CD39high-CD73+ cells were quiescent cells. Hence, we speculated that they were mostly memory B cells.

Accordingly, we show here that the frequency of IgG secreting cells (ISCs) resulted statistically higher in the CD39highCD73+ fraction when compared to the CD39lowCD73- one, as measured by enzyme-linked immunosorbent spot (ELISPOT) assay on sorted B cells into the two subsets named above ($p<0.05$, t test, 3 experiments with duplicates). To test whether such tonsillar memory B cell accumulation with age and other age-related immunological changes that we have documented through the last decade have an impact on tonsillar microbiota, we used 16-rRNA sequencing to study the tonsillar microbiota of 20 samples of different ages. Our data suggest that local microbiota composition is strongly age-associated meaning the younger the donor, the lower the diversity. Moreover, we found that tonsillar microbiota continues to mature and develop in adulthood, as opposed to gut microbiota. To conclude, we propose that the tonsillar involution that occurs since puberty, with a re-modelling of the local immune functionality with ageing, has an impact on the bacteriological diversity until later stages of life in contrast to the situation observed in niches that remain immunologically stable.

Key words: tonsils; microbiota; immunoregulation; ectoenzymes

MR-090

Pro-inflammatory and lytic profile on DC and their impact on endometrial receptivity

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During the implantation window, endometrial stromal cells adopt a secretory phenotype linked to endoplasmic reticulum (ER) expansion, leading to physiological ER stress (ERS) and activation of the unfolded protein response (UPR). ERS/UPR signaling pathways are known to initiate sterile inflammation, a process essential for endometrial receptivity and embryo implantation. We previously demonstrated that stromal cells can transmit ERS signals to monocytes/dendritic cells, inducing a pro-inflammatory and lytic phenotype that may impair the embryo implantation. The present study aims to explore the ERS/UPR pathways in stromal cells that lead to the induction of a lytic and inflammatory phenotype in dendritic cells. Therefore, a human endometrial stromal cell line (HESC) was exposed to an IRE1α inhibitor (STF-083010) or a PERK inhibitor (GSK2606414) for 1h and subsequently treated with thapsigargin (Tg, a potent ERS inducer) for 4 h. Conditioned media (CM) were collected after 48h. Monocytes of healthy women were cultivated with rh-GM-CSF+rhIL-4 for 2 or 5 days in the absence/presence of CM. On the second day of differentiation, an increase in cells exhibiting lytic cell death was observed ($p < 0.05$) with HESC+Tg conditioned media. However, no changes were observed in early apoptosis. In addition, an increase in LDH released was determined confirming lytic death. The inhibition of IRE1a, but not of the PERK pathway, prevents the effect of stressed stromal cells on the lytic death of monocyte-derived cells. Furthermore, using a model of blastocyst-like spheroid expansion, we observed that HESC+STF+Tg CM prevents the negative effect of stressed DC cell supernatant on trophoblast cell expansion. On the contrary, on the fifth day of differentiation HESC+STF+Tg CM further increased the rate of lytic cell death, indicating that inhibition of IRE1a only prevents the effect on stromal cells in the short term. Concerning dendritic cell phenotype, despite no changes in differentiation, the acquisition of an inflammatory phenotype was prevented, evidenced by a decrease in CD83 and CD86 expression, as well as in the CD86hi cell population ($p<0.05$). These results demonstrate that the IRE1α pathway mediates ER stress transmission from stromal to immune cells, promoting an inflammatory and lytic dendritic cell phenotype that can impair trophoblast expansion.

Key words: Implantation; Dendritic Cells; Endoplasmic Reticulum Stress

MR-104

Probiotic strains enhance resistance to Listeria monocytogenes through innate immune stimulation in immunosuppressed mice

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Listeria monocytogenes is a foodborne pathogen responsible for severe listeriosis in immunocompromised hosts. Current therapy relies on antibiotics, whose overuse promotes resistance and gut dysbiosis. *Lactiplantibacillus paraplantarum* SC291, SC411, and *Latilactobacillus curvatus* SC076 have emerged as promising alternatives with anti-listerial properties. In this study, we investigated whether the strains SC291, SC411 and SC076 enhance resistance against L. *monocytogenes* through innate immune stimulation in immunosuppressed mice.

Adult C57BL/6 mice were orally administered SC291, SC411 or SC076 for 7 consecutive days (108 CFU/mouse/day). Control groups included mice treated with *Lacticaseibacillus rhamnosus* CRL1505 (positive control for immunomodulatory effects), *Latilactobacillus curvatus* CRL705 (positive control for anti-listerial activity), and untreated mice. On day 8, all mice received one intraperitoneal dose of cyclophosphamide (Cy 150mg/kg). On day 11 post-Cy injection, mice were challenged orally with L. *monocytogenes* (1012 UFC/mice). Following infection, we evaluated clinical severity, total and differential leukocyte counts in blood and peritoneal lavage, neutrophil functionality, and TNF-α and IL-10 levels in serum and peritoneal lavage. Untreated mice showed high susceptibility to listerial infection, characterized by impaired innate immune response in peritoneal lavage, and reduced phagocytic cell counts. Positive control groups showed no bacterial recovery from the liver or spleen. The experimental strains yielded intermediate bacterial loads in the liver compared with the controls, whereas SC411 and SC076 completely eliminated bacterial counts in the spleen. Notably, treatment with SC411 and SC076 significantly increased peritoneal neutrophils and macrophages, circulating neutrophils, and peroxidase+ cells with respect to the untreated mice. In addition, these treatments were associated with higher TNF-α levels, and increased IL-10 production, indicating a balanced regulatory response that may prevent excessive inflammation.

In conclusion, strains SC411 and SC076 promoted resistance to L. *monocytogenes* in immunosuppressed mice by enhancing phagocytic cell recruitment and functionality, while also modulating cytokine responses. These findings support their potential use as postbiotic candidates or probiotic adjuvants to improve host defense against listerial infection under conditions of immunosuppression.

Key words: Probiotics; infection; innate immune response

MR-108

Imbalance of the immune cell's populations in the aging human ovary

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Inflammaging is a systemic and chronic inflammatory response linked with advanced age. In the ovary, inflammaging leads to decreased ovarian reserve and oocyte quality, causing infertility. Here, we investigate the mechanisms underlying the exacerbated inflammatory response during ovarian aging, focusing on the metabolic profile of follicular macrophages and a novel T lymphocyte regulatory subpopulation, CD3+CD4-CD8- (DNT), and the in vitro treatment with platelet-rich plasma (PRPr) in order to revert inflammaging. We studied FF (n=102) from women from ovodonation (21-29y) and Assisted Reproductive Treatment indication (36-42y). Mononuclear cells were isolated using Ficoll-Hypaque and adherent macrophages profile and DNT cell subpopulation tested by flow cytometry. FF macrophages from aged women showed a significant increase in IL-1β production ($p<0.05$) in comparison with the youngest group, IL-1β production also correlates negatively with the number of M2 oocytes recovered. Focusing on macrophages' (CD68+) metabolic profile, we found increased accumulation of cytoplasmic lipid droplets in mac-

rophages from aged women in comparison with ovodonors. Moreover, these macrophages had hyperpolarization of the mitochondrial membrane and, as we expected, higher reactive oxygen species (ROS) and lactate production. This leads us to infer that these M1 macrophages undergo glycolysis. Then, considering that PRPr therapy is being widely explored to improve ovarian function and increase the chances of pregnancy, the isolated follicular macrophages from aged women were treated in vitro with PRPr from fertile women to evaluate its modulatory effects. We observed a reduction in the frequency of CD68+IL-1β+ cells in comparison with those cultured without treatment. Also, treated macrophages showed a reduction in the levels of ROS and lactate production and lower lipid droplets accumulation. Finally, we focused on a novel DNT lymphocyte subpopulation that is emerging as effector cells capable of mediating immune tolerance in the female reproductive system. We found that FF from aged women displayed a reduced frequency of DNT, in comparison with FF from younger women ($p<0.05$).

Based on these results, we conclude that women of advanced reproductive age show a dysregulated ovarian immune microenvironment that may contribute to chronic inflammation and alter oocyte quality.

Key words: Inflammaging; foamy macrophages; immunometabolism; double negative T cells; aging

MR-148

Indole-3-propionic acid (IPA) promotes T cell migration into the intestinal intraepithelial compartment

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Background

Inflammatory bowel disease (IBD) is characterized by aberrant immune responses that compromise the epithelial barrier. Current therapies focus on reducing inflammation but do not offer definitive cures. Intestinal epithelial cells (IECs) play a crucial role in orchestrating immune responses within the intestinal mucosa by sensing metabolites produced by the microbiota. Tryptophan metabolites have been shown to influence IECs and promote intestinal mucosal homeostasis, although the precise mechanisms remain unclear. This study investigates the cellular and molecular mechanisms by which indole-3-propionic acid (IPA), a microbiota-derived tryptophan metabolite, promotes intestinal mucosal integrity. We hypothesized that IPA enhances T cell migration and recruitment to the intestinal intraepithelial compartment mediated by E-cadherin upregulation, thereby strengthening intestinal mucosal barrier integrity.

Methods: Wild-type C57BL/6 mice received oral IPA treatment (0.5 mg/100 µL) daily for four consecutive days, with lymphocyte isolation from intestinal mucosa performed on day five. Flow cytometry analysis was conducted following IPA treatment to evaluate immune cell populations in mesenteric lymph nodes, small intestine, large intestine, and Peyer's patches. Confocal microscopy was employed to assess E-cadherin expression in IECs and immune cell distribution within the intestinal mucosa.

Results: IPA treatment significantly increased TCRb CD4 (Mean Control= 2021 vs. Mean IPA= 3481; p=0,0165), TCRb CD8aa (Mean Control= 260.8 vs. Mean IPA= 1021; p=0,0286) and TCRgd CD8aa (Mean Control= 5922 vs. Mean IPA= 29447; p= 0,0286) cell populations in the intraepithelial compartment of the small intestine. Confocal microscopy analysis revealed enhanced E-cadherin expression in the duodenum of IPA-treated mice.

Conclusion: IPA treatment increased TCRb CD4, TCRb CD8aa and TCRgd CD8aa cell recruitment to the epithelium. This appears facilitated by increased E-cadherin expression. Results suggest IPA promotes intestinal barrier integrity through immune cell modulation, offering insights for IBD therapeutic strategies.

Key words: Microbiota; metabolites; Intraepithelial; lymphocites

MR-151

Pregnancy shapes maternal monocyte immunometabolic profile with higher fatty acid utilization: The trophoblast-macrophage communication for M2 polarization

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To sustain immune homeostasis at the maternal-fetal interface, cytotrophoblast cells (Tb) contribute to the polarization of monocytes into M2-like decidual macrophages, a process tightly linked to immunometabolic reprogramming. We previously showed that pregnancy induces immunometabolic adaptation of maternal monocytes, modulating their phenotype at early gestation. Moreover, trophoblast-derived soluble factors modulate nutrient uptake. Here we investigated how Tb factors regulate macrophage metabolism and their association with the acquisition of an M2-like profile. Monocytes from non-pregnant and 16-20w pregnant women were analysed by flow cytometry for long-chain fatty acid (LCFA) uptake, lipid droplets (LD), and mitochondrial mass/potential using Bodipy-FL-C12, Bodipy493/503, MitoSpyGreen, and CMXRos probes. Efferocytosis was assessed with FITC-labeled latex beads and flow cytometry. Monocyte-derived macrophages (M0) from non-pregnant women were cultured with conditioned media from the HTR8/SVneo cell line (Tb-CM) with/without metabolic inhibitors (2DG, oligomycin, Etomoxir). Gene expression, LD–mitochondria colocalization, and phenotype were assessed by RT-qPCR, confocal microscopy, and flow cytometry. Pregnant women exhibited higher plasma lactate levels ($p<0.05$) compared with non-pregnant controls, without changes in glucose concentration. Monocytes from pregnant women produced more lactate ex vivo and displayed significantly increased LCFA uptake ($p<0.05$), with no changes in mitochondrial mass/potential or LD content. FAO inhibition with Etomoxir abrogated the pregnancy-associated enhancement of efferocytosis. Consistently, Tb-CM increased CPT1 and DGAT expression ($p<0.05$) in macrophages, key regulators of FAO and lipid droplet synthesis, promoted LD–mitochondria colocalization (Manders M1/M2, $p<0.05$) and increased RARα transcripts ($p<0.05$), a retinoic acid-activated transcription factor linked to FAO and mitochondrial respiration. Eto led to a greater increase in LD formation compared to non-stimulated M0, impaired Tb-CM–induced upregulation of CD209 and CD163, and enhanced CD86 expression. These findings demonstrate that pregnancy reshapes monocyte and macrophage lipid metabolism and support a role for trophoblast-derived factors in driving FAO and lipid storage programs that favor anti-inflammatory macrophage polarization.
Key words: Immunometabolism; pregnancy; Monocyte

MR-160

Probiotic Lentilactobacillus kefiri CIDCA8348 Attenuates Inflammation in a Murine Model of Endometritis

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Endometritis (E) is the inflammation of the uterine lining (endometrium) as an immune response usually associated with an infectious process. The presence of bacterial endotoxins in the uterine cavity triggers immune responses such as leukocyte recruitment and the production of pro-inflammatory cytokines, affecting normal endometrial functions and leading to reproductive disorders. We previously demonstrated that prophylactic treatment with the potentially probiotic *Lentilactobacillus kefiri* CIDCA8348 (Lk48) reduces fetal resorption rates and attenuates apoptosis and inflammatory cell infiltration in both uterus and cervix in a murine model of LPS-induced endometritis. Here we aimed to evaluate the ability of Lk48 to attenuate the uterine inflammatory response induced by LPS.

Virgin C57BL/6 female mice, aged 8-12 weeks, were pretreated intravaginally with Lk48 (10^8 CFU/ml) or milk (control) every 72 hours for two weeks. 24-72 hours after the last administration, diestrus females were challenged intravaginally with LPS (50 µg per mouse) to induce E. Two experimental groups were studied. In the first one, uteri were collected 24 hours post-E induction and neutrophil infiltrate was measured by flow cytometry. In the other group, females were mated with BALB/c males 24 hours after E induction and sacrificed on day 12 of gestation. Both groups were used to measure uterine MPO specific activity and pro-inflammatory cytokine expression (IL-6, TNF, IL-1β) by qPCR.

Our results show that LPS-induced endometritis increased relative mRNA expression of IL-6, IL-1β, TNF, as well as MPO activity, and neutrophil recruitment in mouse uteri. Although no statistically significant differences were found, Lk48-treated mice consistently showed lower uterine levels of pro-inflammatory cytokines and reduced neutrophil infiltration (MPO and $CD45^{+}Ly6G^{+}$) compared to controls.

In summary, *Lentilactobacillus kefiri* CIDCA8348 (Lk48) shows promise in alleviating LPS-induced uterine inflammation in a mouse model of Endometritis.

Key words: Endometritis; Lentilactobacillus kefiri; uterine inflammation; pro-inflammatory cytokines; neutrophils

MR-162

Chronic exposure to glyphosate-based herbicides associates with reduced semen quality, semen inflammation and oxidative stress. A new threat for human reproduction?

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The extensive application of glyphosate-based herbicides (GBHs) has raised substantial concerns regarding their potential impact on reproductive health. However, the existing evidence remains scarce and inconclusive. Notably, to the best of our knowledge, no prior studies have directly assessed the potential effects of GBHs exposure on male fertility in humans. Thus, we conducted a prospective study to evaluate sperm quality, as well as inflammatory and oxidative stress biomarkers in the semen of men chronically exposed to GBHs and of non-exposed controls. A total of 39 reproductive-aged men reporting occupational chronic exposure to GBHs and 25 non-exposed control individuals were enrolled. Semen samples were collected by masturbation, and sperm quality was assessed following the World Health Organization (WHO) guidelines. Levels of various cytokines and chemokines, total leukocytes and their subsets, sperm oxidative stress,

and sperm apoptosis/necrosis were quantified using flow cytometry. Men chronically exposed to GBHs showed significantly lower total sperm counts and reduced normal morphology compared to non-exposed controls. However, sperm motility, viability, and rates of apoptosis/necrosis were comparable between groups. Additionally, semen samples from GBH-exposed individuals showed significantly elevated levels of IL-6 and TGF-β, increased frequencies of sperm producing reactive oxygen species (ROS), and higher counts of total leukocytes and granulocytes compared to controls. Our results indicate that chronic exposure to GBHs is associated with impaired sperm quality, semen inflammation, and oxidative stress, suggesting that GBH exposure may constitute a significant risk factor for male infertility.

Key words: Glyphosate; Semen; Seminal Plasma; Inflammation

MR-165

NR4A Nuclear Receptor Expression and Differential Cytokine Profiles in Paternal-Maternal PBMC Co-cultures from URSA Patients

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Spontaneous abortion is the most common complication in the early stages of pregnancy. Recurrent Spontaneous Abortion (RSA) is defined as the occurrence of three or more consecutive losses before 20 weeks of gestation. For a majority of these patients there is no medical explanation, leading to their classification as Unexplained Recurrent Spontaneous Abortion (URSA).NR4A receptors are a family of ligand-independent nuclear receptors that act as transcription factors. These receptors are implicated in diverse cellular functions, including apoptosis, proliferation, angiogenesis, inflammation, metabolism, and developmental processes. Our previous studies have reported that URSA patients present significantly lower levels of NR4A2 and NR4A3 expression compared to fertile control women.In this study, to investigate whether these receptors might play a role in early reproductive stages like fertilization and implantation, we performed co-cultures of Peripheral Blood Mononuclear Cells (PBMC) from URSA couples and fertile control couples. These cultures were conducted under in vitro conditions that simulated early reproductive interactions. The URSA co-cultures showed a notable increase in NR4A1, NR4A2, and NR4A3 expression, as well as TNFα and IL10, when compared to the fertile control group.We also analyzed the expression of NR4A1, NR4A2, and NR4A3 mRNA in 60 URSA patients, and evaluated their relationship with the application of immunoparental therapy (IPT), the occurrence of a new pregnancy, and its successful outcome. Statistical analyses showed that the combined basal expression of NR4A did not significantly predict pregnancy or live birth.However, patients who underwent IPT exhibited a significantly higher probability of achieving pregnancy compared to those who did not receive this intervention.These findings suggest that NR4A receptors might contribute to immunological tolerance during the early stages of human reproduction.

Key words: URSA; NR4A; FERTILITY

MR-176

Memory T Cell Dynamics in Clostridioides difficile infection: The Role of BLIMP-1

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Clostridioides difficile infection (CDI) arises following disruption of the intestinal microbiota and is primarily treated with antibiotics, which further exacerbate microbial imbalance. The high recurrence rate of CDI may be related to suboptimal generation or maintenance of immunological memory. Memory T cell subsets—central memory (TCM), effector memory (TEM), and tissue-resident memory (TRM)—play pivotal roles in mucosal immunity. TRM cell regulation by transcription factors such as B lymphocyte–induced maturation protein-1 (BLIMP-1) has been linked to protection against colitis by reducing pro-inflammatory responses. Moreover, BLIMP-1 expression in Treg cells limits the production of Th17-associated cytokines, preserving suppressive function. In CDI, a type 3 immune response is triggered, in which BLIMP-1 may help limit excessive inflammation. This study aimed to characterize memory T cell subsets during CDI and evaluate the role of BLIMP-1 in this context. A murine model of CDI was used. Mice were clinically scored daily and sacrificed at 2, 5 and 8 days post-infection (dpi). Colon and cecum were homogenized for western blotting (BLIMP-1), and lamina propria mononuclear cells (LPMCs) and mesenteric lymph node cells (MLNCs) were isolated and used for multiparametric flow cytometry (CD3, CD4, CD8, CD69, CD44, CD62L, BLIMP-1, IL-17). Data were analyzed using ImageJ and FlowJo V10 software, respectively. Significance was determined by unpaired t test and Two Way ANOVA (Bonferroni post hoc) ($p<0.05$). We observed a predominance of CD8+ T cells across TRM, TCM, and TEM subsets in LPMCs from infected mice at 8 dpi. At 2 dpi, TCM cells were more abundant in both MLNCs and LPMCs, while TEM cells predominated at 8 dpi. BLIMP-1 expression in the colon increased during the acute phase of CDI and progressively decreased by 5 dpi. Infected mice showed no significant rise in IL-17$^+$ TRM frequency compared to controls, independently of BLIMP-1 expression. Within the BLIMP-1+ TRM subset, over a 75% cells were negative for IL-17, suggesting a suppressor phenotype.

Our findings indicate that intestinal TRM cells are dynamically modulated during CDI and could play a predominantly suppressive role influenced by BLIMP-1 expression. Further studies are required to determine whether BLIMP-1 acts as a marker of inflammation or of infection control.

Key words: Clostridioides difficile; Tissue-resident memory T cells; BLIMP-1

MR-201

Semen inflammation disrupts the uterine gene expression program both shortly after copulation and during the peri-implantation stage, leading to decreased pregnancy outcomes

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Seminal fluid delivers essential signals to the female genital tract, stimulating the expression of factors that modulate the immune environment and promote embryo implantation and pregnancy development. Consequently, conditions that impair semen quality, such as chronic prostatitis, may compromise this ability. Using an animal model of Experimental Autoimmune Prostatitis (EAP), we evaluated how semen inflammation affects the seminal fluid ability to induce uterine gene expression of cytokines, chemokines and growth factors shortly after copulation and during the peri-implantation stage, as well as its effects on male fertility. C57BL/6 male mice were immunized with prostate antigens plus adjuvants to induce EAP, while control animals received saline. Twenty-four days later, mating experiments were conducted with BALB/c females. Females were euthanized at 8–16 hours, 4.5 days, and 19 days post-copulation, and the uterine expression of different cytokines, chemokines, and embryotropic factors, along with fertility parameters, were evaluated. Statistical analyses were performed using the Mann-Whitney test or ANOVA. At 8-16 h post-copulation, females mated with EAP males showed

a marked uterine pro-inflammatory signature with respect to females mated with controls, revealed by elevated expression of Il1β, Ifng, Il17a, Cxcl1 and Cxcl2, whereas reduced expression of Il10. Interestingly, this altered pattern persisted during the peri-implantation stage (day 4.5 after copulation), with additional upregulation of Cxcl5, Cxcl7, Cxcl10, and Cox2. Additionally, females mated with EAP males showed significantly decreased uterine expression of key implantation and decidualization factors, including Lif, Il6, Vegf, Igfbp1, and Prl, while the blastomere pro-apoptotic factor Trail was increased. Moreover, these females showed significantly lower fertility indices and higher rates of pre- and post-implantation embryo loss compared with controls.

Our results indicate that semen inflammation, consequence of chronic prostatitis, disrupts the transcriptional program of the uterine mucosa induced by seminal fluid early after copulation as well as during the peri-implantation stage. The shift toward a sustained pro-inflammatory state, accompanied by reduced expression of embryotrophic and decidualization signals, impairs embryo implantation and development resulting in decreased pregnancy outcomes.

Key words: Chronic prostatitis; seminal plasma; uterus; immune tolerance; implantation.

MR-208

Lactate treatment ameliorates remote organ damage induced by intestinal ischemia- reperfusion injury.

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Intestinal ischemia/reperfusion (I/R) injury is a common clinical event with high mortality in patients undergoing surgery, trauma or transplantation and is often associated to multi-organ dysfunction. Lactate, previously considered a metabolic waste product, has shown several biological properties including beneficial effects in pathology models involving inflammatory and oxidative stress. The aim of this study was to evaluate the protective potential of lactate administration in an intestinal I/R murine model, where inflammation and oxidative stress contributes to tissue damage. Adult male BALB/c mice were subjected to intestinal ischemia-reperfusion injury by reversible occlusion of the superior mesenteric artery, consisting of 45 minutes of ischemia followed by 4hs of reperfusion. Lactate (200mM) was administered either in drinking water for 96hs prior to ischemia or via intraperitoneal route (445mM - 10ml/Kg) 30 minutes before ischemia. Intestine, lung and kidney samples were collected for histological analysis and evaluation of inflammatory markers. Lactate pre-treatment by oral or systemic route showed reduced effect on intestinal histological damage evaluated by Park/Chiu score. Nevertheless histological analysis showed lower lung histopathological score (I/R vs. I/R+oral lactate, $p< 0.0001$; I/R vs. IR +systemic lactate, $p< 0.0005$) and renal histopathological score (I/R vs. I/R+ oral lactate, $p< 0.0001$; I/R vs. IR + systemic lactate, $p< 0.0005$). Assessment of intestinal cytokines showed increased IL-10 levels in systemic lactate administration group ($p<0.05$ vs. I/R group) whereas no differences were found for oral lactate administration group. Our preliminary results indicated that lactate pretreatment, administered either by oral or systemic route, reduced remote organ damage in an intestinal ischemia reperfusion murine model. Further studies are needed to address the underlying mechanisms.

Key words: Lactate; Intestine; ischemia; reperfusion

Onco Immunology

OI-004

Tumor cells and TAM-like macrophages cooperate to induce TIM-3 expression and functional exhaustion in NK cells

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Natural killer (NK) cells have recently gained attention as promising tools in cancer immunotherapy due to their potent antitumor activity. However, within the tumor microenvironment (TME), NK cells often become exhausted, displaying a phenotype characterized by the upregulation of inhibitory immune checkpoints (NKCIC) such as TIM-3, TIGIT, LAG-3 and PD-1 and impaired effector functions. While blocking certain NKCIC can reinvigorate NK cell activity, the stimuli and mechanisms leading to the upregulation of NKCIC expression in the TME remain poorly understood. Therefore, this study focused on identifying the factors within the TME that can promote the expression of the NKCIC TIM-3 in NK cells and determining the impact of this upregulation on their effector functions. We performed in vitro co-cultures of peripheral blood mononuclear cells from healthy donors with the human clear cell renal carcinoma cell line 786-O. Also, triple co-cultures were established with 786-O cells, NK cells, and macrophages polarized toward a tumor-associated phenotype (TAM-like). After 2 days of culture, TIM-3 expression and NK cell functionality (degranulation and IFN-γ production) were assessed by flow cytometry. We observed that co-culture with 786-O tumor cells induced TIM-3 expression on NK cells only when direct cell-cell contact was maintained. This interaction also led to an overall and permanent loss of effector functions in the NK cell population. However, within this impaired landscape, TIM-3$^+$ NK cells retained higher metabolic and functional activity, including increased glucose uptake, degranulation, and IFN-γ production, compared to the TIM-3$^-$ population. Furthermore, the presence of TAM-like macrophages amplified the upregulation of TIM-3 triggered by 786-O cells. These findings suggest that TIM-3 may initially act as a marker of early NK cell activation. However, its sustained expression, driven by continuous contact with tumor cells and TAM-like cells in the presence of increasing amounts of its ligands in the TME, might contribute to a progressive functional exhaustion. We propose that this mechanism constitutes a tumor-driven immune escape strategy that selectively impairs the antitumor response of NK cells via TIM-3.
Key words: Natural killer cells; Tumor microenvironment; Inhibitory immune checkpoints

OI-007

Key role of the adaptive immune response in the anti-tumoral effect of the histamine H4 receptor inhibition in murine breast cancer

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The physiological and pathological effects of histamine (HIS) involve four receptors (H1R-H4R). These as well as elevated levels of HIS were reported in different tumors. We previously showed the antitumor effect of the H4R blockade employing the specific antagonist JNJ-7777120 (JNJ) on the 4T1 murine breast cancer cell line, both in vitro and in a syngeneic model in vivo. In the latter,

such activity was coupled by an early increased recruitment of CD8 lymphocytes in the neoplastic tissue. In this work, we attempted to shed light on the mechanisms by which JNJ directly acts on malignant cells and those implicated in the modulation of the immune response. In vitro, we found that the JNJ (10 µM) treatment induced a substantial reduction ($p < 0.01$, $n = 4$) in the migratory capacity of 4T1 cells, evaluated by the wound healing assay. Also, we noticed a marked decrease ($p < 0.01$, $n = 3$) in the oxidative metabolism, as determined by DCFH-DA oxidation measured by flow cytometry (FC). These effects were accompanied by an increase in lactate ($p < 0.01$, $n = 3$) release, colorimetricaly assayed, and a prompt activation of the ERK signaling pathway ($p < 0.05$, $n = 4$), as evaluated by Western blot. In vivo studies were carried out by subcutaneous inoculation of JNJ-treated or control 4T1 cells (105) in BALB/C or lymphocyte-lacking Rag1 mice. In the former, ex vivo studies with JNJ-treated tumor-derived cells showed augmented proliferation of CD4+ and CD8+ lymphocytes ($p < 0.001$) compared to control tumors, as determined by CFSE staining and FC analysis in at least 3 independent experiments. Of note, after a 24-h ex vivo coculture with 4T1 cells, JNJ-tumor-derived CD8+ lymphocytes exhibited, although not statistically significant, a higher trend in cytolytic capacity, as determined by Lamp1 staining by an FC analysis, respect to control-tumor-isolated ones. As in vitro, supernatants of tumor cells from JNJ-treated BALB/C animals presented increased levels of lactate ($p < 0.05$, $n = 3$). The most relevant thing was to verify a comparable tumor growth in both groups in Rag1 mice ($n = 3$), indicating the importance of adaptive immune response on JNJ effect. In conclusion, our results showed the dual effect of HIS through H4R modulating not only tumor cells development, but also the immune microenvironment.

Key words: Breast cancer; 4T1 cells; Histamine; H4R; CD8+ lymphocytes

OI-008

Humoral Immune Response and B Cell Contribution to Tumor Immunity in a Mouse Melanoma Model

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The role of B cells in tumor immunity remains controversial. Depending on their phenotype and the microenvironment, B cells can exert either pro- or anti-tumorigenic functions. In this study, using a murine melanoma model, we evaluated the localization and effector functions of tumor-infiltrating (TI) B lymphocytes. C57BL/6 mice were intraperitoneally injected with 4×10^5 B16F10-OVA tumor cells, and on day 13, tumors, spleens, and bone marrow were collected. Using immunofluorescence microscopy, we observed B cells clustered in defined regions within the tumor, most of them displaying a naïve phenotype (IgD^+) and located near $CD4^+$ T lymphocytes. As antibody production is a unique function of terminally differentiated B cells, we assessed the humoral response by evaluating antibody-secreting cells (ASCs) by ELISpot in all tissues. Although tumor-specific ASCs in tumors were predominantly IgM ($p<0.01$), analysis of total ASCs revealed a larger infiltration of cells expressing IgG and IgA isotypes compared to IgM. We also identified tumor-specific ASCs in the spleen, mainly of the IgM isotype, whereas in the bone marrow there were no significant differences in the numbers of tumor-specific ASCs among the three isotypes. Analysis of secreted immunoglobulins in culture supernatants by LEGENDplex demonstrated that TI B cells mainly produced IgG2c and IgG2b, while IgM was more abundant in spleen cultures after 24 hours of incubation with either complete medium or CpG-ODN. To assess the impact of B cells on tumor progression, we performed the same model in µMT mice. No significant differences were observed in tumor weight ($p=0.0967$) or leukocyte infiltration between µMT and C57BL/6 mice. However, the absence of B cells resulted in a reduced frequency of TI OVA-specific $CD8^+$ T cells ($p<0.05$), without affecting the exhaustion profile of this population. Interestingly, µMT mice exhibited a higher frequency of $TNF\alpha^+$ and $IFN\gamma^+$ TI T and NK cells following PMA-ionomycin stimulation compared to C57BL/6 mice. These findings suggest that B cells may modulate

the local immune response through interactions with other immune cell populations. Further studies are needed to determine whether targeting specific B cell interactions or functions within the tumor microenvironment could influence tumor growth.

Key words: B cell; Tumor microenvironment; Antibody-secreting cells

OI-014

SENESCENT FIBROBLASTS IMPAIR NK CELL FUNCTION AND PROMOTE A PRO-TUMORAL MYELOID SHIFT IN THE TUMOR MICROENVIRONMENT

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Senescence constitutes a key tumor suppressor mechanism. However, accumulation of senescent cells in tissues during aging contributes to immune dysfunction and cancer development. We previously reported that murine senescent fibroblasts (SenFb) reduced the frequency of IFN-γ-producing NK cells, skewed macrophages toward an M2-like phenotype, and enhanced CT26 tumor growth in vivo. Also, SenFb-co-injected tumors displayed reduced frequencies of tumor-infiltrating NK cells that displayed decreased NKG2D expression. In this study, we further investigated the impact of SenFb on the tumor microenvironment (TME) and explored their effects in a human model. CT26 tumor cells (2.2×10^5) were co-injected subcutaneously with SenFb or control fibroblasts (ConFb) (1×10^6) into 11-week-old BALB/c mice. On day 12, tumors were processed for flow cytometry. We observed a trend toward higher percentages of $CD11b^+$ cells and macrophages ($CD11b^+F4/80^+$) in intratumoral $CD45^+$ leukocytes in the SenFb group. Within the $CD11b^+$ population, we observed a significant increase in monocytic-myeloid-derived suppressor cells (M-MDSC: $CD11b^+Ly6G^-Ly6Chigh$). Macrophages ($CD11b^+F4/80^+$) from SenFb coinjected tumors showed trends toward reduced expression of CD86 and increased MARCO levels, with no differences in CD206 or CD274. Accordingly, the MARCO/CD86 ratio was significantly increased ($p<0.05$), consistent with a shift toward an M2-like profile. To assess the impact of senescence in a human model, we induced senescence in MRC-5 fibroblasts using 1 µM etoposide for 48 h, followed by a 5-day recovery. Peripheral blood mononuclear cells (PBMC) were cultured in the presence of control or senescent MRC-5 cells or their respective conditioned media (ConCM, SenCM) overnight. K562 target cells were added during the last 4 h to evaluate NK cell degranulation by flow cytometry (gated as $CD3^-CD56^+CD107a^+$). SenFb and their conditioned media significantly impaired NK cell degranulation compared to ConFb ($p<0.05$).

These results suggest that SenFb reshape the TME by promoting the accumulation of suppressive myeloid subsets and functionally impairing NK cells, highlighting senescent cells as potential therapeutic targets to enhance antitumor immunity.

Key words: senescence; macrophages; NK cell

OI-023

Divergent Prognostic Implications and Cellular Expression Patterns of Vav proteins in Melanoma

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The most dangerous form of skin cancer is melanoma, which is associated with an increasing incidence in the population. Vav proteins are guanosine nucleotide exchange factors (GEFs) of the Rho GTPase family. As GEFs, they modulate processes highly associated with the development of cancer and metastasis, mostly through their GTPase regulatory function, but their involvement in melanoma is yet to be elucidated. Using bioinformatic approaches and publicly available datasets, we analyzed bulk RNA-seq data from The Cancer Genome Atlas (TCGA) to assess survival and immune-related transcriptional correlations. High expression of Vav1 or Vav3 was significantly associated with better overall survival compared to low expression of these proteins ($p<0.01$ and $p<0.001$, respectively), whereas high Vav2 expression correlated with worse survival ($p<0.05$). Bulk RNA-seq analysis revealed a strong correlation between Vav1 expression and immune-related transcriptional signatures, suggesting predominant expression in immune cells. To confirm this, we analyzed melanoma single-cell RNA-seq data (GSE115978). Vav1 expression was almost exclusively restricted to immune cells, whereas Vav2 and Vav3 were expressed in both tumor and immune cells. A significantly higher proportion of tumor cells expressed Vav2 or Vav3 compared to Vav1 ($p<0.001$). Over-representation analysis (ORA) revealed that $Vav1^+$ immune cells were enriched in pathways related to antigen presentation, T cell receptor signaling, interleukin signaling, and neutrophil activity, while $Vav1^+$ tumor cells retained a predominantly immune-interaction profile. In contrast, $Vav2^+$ tumor cells were mainly associated with cell cycle regulation, DNA repair, and RNA processing, whereas $Vav3^+$ tumor cells showed enrichment in extracellular matrix organization, adhesion, and related structural pathways.

These results indicate that the favorable prognosis linked to high Vav1 or Vav3 expression arises from different mechanisms. $Vav1^+$ cells are mainly immune and enriched in antigen presentation and T cell pathways, suggesting an immune-driven effect. $Vav3^+$ tumor cells are enriched in extracellular matrix and adhesion pathways, consistent with a tumor-suppressive role. In contrast, high Vav2 expression correlates with poor survival, with $Vav2^+$ tumor cells showing proliferative and cell-cycle signatures, supporting a tumor-promoting function.

Key words: melanoma; Rho GTPases; Vav proteins

OI-026

UNRAVELLING THE COMPLEXITY OF THE INTERACTIONS AMONG VACCIMEL, BCG AND BLOOD MONOCYTES

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We developed VACCIMEL, an adjuvant immunotherapy (IT) composed of four irradiated cutaneous melanoma (CM) cell lines plus BCG and GM-CSF. Phase I/II trials showed safety and improved distant metastasis-free survival (DMFS) in stages IIB–III CM patients compared to IFN-α2b. A 5-year follow-up confirmed longer DMFS, with outcomes comparable to anti-PD-1 IT. Notably, five pts relapsing after VACCIMEL achieved complete responses with anti-PD-1 IT, without added toxicity. We demonstrated that VACCIMEL induced a polyclonal cellular immune response against melanocytic differentiation antigens (MDA), cancer-testis antigens (CTA), and neoantigens derived from both the patient's tumor and VACCIMEL. To explore early steps of immunization with VACCIMEL, BCG, and GM-CSF, we studied blood monocytes (Bl-Mo), which rapidly migrate to inflamed sites and display functional plasticity. In vitro cocultures showed that ~70% of Bl-Mo promptly captured BCG and VACCIMEL-derived material, confirmed by transmission electron microscopy and flow cytometry. Phagosomes evolved over time from small vesicles to large phagolysosomes with detectable debris, and resolved by 96 h into lysosomal structures. After 24–48 h of VACCIMEL phagocytosis, and in a MOI-dependent manner (0.4–0.04), BCG addition blocked the transition of Bl-Mo from classical (CD14+CD16–) to intermediate phenotype (CD14+CD16+) and markedly reduced expression of antigen presentation molecules (CD86, HLA-I/II, CD11c). The true BCG MOI in situ remains uncertain, since BCG is also

phagocytosed by neutrophils, draining them from the injection site. In cross-presentation assays for gp100 MDA to a specific T cell clone (readout: IFNγ release), Bl-Mo captured and processed gp100 and presented the peptide to T cells, a process diminished by BCG plus GM-CSF.

We suggest that Bl-Mo not phagocytosing BCG retain antigen presentation capacity, while BCG-loaded Bl-Mo provide inflammatory stimuli, jointly sustaining immunity. The interaction between VACCIMEL and the immune system is a dynamic process, and previous clinical studies showed that immunity to tumor antigens built with successive vaccinations. Thus, a limitation of this work is that it only characterized the first encounter between VACCIMEL and Bl-Mo of the innate immune system.

Key words: VACCIMEL; Monocytes; BCG; Phagocytosis; Cross-presentation

OI-033

Adaptive NK Cells Enhance Dendritic Cell Maturation Markers in a HER2+ Breast Cancer Model

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A subpopulation of Natural Killer (NK) cells, termed adaptive NK cells (adNK), emerged in response to human cytomegalovirus infection. This subpopulation exhibits greater CD16-mediated cytokine production, as well as increased longevity and persistence compared to conventional NK cells (cNK), making them promising candidates for cancer immunotherapy. In previous studies, we demonstrated a higher functionality of adNK cells (higher IFN-γ, TNF-α, and CD107a expression) compared to cNK, employing HER2+ breast cancer (BC) cells and clinically used monoclonal antibodies (mAbs) such as Trastuzumab (TRZ) and Pertuzumab (PER) as a model. However, it remains unknown whether the enhanced functionality of adNK cells leads to an increased release of tumor antigens, thereby promoting the activation of dendritic cells (DCs) and, consequently, naïve T lymphocytes. In this study, we analyzed the interaction between NK cells and CD14+ purified monocytes-derived DCs in the same HER2+ BC model, using either total purified NK cells or the individually sorted subpopulations. When NK cells were exposed to tumor cells opsonized with TRZ, the proportion of activation markers CD25 ($p<0.0001$), CD69 ($p<0.0001$), and CD137 ($p<0.0001$) increased, along with a decrease in CD16 ($p<0.0001$), in total NK cells and in both subpopulations. NK cells, in turn, promoted an increase in the expression of maturation markers CD80 ($p=0.002$), CD83 ($p<0.0001$), and CD86 ($p<0.0001$) in DCs. Moreover, adNK cells promoted the upregulation of all three maturation markers in DCs, whereas cNK cells induced only CD80 expression ($p<0.0001$). Comparatively, adNK cells induced greater CD83 expression than cNK cells ($p=0.0285$). Reciprocally, DCs induced an increase in the expression of HLA-II in total NK cells ($p<0.0001$) and in both subpopulations, as well as in CD25 expression in cNK cells ($p=0.0033$). These preliminary findings suggest greater maturation of DCs in the presence of adNK cells, potentially leading to a more efficient activation of the adaptive immune response.

Key words: Adaptive NK cells; Dendritic cells; HER2+ breast cancer

OI-037

POTENTIATION OF VACCIMEL IMMUNOTHERAPY WITH ANTI-PD-1 MONOCLONAL ANTIBODIES

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We developed VACCIMEL, an adjuvant immunotherapy (IT) administered with BCG and GM-CSF to foster the immune process. In phase I/II trials, VACCIMEL showed safety and prolonged distant metastasis-free survival (DMFS) in stages IIB–III cutaneous melanoma (CM) patients (pts) compared to medium-dose IFN-α2b. A 5-year follow-up confirmed longer DMFS, with outcomes comparable to anti-PD-1 IT. Remarkably, 5 pts relapsing after VACCIMEL achieved complete responses (CR) with anti-PD-1 IT without added toxicity. VACCIMEL expanded T cells against shared tumor-associated antigens (TAA) and neoantigens, as it was demonstrated in PRE and POST-VAC PBMC cultured ex vivo with HLA-restricted peptides for 12 days and quantified by IFNγ ELISPOT. Assuming that anti-PD-1 synergized VACCIMEL responses, we performed analogous ex vivo assays, stimulating available PBMC from 8 pts treated with VACCIMEL IT, with HLA-restricted peptides ± nivolumab (10 µg/ml) and evaluated their response by IFNγ ELISPOT. Two pts (#2, #5) were studied in depth. Overall, IFNγ+ responses increased for 13/30 peptides tested. Pt#2 who relapsed 29 months after VACCIMEL, received pembrolizumab (200 mg Q3W), and achieved CR lasting >24 months. PBMC collected after 10 months of therapy (anti-PD-1 IT-PBMC) retained reactivity to Tyrosinase and gp100, which was induced only after vaccination, since they had been detected in POST but not in PRE-VAC PBMC. Compared to PRE and POST-VAC PBMC, anti-PD-1 IT-PBMC had higher Ki67+ CD4 and CD8 T cells, more HLA-DR+, CD69+, CD137+ activation markers, the highest effector memory and lowest TEMRA fractions. Upon peptide stimulation, POST-VAC and anti-PD-1 IT-PBMC similarly increased effector memory and reduced central memory/naïve T cells, which prevailed in PRE-VAC PBMC. Anti-PD-1 IT PBMC after ex vivo TAA stimulation enhanced their activation phenotype and showed the lowest proportion of PD-1+ T cells. In Pt#5 (HLA-A0201+), PRE and POST-VAC PBMC were cultured ± nivolumab and tested in a calcein-lysis assay targeting an HLA-A0201+ CM line expressing relevant TAA. Nivolumab increased POST-VAC PBMC lysis from 11.5% to 24.2% (E:T 30:1), while PRE-VAC PBMC remained weakly cytotoxic. These results show that VACCIMEL-induced T cell responses persist long after vaccination and remain functional under anti-PD-1 IT, suggesting that VACCIMEL expands clones whose enhanced lytic activity may contribute to favorable outcomes with checkpoint blockade.

Key words: VACCIMEL; Immunotherapy; anti-PD-1; Cutaneous melanoma

OI-041

Cutaneous Melanoma Patients with Longer Distant Metastasis-Free Survival After Adjuvant Immunotherapy present primary Lesions enriched in Dendritic Cells and Naïve T Lymphocytes

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Introduction: Analysis of the tumor immune microenvironment in primary cutaneous melanoma can help identify early tissue biomarkers associated with response to adjuvant immunotherapy. Methods: This retrospective study included a cohort of stage II-III cutaneous melanoma (CM) patients with available primary biopsies, who had enrolled for VACCIMEL clinical trials in the adjuvant setting (n=24). Distant Metastasis-Free Survival (DMFS) was selected as the primary endpoint. Two patient cohorts were defined: one with a favorable outcome (GO, median DMFS 130.0 months) and another with an unfavorable outcome (BO, median DMFS 8.5 months). Immunohistochemistry analysis was performed to determine the expression of relevant biomarkers related to tumor cell biology and immune cells, both in the tumor and peritumoral areas. Results: BO patients exhibited highly proliferative Ki-67+ tumor cells (p=0.03). In contrast, GO patients showed the most favorable biomarkers for prolonged DMFS, including higher abundance of PNAd+ High Endothelial Venules (HEVs) (p=0.02), peri- and intra-tumoral CD11c+ cells (p=0.002 and p=0.06), and increased levels of CD8+ and CD20+ lymphocytes (p=0.003 and p=0.043). Interestingly, GO patients were also significantly enriched in CD62L+ cells in the peritumoral area (p=0.008), indicating a difference in the infiltration of naïve lymphocytes into primary CM. Discussion: The comparative analysis of primary CM in GO and BO patients revealed notable differences in immune cell composition. This study underscores the importance of evaluating resected primary tumors from CM patients and suggests a potential link between tumor and immune microenvironment features and long DMFS, either spontaneously or vaccine-in-

duced. Further prospective studies are needed to validate these findings.

Key words: Cutaneous melanoma; primary tumors; dendritic cells; naïve T cells

OI-048

Urinary cytotoxic cells mark BCG-Induced immunity in Bladder Cancer

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For four decades, intravesical BCG has been the standard for non–muscle invasive bladder cancer (NMIBC), yet its mechanisms remain unclear and predictive biomarkers are lacking. With only ~60% response rates, early identification of non-responders is a clinical priority. We previously developed a Th2-score from pre-BCG biopsies—combining GATA-3$^+$ (Th2)/T-bet$^+$ (Th1) ratio with eosinophil density/activation—which correlated with outcome. We proposed that a high score (HS) would prime the tumor microenvironment (TME) for a BCG-driven Th1 shift, while a low score (LS) would indicate a suppressive TME limiting benefit. In this prospective study of 30 NMIBC patients on BCG, we profiled immune cells and cytokines in blood, serum, and urine at baseline and during therapy. Strikingly, functional cytotoxic lymphocytes were detected in urine after BCG. Urine-derived lymphocytes (UDL), though scarce, degranulated (CD107a) and released granzyme B against J82 bladder cancer cells, with responses enhanced by BCG preincubation of targets. PBMC collected post-induction also responded functionally to J82 in vitro. These targeted experiments support the presence of effector CD4 subsets with antitumor potential during BCG. In vitro, we observed degranulation of CD4, CD8, DN T cells, NK and NKT cells isolated from urine in response to J82 cells, further increased by BCG. Among CD4 T cells, an NKG2D$^+$ subset stood out, showing stronger degranulation to PMA, higher IFN-γ production, and a trend toward CD8 acquisition—a phenotype consistent with highly cytotoxic, terminally differentiated CD4 T cells. These were enriched in HS versus LS patients. Moreover, brief BCG exposure (24 h) upregulated MICA/B on J82 cells, potentially providing co-stimulatory signals for NKG2D$^+$ CD4$^+$ activation.

Our findings demonstrate, for the first time in NMIBC, that BCG can elicit functional cytotoxic CD4 T cells detectable in urine. Integrating this immune signature with the Th2-score may refine patient stratification and guide personalized therapy, identifying those unlikely to respond to BCG immunotherapy.

Key words: Bladder Cancer; BCG; Biomarkers

OI-059

CD20+ EVs from B-cell lymphoma bind RTX and activates NK cells

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Background: Current treatment for B-cell lymphomas (BCL) combines chemotherapy with rituximab (RTX), an anti-CD20 monoclonal antibody. NK cells are a key component of cancer immune surveillance. During BCL progression, CD20+ extracellular vesicles (EVs) are produced, but it has not yet been determined whether these EVs can bind to RTX to form immune complexes (IC), and if so, what function such complexes might exert.

Aims: -To characterize EVs derived from lymph node (tEVs) and plasma (pEVs) of BCL and healthy donors (HD), -To determine the binding of RTX to tEVs and pEVs expressing CD20 antigen, -To determine the functional consequences of CD20+ EVs and RTX immune complexes on NK cells. Methods: tEV were isolated through ultracentrifugation, while pEVs were isolated by (SEC). Characterization included western blot (WB) and flow cytometry (FC). IC formation was confirmed by protein A–immunogold labeling. Activation of NK was assessed by FC. Results: Isolated tEVs and pEVs from BCL and HD exhibited an enrichment of EV markers CD81/CD107a. As expected, BCL tEVs had higher CD20 expression compared to HD tEVs ($p<0.05$, n=6) by FC. Notably, BCL pEVs had higher CD20 expression compared to HD pEVs, evidenced by an increased CD20/CD81 index in WB ($p<0.0001$, n=13), mainly in advanced BCL patients. We also observed that pEVs from BCL, upon exposure to RTX, formed IC, as revealed by TEM (n=2). A higher frequency of gold-labeled EVs was observed in BCL compared to HD ($p<0.0001$). The addition of tEVs derived from BCL to NK cells did not modify NK cell activation, as revealed by CD69 expression. Notably, when NK cells were exposed to IC, CD69 surface expression was upregulated ($p<0.05$), while RTX alone had no such effect (n=5). Interestingly, when tEVs derived from HD were added to NK cells, the percentage of CD56+ cells expressing CD69 remained stable among different conditions. Similar trends were observed when the IC was formed by CD20+ pEVs and RTX (n=14).

Conclusion: EVs released by lymph nodes are found in plasma and reflect the CD20 protein expression of the parental cells. Moreover, RTX, by forming immune complexes with CD20+ EVs, promotes NK cell activation and may boost therapeutic efficacy.

Key words: EVs; Lymphoma B; Natural Killer; Rituximab

OI-072

Multi-omics data integration for immunotherapy response prediction in colorectal cancer

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Immunotherapies (IT) are effective antitumoral therapies, yet predicting patient outcomes remains challenging. While biomarkers are relevant in the clinics, their efficacy is limited. Notably, aberrant glycosylation is linked to tumor development, but its role in predicting IT outcomes is underexplored. In this study we integrate glycomics, transcriptomics, and proteomics data using Machine Learning to develop a new biomarker of response to IT in colorectal cancer (CRC). Previous analyses led to the development of the GlycoImmune Signature (GIS), an 18 genes expression signature associated with response to IT in melanoma. We applied the GIS in CRC samples from the TCGA-COAD project and found that MSI-H patients and responders to IT showed higher GIS scores. High GIS-scoring patients also presented a “hot” TME and higher scores of immune-related signatures. Interestingly, ~40% of MSI-L/MSS patients showed high GIS scores, showing that this signature may aid in the recognition of patients prone to respond to IT. We then analysed available proteomics data form 65 patients and found 29 upregulated proteins associated with immune response and cell killing in high-GIS scoring patients. Moreover, we found 9 downregulated proteins associated with metabolism regulation, revealing a metabolic reprogramming. To further our results at a single cell resolution we used a dataset containing data from both the TME and tumour cells (GSE200997) to generate pseudobulk counts and classified samples in high and low GIS scorers. Analyses of the TME composition showed that high GIS-scoring patients were enriched in effector CD8 T cells, and reduced in infiltration of Tregs, T CD4 annexin-1+ and Th17

cells. Next, we used the transcriptomic profile of the different cell types associated with high and low scorers as a reference to map them onto cells from patients treated with IT (GSE205506) using a Canonical Correlation Analysis approach. The transcriptomic profile of effector CD8 T cells from high scorers was highly cytotoxic and poorly exhausted, while Tregs from these patients showed higher dysfunctionality. These results support the role of the GIS as a surrogate marker of IT response in CRC patients at different molecular levels. This signature was also found to be correlated with an increased capacity of triggering anti-tumour immune responses, including in MSI-L/MSS patients who lack approved IT options but show promising glycosignature profiles.

Key words: Multi-omics; Immunotherapy; Glycosylation; Colorectal cancer; Biomarker

OI-100

Immune Modulation by Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia

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Introduction: Chronic myeloid leukemia (CML) is characterized by the BCR-ABL1 oncogene, which drives leukemogenesis and impairs immune surveillance. Tyrosine kinase inhibitors (TKIs) not only inhibit oncogenic signaling but also modulate immune recovery. The extent and pattern of immune reconstitution may differ among TKIs, with potential implications for treatment-free remission (TFR). Objectives: To assess the effect of imatinib and dasatinib on T, B, and NK lymphocytes in CML patients. Materials and Methods: Peripheral blood from 45 CML patients was analyzed: 6 untreated (G0), 25 on imatinib (6 for 12–24 months, 19 >24 months), and 14 on dasatinib (4 for 12–24 months, 10 >24 months). Lymphocyte subsets were identified by flow cytometry using T (CD3, CD4, CD8), B (CD19), and NK (CD16/56) markers. Results are expressed as medians and compared by Mann-Whitney U. Results: $CD3^+$ T cells were higher in G0 (2662/µL) compared with G1-Ima (977, $p<0.001$), G2-Ima (1325, $p=0.04$), and G2-Dasa (1480, $p=0.01$). G1-Dasa showed significantly higher $CD3^+$ counts than G1-Ima (1950 vs 977, $p=0.03$). $CD4^+$ T cells were greater in G0 (1629/µL) than in G1-Ima (490, $p<0.001$), G2-Ima (781, $p<0.001$), and G2-Dasa (806, $p=0.01$), while G1-Dasa exceeded G1-Ima (1402 vs 490, $p=0.007$). $CD8^+$ T cells decreased in G1-Ima (483 vs 844 in G0, $p=0.01$), with similar reductions in G2-Ima and G2-Dasa. NK cells declined in G1-Ima (147 vs 437 in G0, $p=0.06$) but increased in G1-Dasa (683, $p=0.007$), whereas G2 groups showed intermediate values. B cells were higher in G0 (478/µL) and significantly reduced in all treated groups, irrespective of TKI or treatment duration.

Conclusions: At diagnosis, CML patients displayed elevated T, NK, and B cells, reflecting nonspecific immune activation against tumor burden, followed by immune rearrangement under therapy. Dasatinib induced a distinct profile, particularly in the first 12–24 months, with higher $CD3^+$ and $CD4^+$ T cells and a marked NK increase compared with imatinib. These findings support that TKI choice influences immune reconstitution, which may have prognostic implications for treatment discontinuation by reflecting the immune system's ability to control residual disease. Although prospective studies with larger patient numbers are required, monitoring of these populations can be performed in a routine laboratory.

Key words: lymphocyte; CML; TKIs

OI-103

Exploring Trypanosoma cruzi antigenic mimicry in murine colorectal cancer through computational analysis

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Trypanosoma cruzi is the etiological agent of Chagas disease. Approximately 15% of infected individuals develop Chagasic megacolon (MC). Although MC causes conditions associated with colorectal cancer (CRC) risk, a negative association has been reported even in animal models. This study hypothesizes that this is the result of CD8+ T cells cross-reactivity, and evaluates whether peptides derived from tumor antigen processing, presented in the context of Major Histocompatibility Complex class I (MHC I) molecules by CRC tumors, also have sequences found in *T. cruzi* antigens, or whether similar sequences exist in the parasite proteome. To this end, whole-exome sequencing, RNA-seq expression and immunopeptidomic data from murine CRC cell lines (MC38 and CT26), as well as corresponding normal tissues whenever available in public repositories were used. During the prediction of potential neoepitopes, the best practice guidelines of the Genome Analysis Toolkit were followed. Differential expression analysis was performed using the edgeR R package. Immunopeptidome analyses were performed with FragPipe 22 using MSFragger 4.1, and the databases employed included the reference proteomes of *Mus musculus* and *T. cruzi*, as well as predicted neopeptides, common contaminants and decoy sequences. pBLAST was used to search for potential cross-reactive peptides. For each peptide, its potential immunogenicity was assessed by analyzing MHC I binding, antigenicity and autoimmunity potential. The evolutionary relationship between tumor antigens and parasite antigens that could potentially share similar or identical sequences was determined according to the Best Reciprocal Hit approach. Peptides presented by tumors, derived from the processing of tumor antigens -including neoantigens and tumor-associated antigens- were found to potentially exhibit cross-reactivity both with peptide sequences of *T. cruzi* proteins through sequence conservation among orthologous proteins, and with highly immunogenic, non-homologous *T. cruzi* surface antigens highly variable due to their role in immune evasion. Cross-reactivity could involve identical or highly similar sequences, with differences of even a single amino acid at the MHC I anchor position. It is likely that the negative association between MC and CRC development may be mediated through antigenic mimicry between *T. cruzi* antigens and tumor antigens, which may contribute to the early immune recognition of neoplastic cells.
Key words: *Trypanosoma cruzi*; cancer; cross-reactivity

OI-105

MHC I-immunopeptidomic evidence of antigenic mimicry between Trypanosoma cruzi and colorectal cancer tumors

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Chagasic megacolon represents the second most common manifestation of the digestive form of Chagas disease (*Trypanosoma cruzi* parasitosis). It causes chronic inflammation, ulceration and hyperplasia, conditions typically associated with colorectal cancer (CRC) risk, yet studies report a negative correlation with CRC incidence. Molecular mimicry could be one of the mechanisms involved in this antitumor effect, given its documented role in the pathophysiology of Chagas disease. This study investigated whether peptides generated from tumor antigen processing and presented on HLA class I molecules by human CRC —but not in matched normal colon tissue— also occur in T. *cruzi* antigens, or whether identical or highly similar sequences are present in the parasite proteome. To this end, immunopeptidomic data from 15 CRC tumors as well as corresponding normal tissues and predicted neo-peptides available in jPOST were used (JPST001069 and JPST001070). Analyses were conducted using FragPipe 22 with MSFragger 4.1. The search database comprised the reference proteomes of *Homo sapiens* and T. *cruzi*, supplemented with predicted neopeptides, common contaminants and decoy sequences. Potential cross-reactive peptides were identified through pBLAST searches. Predicted neo-peptides, some of which displayed low-complexity sequences, were found to potentially cross-react with T. *cruzi* Trans-sialidase and Mucin-Associated Surface Protein antigen families. Identical or highly similar sequences were detected in both tumor and normal colon, corresponding to these and other highly immunogenic parasite antigens, such as Mucins and Dispersed Gene Family-1 (DGF-1), consistent with observations in animal models by our group. Although the HLA class I alleles of patients in our dataset are not prevalent in Chagas disease–endemic regions, the consistency of these observations in this exploratory study suggests that, if cross-reactivity mediated by antigenic mimicry between T. *cruzi* and tumor antigens exists, highly immunogenic T. *cruzi* antigens could be responsible for the initial immune recognition of neoplastic cells. In any case, it remains crucial to experimentally validate the potential sequences involved, as immunization with these antigens may also carry a risk of autoimmunity.

Key words: *Trypanosoma cruzi*; Molecular mimicry; Cancer

OI-120

Influence of TSLP on monocytes and their role in the modulation of the tumor microenvironment

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Gliomas are primary malignant brain tumors that arise from glial cells. Glioblastoma multiforme (GBM), the most aggressive form, is resistant to therapies and generates an immunosuppressive microenvironment that inhibits antitumor immune responses. In this context, thymic stromal lymphopoietin (TSLP) has gained relevance as a potential mediator in the tumor-immune system interaction. Our group has recently demonstrated its involvement in GBM development and immune modulation. The aim of this work is to evaluate the modulation of the tumor microenvironment by TSLP in immune cells, particularly monocytes (Mo). Peripheral blood mononuclear cells (PBMCs) from healthy donors (HD) and patients (GBM-p) were isolated by Ficoll-Hypaque density gradient. Mo were purified from PBMCs by positive CD14 selection using MACS. Cells were cultured for 24 h with or without TSLP, followed by flow cytometry analysis. In addition, GBM-p biopsy samples were digested with collagenase according to standard protocols, then analysed by flow cytometry at baseline (T0).

First, TSLP receptor (TSLPR) expression was evaluated at T0, showing significant differences between HD and GBM-p in both total PBMCs and the $CD14^+$ subset ($p < 0.05$). Next, PD-L1 expression was analysed, revealing a significant increase in $CD14^+$ PBMCs and purified Mo from HD ($p < 0.05$), particularly in the $CD14^+/CD16^+$ subset. A similar trend was observed in both populations from GBM-p. Moreover, GBM-p biopsy samples showed the presence of Mo, both $CD14^+/CD16^+$ and $CD14^+/CD16^-$, with $CD14^+$ cells expressing PD-L1. Consistently, RNA-Seq bulk data from TIMER3.0 revealed that higher infiltration of $CD14^+/CD16^+$ Mo was associated with increased PD-L1 expression ($p < 0.05$). Finally, tubulogenesis assays were performed using supernatants from the U251 cell line, treated or not with TSLP for 24 h. Surprisingly, angiogenesis was significantly reduced in U251 supernatants treated with TSLP compared to control ($p < 0.05$). This reduction correlated with ELISA results for VEGF, showing lower VEGF production by U251 cells upon TSLP treatment compared to control ($p < 0.05$). However, purified HD Mo treated with TSLP produced higher VEGF levels than control ($p < 0.05$), suggesting that Mo may be responsible for the secretion of angiogenic factors within the tumor microenvironment. No significant differences in VEGF production were observed in HD PBMCs.

Our results suggest that Mo in the presence of TSLP may drive tumor growth and immune escape.

Key words: Glioblastoma; TSLP; monocytes

OI-121

PLAGL1 and PLIN2 as Emerging Biomarkers Linking Metabolism, Immune Infiltration, and Aggressiveness in Differentiated Thyroid Cancer

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Thyroid cancer (TC) is the most common endocrine neoplasm, with differentiated thyroid cancer (DTC) accounting for 90–95% of cases. Obesity and metabolic disease have been linked to increased incidence and poor prognosis. The tumor microenvironment (TME) plays a key role in TC progression and is characterized by mononuclear cell infiltration, even at early stages. PLAGL1 is a zinc-finger transcription factor with tumor suppressor functions and regulatory roles in metabolic diseases, whereas PLIN2 is a lipid droplet–associated protein involved in lipid storage and metabolism. We conducted a retrospective cohort study (n=219) to evaluate the association between DTC aggressiveness (tumor size, metastasis, and initial response to treatment) and BMI, age, and gender. In addition, we analyzed the expression profiles of PLAGL1, PLIN2, and immune cell infiltration in DTC samples (n=15). In this cohort, male patients exhibited larger tumors, more frequent lymph node dissections and metastases, and a higher risk of recurrence. Patients over 55 years of age showed a higher frequency of lymph node metastases. Interestingly, the only difference observed by BMI was a higher frequency of tumors >4 cm in overweight compared with obese patients, with no significant differences in other aggressiveness variables. Thus, BMI appears to be a nonspecific marker for assessing the impact of metabolic parameters on tumor progression, underscoring the need for more precise molecular indicators. PLAGL1 showed positive diffuse cytoplasmic staining in tumor cells, while in peritumoral normal thyroid cells it exhibited a granular cytoplasmic pattern. Increased PLIN2 expression was observed in patients with higher lymphocytic infiltration (CD45+) in the TME. Our study suggests that, while BMI is a nonspecific marker of thyroid cancer aggressiveness, molecular markers such as PLAGL1 and PLIN2 may better reflect the metabolic–immune interactions within the tumor microenvironment. These findings support further investigation of PLAGL1 and PLIN2 as candidate biomarkers for disease progression.

Key words: Thyroid Carcinoma; Obesity; PLAGL1; PLIN2

OI-122

Generation of Histone 1 Knockouts in a chronic lymphocytic leukemia murine cell line

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Introduction: Chromatin activation is associated with disease progression in chronic lymphocytic leukemia (CLL). Histones 1 (H1) modulate the conformation of the chromatin and play a key role in the epigenetic regulation of several biological processes. We showed that overexpression of the mutagenic enzyme AID enhances CLL disease aggressiveness and introduces mutations in genes encoding for H1.3 and H1.4, previously identified as disease drivers and highly mutated in different B cell lymphomas. Our goal is to study the role of H1.3 and H1.4 in CLL. We hypothesize that the expression of these H1 impact the phenotype and function of leukemic cells. Methods: We used the murine CLL line TCL1-355 to generate H1.3 and H1.4 stable knock outs (KO) cells by CRISPR/Cas9 technology. End point PCR and gel electrophoresis were used to evaluate the excision of the targeted genomic DNA. Gene expression levels were evaluated for hist1h1d and hist1h1e (encoding murine H1.3 and H1.4, respectively) by quantitative PCR. Proliferation was analysed by cell count. Expression of CD69 was assessed by flow cytometry. Results: We efficiently edited the genomic DNA region of the targeted genes obtaining bulks and, at least, three excised clones by limiting dilution technique, for the following groups of TCL1-355 cells: KO for H1.3, KO for H1.4, and double KO for H1.3+H1.4. Compared to controls, TCL1-355 H1.4 KO cells lacked expression of hist1h1e and upregulated hist1h1d gene levels (n=3, $p<0.05$). TCL1-355 H1.3 KO cells showed significantly lower levels of transcripts for hist1h1d and upregulated hist1h1e gene levels (n=6, $p<0.05$). Impaired expression of hist1h1d led to increased cell proliferation and to enhanced CD69 expression in single H1.3 KO cells (n=6, $p<0.05$). Because the conformation of the chromatin impacts the sensitivity to Ibrutinib, a BTK inhibitor widely used in CLL therapy that decreases cell proliferation, we incubated all the group of cells with Ibrutinib at 1uM for 72 hs. Ibrutinib impaired the proliferation in the control group but, interestingly, this effect was not observed in single H1.3 nor double H1.3+H1.4 KO cells (n=6, $p<0.05$)
Conclusion: H1.3 impairment enhances cell activation, proliferation and suppression of the anti-proliferative effect induced by Ibrutinib. These effects are not rescued by upregulation of H1.4, and are not detected in H1.4 KO cells. We propose that H1.3 could be involved in CLL disease aggressiveness and in loss of sensitivity to BTK inhibition
Key words: Histone 1; chronic lymphocytic leukemia; chromatin

OI-134

Distinct Cytokine Signatures of Toxicity In Anti-PD-1/CTLA-4 Therapy Not Observed With Anti-PD-1 Monotherapy

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Immune checkpoint inhibitors (ICI) have dramatically improved survival outcomes in melanoma. While anti-PD-1 monotherapy prolongs survival, combination regimens such as anti-PD-1/CTLA-4 achieve even greater efficacy but at the cost of substantially higher rates of immune-related adverse events (irAEs). Understanding the biological basis of these toxicities is critical to optimize clinical benefit without compromising patient safety.

To this end, we analyzed the expression of 92 inflammation-related serum proteins (Olink) at baseline in 178 melanoma patients including 111 treated with anti-PD-1 and 67 with anti-PD-1/CTLA-4. Patients were classified as responders (R) or non-responders (NR) based on imaging outcomes three months after treatment initiation, and as with toxicity (T) or without toxicity (NT), according to ESMO clinical practice guidelines.

Comparing anti–PD-1/CTLA-4 with anti–PD-1, we identified cytokines with significantly elevated baseline expression ($p < 0.05$) in the combination cohort. Within this group, distinct signatures emerged: IL-6 (p=0.0271) and IL-8 (p=0.0455) were enriched in R patients, while CCL4 (p=0.0042) was selectively elevated in T patients, highlighting an exclusive toxicity-specific pattern. In contrast, cytokines overlapping between R and T patients (e.g., CCL3: p-R = 0.0022, p-T= 0.0066; ENRAGE: p-R = 0.0102, p-T= 0.0161) suggest shared pathways but limited predictive specificity. These findings point to the existence of a distinct cytokine network driving toxicity in anti-PD-1/CTLA-4 therapy, independent from efficacy, and raise the possibility of targeting these pathways to mitigate irAEs while preserving clinical benefit.

We are now extending our analysis to directly compare anti-PD-1/CTLA-4 with the emerging anti-PD-1/LAG-3 regimen, which has comparable efficacy but a substantially lower risk of irAEs. Having already established this cohort and completed Olink profiling, we are applying a multi-omics approach (flow cytometry, CITE-seq, and TCR-seq) on paired PBMCs and serum to directly compare toxicity and efficacy signatures across both combination regimens. By integrating these datasets, our goal is to identify predictive biomarkers of toxicity that can guide patient stratification, enabling highly effective combination therapy with reduced toxicity and ultimately supporting safer, personalized use of ICI combinations in the clinic.

Key words: melanoma; immunotherapy; biomarkers; cytokines; toxicity

OI-136

Tumor-associated stromal fibroblasts acquire immune-modulatory properties that affect dendritic cells

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The tumor microenvironment (TME) exerts a profound influence on immune responses, with fibroblasts emerging as key regulators. To investigate fibroblast education by melanoma cells, we engineered an NIH3T3-derived fibroblast line expressing iRFP and blasticidin resistance (NF, normal fibroblast) through lentiviral transduction. This strategy allowed NF cells to coexist with melanoma cells in 3D spheroids and subsequently be selectively recovered for downstream analysis. NF cells were cultured as homotypic spheroids (NF only) or heterotypic spheroids (NF + B16 melanoma). After 3 days, homotypic NF spheroids were markedly smaller than heterotypic NF+T spheroids. Following blasticidin selection, fibroblasts from homotypic spheroids were termed NFS (Normal Fibroblasts Selected), and those from heterotypic spheroids were termed EFS (Educated Fibroblasts Selected). While NFS and EFS spheroids showed comparable size after selection, EFS exhibited reduced migratory capacity in 3D assays, indicating functional reprogramming. Importantly, EFS influenced dendritic cell (DC) behavior: in transwell assays, EFS enhanced DC migration, and upon stimulation, EFS-exposed DCs displayed an altered profile of co-stimulatory and inhibitory molecules compared to NFS. Altogether, our findings reveal that melanoma rapidly educates stromal fibroblasts toward a durable immunomodulatory phenotype that persists even in the absence of tumor cells, sustaining their ability to shape dendritic cell behavior beyond

direct tumor contact. Understanding this process may provide new insights into the stromal contribution to tumor immunity.

Key words: Tumor-educated fibroblasts; Dendritic cell modulation; Immunoregulatory stroma

OI-163

Interplay between EGF, TSLP and their receptors in glioblastoma multiforme and immune cell populations

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Glioblastoma multiforme (GBM) is the most frequent and aggressive primary brain tumor. It is characterized by marked cellular heterogeneity and by the induction of a profoundly immunosuppressive microenvironment, which contributes to resistance against conventional therapies. Previous findings from our group highlighted the relevance of thymic stromal lymphopoietin (TSLP) and epidermal growth factor (EGF) in GBM biology such as cell signaling and survival, showing that EGF stimulation increases TSLP expression. Based on these observations, this study aimed to investigate how TSLP and EGF stimulation modulates the expression of their respective receptors in GBM cells and distinct immune cell populations. The epidermal growth factor receptor (EGFR) and thymic stromal lymphopoietin receptor (TSLPR) expression was assessed by flow cytometry after TSLP (25 ng/ml) and/or EGF (20 ng/ml) treatment in U251 cell line, previously identified as representative of GBM cell model. Results, expressed as the percentage increase in mean fluorescence intensity (MFI) relative to control, showed that TSLP treatment significantly upregulated EGFR expression ($p < 0.05$), while EGF treatment significantly increased TSLPR expression ($p < 0.02$) in the cell line. We then analyzed the modulation of vascular endothelial growth factor (VEGF) by EGF in polymorphonuclear cells (PMNs) isolated from GBM patient peripheral blood, tumor biopsies, and healthy donor blood. VEGF levels in culture supernatants were quantified by ELISA after 24 hours of stimulation with TSLP and EGF. Unexpectedly, VEGF concentrations decreased following treatment across all samples ($p < 0.05$). Finally, we examined TSLPR expression in patient-derived neutrophils and lymphocytes after EGF stimulation. Flow cytometry analysis revealed an upregulation of TSLPR in lymphocytes, however, no difference was observed in neutrophils. Together, these results suggest a complex regulatory interplay between TSLP and EGF pathways in GBM and highlight potential mechanisms shaping tumor–immune interactions.

Key words: TSLPR; EGFR; Glioblastoma multiforme; inmune cell populations

OI-180

Peripheral and Tumor Immune Signatures Predict Response to Immunotherapy in Advanced HER2-negative Breast Cancer

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HER2-negative advanced breast cancer (BC) represents an aggressive subtype with poor prognosis and limited benefit from immune checkpoint blockade (ICB). Identifying predictive immune biomarkers is crucial to refine patient stratification and optimize treatment strategies.

We conducted a prospective study in 26 patients with advanced HER2-negative BC treated with anti–PD-L1 plus chemotherapy. Peripheral blood mononuclear cells were analyzed at baseline (week 0) and during treatment (weeks 4, 8, 28) using mass cytometry (CyTOF) and flow cytometry (FACS). In parallel, we integrated weather routine biochemical parameters that could reliably predict response. Their performance was quantified using receiver operating characteristic (ROC) curves, which measure the accuracy of a marker to distinguish responders (R) from non-responders (NR). To extend our findings to the tumor microenvironment, we reanalyzed scRNA-seq data from an independent cohort of patients treated with anti–PD-L1 (Zhang et al.) . At baseline, in peripheral blood, NR exhibited increased frequencies of PD-L1+ monocytes, Ki67– regulatory T cells (Tregs), and naïve B cells compared with R. Survival analysis confirmed that Ki67– Tregs and PD-L1+ monocytes were associated with poorer outcomes, while activated CD4+ T cells correlated with better survival. When these immune signatures were integrated with biochemical variables, predictive models were evaluated using ROC analysis, which plots sensitivity against specificity to assess how well a biomarker can discriminate between R and NR. This approach revealed high discriminatory power, with eosinophils, Ki67– Tregs, and monocytes emerging as the strongest predictors based on the area under the curve (AUC). Analysis of intratumoral scRNA-seq data revealed heterogeneity among FOXP3+ Tregs, identifying distinct phenotypic states. Notably, a naïve-like Treg program was enriched in R, consistent with systemic immune differences and supporting a role for Treg differentiation in ICB response. Together, these results highlight complementary peripheral and tumor immune signatures: expanded Ki67– Tregs and PD-L1+ monocytes in NR, activated CD4+ T cells and naïve-like intratumoral Tregs in R that provide mechanistic insight into ICB efficacy. Integrating immune profiling with routine clinical parameters may enhance biomarker discovery and improve immunotherapy strategies for advanced HER2-negative BC.

Key words: Breast Cancer; Immunotherapy; Biomarkers,

OI-185

A unique glycosylation Signature unveils the potential of the Galectin-1-glycan axis as a Therapeutic Target in Glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by marked heterogeneity and a strongly immunosuppressive microenvironment that limits the efficacy of current therapeutic modalities. Altered glycosylation has emerged as a central regulator of both tumor biology and immune evasion, making lectin-glycan interactions promising targets for immune intervention. To explore the relevance of glycosylation-dependent galectin-driven pathways, we profiled glycosyltransferase expression and analyzed the glycophenotype of four patient-derived GBM stem-like cell lines using a lectin array (SNA, PNA, MAL II, LEL, PHA-L, and Galectin-1 (Gal-1)). Transcriptomic data revealed distinct glycosyltransferase signatures, clustering the cell lines into three groups that correlated with phenotypic features. In parallel, t-SNE analysis of lectin binding distinguished two cell lines clearly, while the other two partially overlapped. Comparative heatmaps with an IDH-mutant high-grade glioma line and non-tumor neural progenitors showed consistently higher lectin binding in GBM cells (one-way ANOVA Gal-1, $p<0.05$; MAL-II, PHA-L, SNA, $p<0.001$; LEL, PNA, $p>0.05$), with Gal-1 displaying the most stable and consistently elevated binding. Given our previous work demonstrating that Gal-1 silencing reduced GBM cell proliferation and viability, we assessed the impact of Gal-1 blockade in vivo using a highly

specific blocking antibody in a murine GBM model. This approach revealed a trend toward reduced tumor volume, together with an increase in CD86 (unpaired t-test, p=0.007) and a trend toward decreased CD206 expression, consistent with a shift toward a pro-inflammatory immune phenotype. Taken together, these findings identify Gal-1 as a common glycosylation-dependent mechanism despite interpatient heterogeneity, and support its potential as a therapeutic target in GBM.

Key words: Glioblastoma; Galectin 1; Glycosylation; Antibody

OI-206

Optimization of a 27-color panel for Immunophenotyping of the TME by Spectral Flow Cytometry

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The introduction of immunotherapy with immune-checkpoint blockade (ICB) as a tool to modulate the immune response signified a paradigm shift in patient treatment. A key determinant of therapeutic efficacy is the tumor microenvironment (TME) composition, where “hot” (immune-inflamed) tumors typically respond better than “cold” or immune-excluded ones. Characterizing the immune components of the TME is therefore essential for developing novel targeted therapies or anticipating response to ICB. To decipher the immune components of the TME we established a 27-color spectral flow cytometry panel to enable high-dimensional analysis of immune infiltrates in the Cytek Aurora cytometer (V/B/YG/R laser), including exhausted or cytotoxic $CD8^+$ T cells, regulatory T cells, B cells, Dendritic cells (DC), macrophages, NK cells and MDCS. The panel was designed using the Cytek Cloud tool, aiming for the optimal complexity index and the lowest spread between fluorochromes. Experimentally, it demonstrated suitability for phenotypic characterization, ensuring reliability and reproducibility of the results. To optimize its performance, we refined sample preparation protocols, demonstrating that Ficoll-Hypaque isolation reduces myeloid recovery and should be avoided when interrogating this population. For staining, which included surface, intracellular, and nuclear markers, we compared different protocols and identified that staining with the TruNuclear Buffer was the most effective. The panel was validated in cell suspension isolated from C4HD tumors (BALB/c mice) treated in vivo with Medroxyprogesterone Acetate (MPA) for 28 days, MPA + Mifepristone (MIFE) or remained untreated. Both MPA and MIFE increased the accumulation of CD8+T cells in the tumor, while MIFE markedly reduced the frequency of terminally exhausted PD1+TIM3+CD8+T cells. MPA treatment increased the frequency of suppressive CD4+Foxp3+CTLA4+ Treg cells, which was reversed by MIFE. MPA treatment induced an enrichment of CD206+F4-80+M2 macrophages, while MIFE treatment promoted a shift towards a CD86+MHCII+M1 phenotype and increased the frequency of mature CD103+MHCII+CD11b+ cross-presenting DCs. In conclusion, this panel suited for spectral cytometry is a powerful tool that facilitated an integrated analysis of the TME immune composition, enabling a deep characterization of the immunological correlates elicited upon treatment, potentially supporting the rational development of precision immunotherapy in oncology.

Key words: spectral flow cytometry; immune infiltrate; TME

OI-210

REPOSITIONING VISMODEGIB FOR CUTANEOUS SQUAMOUS CELL CAR CINOMA: A431 IN-VITRO AND 3D EVIDENCE WITH ULTRADEFORMABLE LIPOSOMAL DELIVERY

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The Hedgehog signaling pathway (HH) is overexpressed in several types of cancer, promoting tumor growth and survival. Vismodegib (VDG), a HH inhibitor approved for unresectable basal cell carcinoma, is currently administered orally and is frequently associated with systemic adverse effects. In this study, we propose its repurposing for cutaneous squamous cell carcinoma (cSCC) and assess topical delivery via ultradeformable liposomes (UDLs) to improve skin penetration, increase local efficacy, and minimize systemic toxicity. Human cSCC A431 cells were analyzed in 2D monolayers and 3D spheroids. Viability and proliferation were measured by Alamar Blue (2D) and spheroid volume quantification (3D). Mitochondrial function was assessed by flow cytometry using MitoSOX Red for superoxide anion (O2●-) and DiOC6 for mitochondrial membrane potential (ΔΨm) detection. Cell migration was evaluated by wound-healing assays. IL-10 levels in culture supernatants were quantified by ELISA. In all of these cases, free VDG, VDG-UDL, and empty UDLs were compared (one-way ANOVA with significance defined as $p<0.05$). The results showed that VDG reduced A431 viability in both 2D and 3D formats ($p<0.05$, $p<0.01$), and impaired wound closure ($p<0.01$). These effects were significantly potentiated when VDG was delivered in UDLs ($p<0.01$, $p<0.01$ and $p<0.01$, respectively). VDG and especially VDG-UDL increased IL-10 secretion by tumor cells (2.3 and 6.4 times basal production). Flow cytometry revealed increased mitochondrial O2●- production (13.9 vs 0.8%) and hyperpolarization (4119 vs 904 FI) after VDG-UDL treatment, consistent with metabolic alterations in tumor cells. VDG demonstrates anti-tumor activity in A431 models, which is enhanced by ultradeformable liposomal delivery. However, it may increase immunomodulation. These findings support further studies on lipid-based nanosystems as a topical strategy to improve VDG efficacy for cSCC.

Key words: cancer immunotherapy; ultradeformable liposomes; Hedgehog pathway

Regulation of the Immune Response

RI-010

Efficient in vitro formation of monocyte–platelet aggregates and subsequent platelet phagocytosis does not significantly modulate the immune profile of monocyte-derived macrophage

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Platelets participate in the regulation of the immune response. Monocyte-platelet aggregates (MPAs) have been observed in vivo, with increased numbers during inflammation and viral infections. We aimed to characterize an in vitro model of platelet-monocyte interaction to evaluate the effect of MPA formation on the immune profile of monocyte-derived macrophages. Human monocytes and platelets were isolated from peripheral blood of healthy donors. Platelets were added to purified monocytes for 1 or 24 h, washed, and then monocytes were differentiated to macrophages by 7-day culture with M-CSF (50 ng/ml). Platelet activation was assessed by expression of CD62p. MPAs were analysed by confocal microscopy and flow cytometry after staining with anti-CD61 and anti-HLA-DR antibodies. Macrophage phenotype was examined by flow cytometry and cytokine production after LPS stimulation was quantified by ELISA. Macrophage efferocytic capacity was assessed by incorporation of CFSE-labeled apoptotic Jurkat cells. Flow cytometry analysis of HLA-DR/CD61 double-positive events revealed that addition of platelets induced MPA formation (11.5% of monocytes in control cultures vs. 43.6% at a platelet:monocyte ratio of 17:1 and 45.2% at 50:1, n = 6). Platelets were found adhered to macrophages even at day 6 of culture (25.9% in control cultures vs. 71.1% at a platelet:monocyte ratio of 50:1, n = 6). Confocal microscopy showed consistent proportions of MPA formation at 24 h: 3.9% of monocytes in control cultures vs. 34.2% at a platelet:monocyte ratio of 17:1 and 51.5% at 50:1, n = 4). MPAs typically involved multiple platelets on a single monocyte. Furthermore, staining platelets with yellow cell tracker before their addition to the culture revealed efficient platelet phagocytosis in the first 24 h. Platelet addition did not affect the number of macrophages at day 7 of culture (n = 5). MPA formation did not modulate

expression of CD16, CD163 and CD206 (n = 4-8), nor the production of TNFα, IL-1β or IL-8 by macrophages after LPS stimulation at day 7 of culture (n = 4-5). Efferocytic capacity of macrophages was unaffected by platelet addition for either 1 or 24 hours (n=5).

Our results indicate that the immune profile of monocyte-derived macrophages remained unaltered despite efficient early in vitro interaction with platelets

Key words: Platelets; macrophages; monocyte-platelet aggregates; efferocytosis

RI-049

NR4A1 Activation in M2 Macrophages Modulates the Response to Mycobacterium tuberculosis.

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Mycobacterium tuberculosis (MTB) infection drives tuberculosis (TB) immunopathology by inducing a persistent and harmful immune response in alveolar macrophages. Nuclear receptors (NRs), a superfamily of transcription factors that coordinate endocrine and immune signals, are critical modulators of this process. Emerging evidence highlights the NR4A subfamily—comprising NR4A1, NR4A2, and NR4A3—as crucial regulators of inflammatory and immune activity, largely through their modulation of pathways such as NF-κB. Supporting their clinical relevance, we and others have observed that all three NR4A members are upregulated in TB patients, and their expression increases with disease severity. To investigate this further, we employed an in vitro model using THP-1 cells differentiated into inflammatory (M1) and antiinflammatory (M2) macrophage phenotypes. Treatment with irradiated MTB (MTBi) revealed that the bacteria differentially modulate NR4A receptor expression, NF-κB family genes, and cytokine production in a manner that is highly dependent on the macrophage's polarization state. In this opportunity, we evaluated by immunoblotting (Western blot) the protein expression of NR4A1 and its phosphorylated form (active form) in cells stimulated or not with MTBi. We observed a statistically significant increase in the phosphorylated (active) form of NR4A1 in MTBi-treated M2 macrophages compared to untreated controls ($p<0.05$). Furthermore, immunofluorescence analysis localized phosphorylated NR4A1 to the perinuclear region in MTBi-stimulated M2 macrophages, suggesting a potential interaction with subcellular structures. Considering NR4A1's established dual role in inflammation, which can shift from antiinflammatory to pro-inflammatory depending on context, our findings indicate a specific immunomodulatory function for this receptor in M2 macrophages responding to mycobacterial infection. This suggests a new mechanism contributing to TB immunopathology to be explored in future work.

Key words: Macrophages; Nuclear receptor NR4A; Tuberculosis

RI-052

Exploring CD163 as a Prognostic Biomarker in Chronic Chagas Cardiomyopathy

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Chronic Chagas cardiomyopathy (CCC) is a severe complication of *Trypanosoma cruzi* infection, causing significant morbidity and mortality years after initial exposure. Characterized by arrhythmias, heart failure, and sudden death risk, CCC develops in about 30% of infected individuals, often following a silent chronic phase. Despite its impact, the immune mechanisms driving progression remain unclear. Evidence points to chronic immune activation and disrupted inflammatory regulation as key factors. Identifying early immune changes linked to disease progression is crucial for better risk assessment and targeted therapies. Among immune cells, monocytes play essential roles in both inflammation and tissue repair. Their changing phenotypes and functions during disease suggest they are pivotal in CCC pathogenesis. We focused on CD163, a scavenger receptor on monocytes/macrophages with anti-inflammatory and antioxidant functions. CD163 exists on the cell surface and as a soluble form (sCD163) in plasma, both reflecting monocyte activation in chronic inflammation. Using flow cytometry, we measured surface CD163 on classical, intermediate, and non-classical monocyte subsets from chronic Chagas disease (CCD) patients without cardiac symptoms (K0), with various cardiac involvement (K1–K3), and uninfected controls (UI). Preliminary findings revealed distinct monocyte subset distributions among groups. K0 patients showed higher CD163 expression, indicating a more regulated, anti-inflammatory state. Conversely, patients with severe cardiomyopathy (K3) had reduced CD163 levels, consistent with a proinflammatory shift. The reduced surface CD163 may result from increased shedding into plasma as sCD163, potentially linked to inflammation and tissue damage. To further explore this, we plan to quantify sCD163 levels in the same cohorts and evaluate their correlation with clinical severity. Altogether, these results highlight CD163, in both membrane-bound and soluble forms, as a promising biomarker to monitor CCD progression and support personalized diagnostic and therapeutic strategies.

Key words: Chagas; monocytes; CD163; Flow Cytometry

RI-192

Differential expression of CD8+PD1+cells in a new fibrin hydrogel model

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Objectives: Peripheral blood and conventional 2D cultures fail to accurately reproduce immune cell dynamics within tissues, limiting their translational relevance. Our laboratory has established 3D fibrin hydrogels (FH) as a model for studying immune cell migration and activation. The aim of this study was to evaluate how immune activation modifies distribution and checkpoint marker expression in our 3D FH model using healthy donor samples under basal and PHA-stimulated conditions. Materials and Methods: Peripheral blood mononuclear cells from 10 healthy donors were isolated and stimulated with PHA or left untreated. Cells were seeded at equal density (5×10^5 cells in 300 µl medium) on top of the FH and incubated for 48 h. After incubation, cells were retrieved from both compartments (supernatant and gels), stained for surface markers (CD45, CD3, CD4, CD8, PD-1), and analyzed by multiparametric flow cytometry. Results: In control conditions there's a decrease in CD3+, CD4+ but not CD8+ cells and an increase in CD3- cells in the hydrogels ($p<0.001$) compared to supernatants. When cells were stimulated with PHA, we didn't observe differences between supernatant and gels for CD3+, CD4+, CD8+ and CD3- populations. The analysis on the expression of PD1shows that there's an increase of PD1 on CD3+,CD4+ and CD8+ expression in supernatants and gels after PHA stimulation, when compared to control conditions. There were no changes for CD4+PD1+ and CD3+PD1+ in between supernatants and gels in any of the conditions analyzed. The analysis of CD8+PD1+ cells showed an increase in FH vs supernatants in the control group ($p<0.01$), but a lower numer of PD1+ cells in gels compared to supernatants with cells stimulated with PHA ($p<0.05$).

Overall these results show that 3D FH model allows for study immune response and reveal a differential pattern in PD1 expression on CD8+ cells.

Key words: CD4; CD8; PD1; Fibrin Hydrogel

RI-215

Immunosenescence-Associated Biomarkers in Pulmonary Tuberculosis, Type 2 Diabetes, and Their Comorbidity

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Immunosenescence is defined as the progressive decline of the immune system associated with aging, characterized by impaired phagocytic activity, reduced antigen presentation, altered T and B cell responses, and increased release of pro-inflammatory cytokines. This process is accelerated by chronic low-grade inflammation, termed inflammaging. Infectious and metabolic diseases such as pulmonary tuberculosis (PTB) and type 2 diabetes mellitus (DM2) are strong inducers of systemic inflammation and may contribute to the premature onset of immunosenescence, while at the same time being aggravated by it.

In this study, we investigated immuno-endocrine markers related to senescence in patients with PTB, DM2, PTB–DM2 comorbidity and healthy controls (HCo), and explored their relationship with age. We also performed a multivariate t-distributed stochastic neighbor embedding (t-SNE) analysis using biochemical, metabolic and immune-endocrine variables from the same study groups. Consistent with previous results, TB patients showed elevated circulating levels of endocrine and inflammatory mediators, including cortisol, IL-6, INF-γ, C-reactive protein, and erythrocyte sedimentation rate, compared with HCo ($p<0.05$), and decreased body mass index (BMI) and Cortisol/DHEA-S ratio ($p<0.01$). These markers correlated with disease severity (assessed by pulmonary involvement; SEV). Additionally, patients with PTB and PTB–DM2 exhibited decreased levels of dehydroepiandrosterone sulfate (DHEA-S) and vitamin D with respect to controls ($p<0.01$). Spearman correlation analyses revealed a negative association between INF-γ and age in TB patients ($r= -0.3$, $p<0.05$) and between age and DHEA-S in HCo ($r= -0.5$, $p<0.001$). Notably, this age-related decline in DHEA-S was absent in TB patients, who presented low DHEA-S levels across all age groups. The t-SNE analysis demonstrated separation between TB patients and controls, with major contributors including erythrocyte sedimentation rate, lymphocyte and neutrophil percentages, BMI, SEV, and cortisol.

Taken together, these findings suggest that TB and TB–DM2 patients may display immunosenescence-like profiles, characterized by systemic inflammation independently of chronological age. This would support the concept of disease-driven acceleration of immune aging and point to a potential bidirectional interplay between chronic inflammation, immunosenescence, and comorbidities.

Key words: Inmune.endocrine regulation; Immunosenescence; ageing; tuberculosis; Type 2 Diabetes

Vaccines and Immunological Memory

VM-001

Recombinant peptidoglycan-associated lipoprotein (rPal) formulated with CpG-ODN/Co-ASC16 induces a specific humoral response in chickens and represents a candidate for a vaccine against infectious coryza

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Avibacterium paragallinarum causes infectious coryza in chickens, an acute upper respiratory disease with major economic impact on poultry production worldwide. Whole-cell inactivated bacterins are the only commercially available vaccine, but they provide incomplete protection, particularly against heterologous strains, underscoring the need for improved immunization strategies. As a first step toward developing a broadly protective subunit vaccine, we used reverse vaccinology to identify antigen candidates from both local and public Av. paragallinarum genomes. An in silico pipeline was designed to predict and rank proteins with favorable features for expression and immunogenicity. Among the top candidates, the peptidoglycan-associated lipoprotein (Pal) emerged as a promising target. For recombinant Pal (rPal) expression, the Pal gene was codon-optimized, synthesized, and cloned into a pET-24a(+) vector. The construct was transformed into Escherichia coli BL21(DE3), and rPal was purified by cobalt-based immobilized metal affinity chromatography, followed by size-exclusion chromatography and endotoxin removal. To assess immunogenicity, 60 birds were randomly assigned into 6 groups of 10 chickens each: control (unvaccinated); rPal alone; rPal with CpG-ODN/Co-ASC16 (a nanoadjuvant previously developed by our group); CpG-ODN/Co-ASC16 alone; a commercial trivalent vaccine; or the commercial vaccine plus rPal/CpG-ODN/Co-ASC16. Chickens were immunized subcutaneously at 6 and 10 weeks of age. Sera were collected before the second dose and 28 days later; rPal-specific IgY levels were measured by a homemade indirect ELISA. Statistical analysis used the Wilcoxon test ($p<0.05$). No antibodies were detected in chickens immunized with rPal or CpG-ODN/Co-ASC16 alone at either time point ($p>0.05$ vs. control). After the first dose, rPal-specific antibodies were detected in groups receiving rPal/CpG-ODN/Co-ASC16, the commercial vaccine, or the combination of both ($p<0.05$ vs. control). After the booster, specific IgY levels were maintained or increased in these groups. The second rPal/CpG-ODN/Co-ASC16 dose significantly enhanced the response, reaching levels comparable to the commercial vaccine. The combination showed no synergistic effect. No adverse effects were observed. These findings support rPal/CpG-ODN/Co-ASC16 as a promising subunit vaccine candidate against infectious coryza.
Key words: vaccines; immunization; chicken; coryza

VM-016

IDENTIFYING A POTENTIAL CORRELATE OF PROTECTION FOR A TRYPANOSOMA CRUZI VACCINE THROUGH MACHINE LEARNING-BASED IMMUNE BIOMARKERS INTEGRATION

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Background: Chagas disease, caused by Trypanosoma cruzi (T. cruzi), remains without an approved vaccine, and immunological correlates of protection (CoPs) have not yet been defined. We developed a vaccine candidate based on a trans-sialidase fragment (TSf) and the ISPA adjuvant. We observed that $CD11b^{+}Gr\text{-}1^{+}$ myeloid-derived suppressor cells (MDSCs) play a key regulatory role during immu-

nization and infection. Depleting MDSCs with 5-fluorouracil (5-FU) affected $CD4^+$ and $CD8^+$ cells in peripheral blood and spleen, and enhanced vaccine efficacy. In this context, machine learning represents a valuable tool to integrate immune data and identify potential CoPs to guide vaccine development. Objective: To identify potential CoPs for a T. cruzi vaccine using machine-learning analysis of immune biomarkers. Methods: BALB/c mice were vaccinated with protocols based on TSf-ISPA immunization with 5FU administration (n=31). Percentage of CD11b+Gr-1+, CD4+, CD8+ cells in peripheral blood were measured by flow cytometry. Mice were challenged with 1600 T. cruzi parasites, and survival was recorded until day 35 post-infection. Flow cytometry-derived immune biomarkers were evaluated both individually and as linear combinations, and used as input variables for training a decision tree classification model aimed at identifying potential associations between immune biomarkers and protection outcomes. Results: Integrating $CD8^+$, $CD4^+$, and $CD11b^+Gr\text{-}1^+$ biomarkers —an approach named biomarker engineering— into a single-split decision tree model resulted in higher predictive accuracy for survival, compared to models using individual variables. Biomarker engineering was implemented through two strategies: a rational design based on biological insight, and a computational search in which the algorithm systematically evaluated a wide range of linear combinations of the input variables within a defined range of coefficient assignments. The optimal model achieved an average AUC-ROC of 0.87 and an average accuracy of 0.86 for survival prediction, with all metrics significantly outperforming individual-biomarker models ($p < 0.05$). Discussion: These findings support the utility of machine learning as a valuable tool for identifying potential CoPs in the context of T. cruzi vaccination. The integration of immune biomarkers substantially enhanced the predictive performance of the models.

Key words: Vaccine; Machine Learning; Trypanosoma cruzi; Myeloid-Derived Suppressor Cells

VM-030

Development of a prophylactic method for immunization against human metapneumovirus based on the encapsulation of a chimeric immunogenic protein in lipid nanoparticles

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ABSTRACT

Human metapneumovirus (hMPV) is a respiratory pathogen that infects both the upper and lower respiratory tracts, primarily affecting young children, older adults, and individuals with compromised immune systems. Given its high prevalence and potential to cause severe respiratory disease, multiple vaccine prototypes have been developed, yet no candidate is currently approved for human use. The lack of an effective vaccine represents a significant public health concern, as hMPV can lead to respiratory complications such as bronchitis, pneumonia, and exacerbations of chronic respiratory diseases.

This study proposes an innovative approach to address this gap by developing a lipid nanoparticle-based vaccine. The design of our vaccine incorporates a chimeric protein composed of the F (fusion) and N (nucleoprotein) fragments of hMPV, encapsulated within lipid nanoparticles, which play a key role in viral entry and pathogen replication.

The lipid nanoparticles were generated using the lipid film hydration method and physicochemical characterized by dynamic light scattering (DLS), confirming their uniform size and stability. Following in vitro experimental administration, dendritic cell maturation and activation were observed via flow cytometry. Our results suggest that the liposomal formulation promotes in vitro dendritic cell maturation and activation, highlighting its immunogenic potential for future in vivo immune protection studies. Funding: Fondecyt Regular 1231866, ANID, Chile.

Key words: Human metapneumovirus (hMPV); Lipid nanoparticles; Chimeric protein; Dendritic cell activation; Vaccine development

VM-035

Immunopeptidomic analysis of murine dendritic cells reveals novel Trypanosoma cruzi epitopes presented by MHC class I and II during infection

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Trypanosoma cruzi is the causative agent of Chagas disease. During infection, both $CD8^+$ and $CD4^+$ T lymphocytes play a critical role in controlling parasite load. Therefore, vaccines capable of eliciting robust cellular immune responses are expected to contribute to the infection control. This study aims to identify potentially immunogenic peptides derived from the processing of parasitic antigens presented by Major Histocompatibility Complex (MHC) molecules, following the internalization of live or dead parasites (Y strain). To this end, the immunopeptidome of Bone Marrow-derived Dendritic Cells (BMDCs) from C57BL/6 mice was analyzed under two experimental settings: (i) co-cultured with T. cruzi-infected fibroblasts (1 parasite per 5 cells), or (ii) exposed to dead trypomastigotes obtained by freeze–thaw cycles (2 parasites per cell). Peptide–MHC complexes were isolated by immunoaffinity capture after BMDC lysis. Peptides were eluted and identified by mass spectrometry. Data were analyzed with FragPipe 22 (MSFragger 4.1), comparing acquired fragmentation spectra with theoretical ones, using the reference proteomes of T. cruzi Y strain clone 6 and Mus musculus from UniProt, plus decoys and contaminants. The "HLA nonspecific" workflow was used for peptide identification (8–15 amino acids for MHC I and 8–25 for MHC II) with a 1% false discovery rate. Peptides presented by both MHC I and II were identified in BMDCs under the two conditions, most likely derived from proteins of the mucin, MASP, trans-sialidase, cruzipain and calreticulin families. Some of these peptides exhibited low-complexity sequences and mapped to surface proteins, which is relevant since repetitive motifs are often the most immunogenic. Peptides presented by MHC II molecules in BMDCs were predominantly found in dead parasites, suggesting that the type of stimulus influences the response, considering the complexity underlying the processing not only of parasitic peptides but also of whole cells. These results suggest that BMDCs are capable of processing and presenting peptides from antigens already known for their immunological relevance, supporting their importance, as well as from other proteins not yet explored. The absence so far of immunogenicity studies of these sequences highlights the need for their experimental validation as T cell epitopes.
Key words: immunopeptidome; epitopes; Trypanosoma cruzi; dendritic cells; vaccine

VM-045

MAte-Vac: A Bioinformatics tool for selecting Vaccine Antigens through Epitope Conservation Analysis

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Immunoinformatics tools facilitate rational vaccine design by prioritizing antigens according to predicted epitopes, conservation across pathogens, and potential population coverage. However, integrating these resources at the early stages of vaccine development remains challenging. To address this, we developed MAte-Vac, a platform that mines complete pathogen genomes to discover vaccine candidates based on conserved epitopes. Implemented in Python with libraries such as NumPy, pandas, and Biopython, MAte-Vac integrates BepiPred 3.0, NetMHC, NetMHCIIpan, BLAST, NCBI Datasets, and WebLogo. The workflow is organized into modular steps: (i) starting from a protein of interest, it retrieves target species genomes and runs BLAST to assess presence/absence, sequence identity, and coverage; from these data, it computes conservation metrics, clusters variants, and generates variability maps; (ii) across these variants, it predicts T-cell (MHC I/II) and linear B-cell epitopes, evaluates their conservation throughout the pangenome, and removes redundancies to prioritize robust candidates. As proof of concept, we analyzed group I trans-sialidase (TS-GI) from Trypanosoma cruzi, a well-established vaccine target previously examined manually by our group. The workflow identified over 150 high-affinity T-cell epitopes across the most representative human and mouse MHC molecules, including those from BALB/c and C57BL/6 strains commonly used in vaccine evaluation. It also detected several B-cell epitopes, with both sets exhibiting high conservation among TS-GI protein variants (99% amino acid identity, 100% coverage), consistent with our previous manual analysis (Pancini et al., 2023). The platform generates reproducible reports including ranked antigen and epitope lists, comparative conservation metrics, and sequence logos. Our preliminary results indicate that MAte-Vac provides a complete workflow (from raw sequence data to prioritized vaccine candidates) substantially accelerating the design of robust multi-epitope vaccines that address pathogen genomic diversity.
Key words: Vaccine; epitope; Immunoinformatic; platform; conservation

VM-051

Adult tonsils are a survival niche for human memory B cells

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Long-lived plasma cells (PC) and memory B cells (mBc) constitute the two arms of humoral immune memory. Long-lived PC reside in the bone marrow, although they can also be detected in the spleen and tonsils. The survival niches of mBc remain unknown. We have recently shown an age-dependent reduction in the proportion of tonsillar germinal center B cell population and its T cell counterpart. Taking into account those previous observations, we hypothesized that adult tonsils constitute a niche for human mBc. Here, we report our initial findings supporting this claim. We used tonsils from donors at different ages and analysed them using a number of techniques like multiparametric flow cytometry, human primary cell cultures and ELISA. We found an age-correlated increment in the ratio mBc/naïve B cells in tonsils (N=60, r=0.3, p<0.05; r & p values are from the Pearson correlation analysis from now on). Additionally, we established a positive linear correlation between a particular mBc population associated with aging (ABC, CD19highCD27+CD11c+Tbet+ cells) and the age of the patients (N=81, r=0.5, p<0.0001). In order to elucidate whether such mBc accumulation comprised mBc raised distantly, we developed an assay to test toxoid-specific mBc (TT+mBC) *ex vivo*, within tonsillar mononuclear cells (TMC). Briefly, we cultured TMC from vaccinated individuals (N=12) for 3 days in media supplemented with tetanus toxoid (TT) and tested for anti-TT Igs in the culture supernatant. The presence and functionality of TT+mBC

as well as their reactivation capabilities within TMC was demonstrated through this assay. We concluded that mBc displace other B cell subsets in the tonsils with advancing age and more importantly, at least a fraction of those mBc were generated by parenteral TT administration at a distant location. Whether these were re-circulating mBc that were caught at the moment they were patrolling tonsils or they were established tissue residents remains to be understood.

Key words: Memory; Tonsils; B cells

VM-060

Humoral immune response by oral attenuated Salmonella Vaccine for SARS-CoV-2 infection

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Considering that SARS-CoV-2 infection mainly generates a disease of the respiratory tract, and that the main route of infection is at the mucosal level (respiratory, oral and ocular), mucosal protection is essential. The aim of this study was to develop vaccine candidates capable of inducing mucosal immunity for the prevention and control of SARS-CoV-2 infection. For that, pcDNA3.1 (+) plasmid encoding two antigens of interest: RBD and nucleocapsid protein (NuC) were cloned. Attenuated *Salmonella* enterica serovar Typhimurium aro A SL7207 were then transformed with each pcDNA3.1 (+) plasmid. RBD and NuC proteins were produced in eukaryotic cells and prokaryotic E. coli, respectively and their identity was verified by Western blot using sera from SARS-COV-2 infected patients (showing positive reactivity for both proteins). Mice were immunized with four oral doses (one every ten days) as follows: G1: PBS; G2: 1x109 CFU *Salmonella* with empty plasmid; G3 and G4: two oral doses of 1x109 CFU *Salmonella* with NuC or RBD plasmid + two intramuscular doses of each protein + CpG (Prime boost groups, PB); G5: four doses of 1x109 CFU *Salmonella* with NuC + RBD plasmids; G6: four doses of 1x109 CFU *Salmonella* with NuC plasmid; G7: four doses of 1x109 CFU *Salmonella* with RBD plasmid. Two weeks after the final immunization, mice were euthanized and sera and intestinal fluid (IF) samples were obtained. Sera and IF were evaluated by dot-blot and surface plasmon resonance (SPR) to detect specific antibodies. Sera from the PB groups tested positive for specific antibodies against each candidate, as determined by dot blot and SPR (NuC: G2 *vs*. G3, 2.60 ± 2.07 *vs*. 227.30 ± 19.06 RU, t-test, $p < 0.05$; RBD: G2 *vs*. G4, 8.45 ± 1.68 *vs*. 47.93 ± 6.19 RU, t-test, $p < 0.05$). In IF samples, specific antibodies were detected against NuC (G3 and G6) and RBD (G4 and G7) by dot blot. These findings demonstrate that the vaccine candidates elicit specific antibody responses at both systemic and mucosal levels, which could play an important role in the prevention and control of SARS-CoV-2 infection.

Key words: Vaccine; SARS-Cov-2 Infection; Humoral Response

VM-064

A Tailored Coated Bacterial Vaccine with the S-layer Protein from Lenti lactobacillus kefiri as Immunomodulator

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The development of new vaccine platforms capable of efficiently presenting antigens remains a major challenge in immunology. In this context, our group has developed the Coated Bacterial Vaccines (CBVs) system, in which the antigen of interest is fused to the dSLPA domain of the S-layer protein SlpA from *Lactobacillus spp.*, which exhibits high affinity for teichoic acids of Gram-positive bacteria. The chimeric antigen is produced in heterologous systems and used to coat chemically inactivated bacteria, which act as antigen-presenting carriers. We previously demonstrated the efficacy of the CBVs platform using the tetanus toxin fragment C (TTFC) as a model antigen. On the other hand, S-layer proteins from *Lentilactobacillus kefiri* are recognized for their immunomodulatory properties and as key mediators of interactions between bacteria and host immune cells. Based on this, the aim of this work was to tailor the CBVs to enhance immune modulation by co-formulating the TTFC antigen with the S-layer protein from *L. kefiri* (SLP-83111). To this end, TTFC-dSLPA was produced in *E. coli*, while SLP-83111 was isolated from *L. kefiri* cells by chaotropic method. Purified TTFC or an equimolar TTFC/SLP-83111 mixture were then used to coat the surface of chemically inactivated *B. subtilis* to obtain TTFC-CBVs or SLP-83111-TTFC-CBVs. The interaction between the antigen, S-layer protein, and bacterial carrier was confirmed by SDS-PAGE and Western blot, showing simultaneous, specific, and homogeneous coating. To evaluate the effect of tailored CBVs on immune response, BALB/c mice were immunized with three doses (2.5 µg TTFC/dose) of TTFC-CBVs or SLP-83111-TTFC-CBVs, and antigen-specific IgG and IgG1/IgG2a isotypes were evaluated by ELISA. Anti-TTFC IgG titers reached 3.9 and 3.5 log10 for TTFC-CBVs and SLP-83111-TTFC-CBVs, respectively. Consistent with previous reports, TTFC-CBVs induced a higher proportion of IgG1 antibodies, whereas SLP-83111-TTFC-CBVs elicited similar levels of IgG1 and IgG2a, indicating a more balanced immune response. Finally, mice were challenged with 75xLD50 of tetanus toxin, showing delayed symptom onset and increased survival in animals immunized with SLP-83111-TTFC-CBVs. These results suggest that incorporating S-layer proteins into the CBVs platform can modulate the induced immune profile, favoring a more balanced response and enhancing its use as a vaccine platform.

Key words: Antigen surface display; Tetanus; Bacillus subtilis; Lentilactobacillus kefiri; S-layer protein

VM-074

Preliminary efficacy results of an oral bivalent inactivated ETEC F4+/F18+ vaccine combined with a natural nanoadjuvant in weaned piglets

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Enterotoxigenic Escherichia coli (ETEC) strains expressing F4 and F18 fimbriae represent the primary cause of diarrhea in neonatal and post-weaning piglets. The aim of this study was to evaluate the efficacy of an oral vaccine formulated with inactivated ETEC strains (F4+/F18+) and a nanoadjuvant based on the essential oil of Minthostachys verticillata (EO) nanoemulsion. EO was extracted by hydrodistillation and the nanoemulsion (NE15) was performed by high-energy method using Tween 80/Span80 as surfactants. NE15 were chemically analyzed by gas chromatography-mass spectrometry (GC-MS), and their cytotoxic effects were evaluated on Caco-2 cell line. ETEC strains grown in modified Minca medium or CTS to increase expression of F4 and F18 fimbriae and inactivated with formaldehyde (0.5%) were used as immunogens. The immunogens were combined with NE15 (with 0.5 and 1 mg/ml of EO) to develop the vaccines (Vax1 and Vax 2). For in vivo assay, male weaned piglets (age: 28 days, mean initial body weight: 11.63 ± 0.37 kg) were randomly distributed in four groups of six animals each (n = 6) and were received orally immunogens alone, Vax1 and Vax 2 with four doses administered every 14 days. Animals not treated were evaluated as control. GC-MS analysis demonstrated that NE15 maintained the pulegone/menthone chemotype of EO. In addition, NE15 was not toxic in Caco-2 cells until 750 µg/ml. Vax1 improved growth performance compared to control and immunogens groups ($p < 0.05$). An in-

crease in leukocyte count was observed in the group receiving Vax1 compared to the control and Vax2 groups ($p<0.05$). The neutrophil to lymphocyte ratio (NLR) was increased in all vaccinated groups compared to the control group ($p<0.05$) being higher in the group that received the immunogens alone. The piglets receiving Vax1 and Vax2 showed an increase in percentage of CD4+ T cells compared to the control and immunogens groups ($p<0.05$). The percentage of CD8+ T cells was not altered in any group. Serum albumin, glucose, cholesterol, GPT and GOT were not altered and triglycerides showed a tendency to decrease. In conclusion, preliminary results suggest that Vax1 and Vax2 could activate the intestinal mucosa with a less inflammatory profile oriented towards adaptive immunity with a predominance of CD4+ T cells.

Key words: Enterotoxigenic Escherichia coli; Post-weaning diarrhea; Essential oil; Nanoadjuvant; Vaccine

VM-076

Increased expression of F4 and F18 fimbriae in enterotoxigenic Escherichia coli (ETEC) strains as immunogens for a bivalent vaccine to prevent post-weaning diarrhea in piglets

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Post-weaning diarrhea in pigs is one of the leading enteric diseases worldwide, causing significant economic losses and promoting antimicrobials use, which contributes to the development and spread of antimicrobial-resistant strains. Its main etiological agent is enterotoxigenic Escherichia coli (ETEC), being F4 and F18 fimbriae associated with neonatal and post-weaning diarrhea, respectively. Oral vaccines represent a promising alternative for their control and for reduction of antibiotic use. The aim of this study was to optimize and standardize the culture conditions of two ETEC strains, F4+ and F18+, to enhance fimbriae expression for their use as immunogens in a bivalent vaccine. Strains were previously characterized by conventional bacteriological tests (SIM– –/+/+; MRVP– +/–; citrate –; arginine –, lysine +; ornithine +; oxidase –; catalase +; TSI +/+). PCR analysis showed STb/LT/F4/EAST1 and STb/LT/F18/St×1/East1/AIDA-I as virulence factors. Bacteria were cultured at 37°C for 24 h in Trypticase Soy Broth (TSB), Minca broth, and a modified Minca broth supplemented with 1% fetal bovine serum (Minca-FBS) instead IsoVitalex. In addition, F18 strain was cultured in iron-free Minca broth. Colony- forming units (CFU/ml) were determined using the microdrop method, and optical density (OD600nm) was recorded. Relative expressions of genes encoding F4 and F18 fimbriae were evaluated by quantitative real-time PCR (qPCR). Counts of 1.3×10^9 and 1.25×10^9 CFU/ml were observed in TBS for ETEC $F4^+$ and $F18^+$, respectively. In contrast, in Minca broth the counts were 4.2×10^8 and 2.5×10^8 CFU/ml, while in Minca–FBS they reached 5.6×10^8 and 2.7×10^8 CFU/ml for ETEC $F4^+$ and $F18^+$, respectively. In iron-free Minca broth, a count of 4.7×10^8 CFU/ml was obtained for ETEC $F18^+$. In terms of relative gene expression, an increase in F4 fimbriae expression was observed in the ETEC $F4^+$ strain grown in Minca-FBS compared with the strain grown in TSB or Minca broth. On contrary, F18 fimbriae expression was higher in the ETEC $F18^+$ strain cultured in TSB compared with the strain grown in Minca broth, Minca-FBS or iron-free Minca broth. The results obtained allow for the selection of appropriate culture media for the expression of virulence factors, such as F4 and F18 fimbriae in ETEC, and provide a basis for the design of immunogens for vaccines aimed at preventing post-weaning diarrhea in piglets.

Key words: Enterotoxigenic Escherichia coli; Post-weaning diarrhea; F4 and F18 fimbriae expression

VM-091

Poly(allylamine hydrochloride)-based Nanoparticles as a Versatile Platform for Systemic and Mucosal Vaccination Strategies

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Nowadays, nanotechnology plays a central role in the development of modern vaccines, offering the possibility to design nanoparticles (NPs) that function both as antigen delivery systems and adjuvants. In this work, we investigated complex coacervate-like NPs composed of poly(allylamine hydrochloride) (PAH) and tripolyphosphate (TPP), as safe carriers and immune enhancers for systemic and mucosal vaccines. To assess their adjuvant potential, an in vivo immunization assay was first performed in which mice were intramuscularly administered NP-OVA. Based on these outcomes, in vitro studies were conducted to investigate underlying mechanisms, including NLRP3 inflammasome activation, unconventional Gasdermin-D–independent IL-1β release via autophagy, and Th1-biased, OVA-specific CD8+ T cell activation. The adjuvant potential of PAH-TPP NPs was subsequently evaluated in a heterologous SARS-CoV-2 vaccination model and in a murine food allergy model. In vivo intramuscular immunization with Np-OVA increased OVA-specific IgG and IgG2a titers, elevated IFN-γ production by splenocytes, and expanded CD4+IFN-γ+ and CD8+IFN-γ+ T cells populations ($p<0.05$). PAH-TPP NPs triggered IL-1β and IL-18 release in dendritic cells co-stimulated with LPS, via the NLRP3 pathway. Further experiments revealed co-localization of IL-1β with LC3B, an autophagosome marker, and autophagy inhibition with 3-methyladenine significantly reduced IL-1β secretion ($p<0,01$), confirming an autophagy-dependent release pathway. When implemented in a heterologous COVID-19 vaccination schedule (Pfizer® prime, NP-based candidate boost), K18 mice challenged with the Omicron BA.5 variant (1×10^4 PFU/10 µL/mouse) exhibited lower viral loads compared to PBS-immunized controls ($p<0.05$). In addition, the formulation demonstrated therapeutic benefits in a murine food allergy model by modulating the immune profile when applied as an adjuvant. In conclusion, these findings highlight PAH-TPP NPs as robust Th1-skewing adjuvants capable of inflammasome activation, pointing out their value as a versatile platform for the design of innovative vaccines targeting both infectious and non-infectious diseases.
Key words: Vaccine; Nanoparticles; COVID-19; immunotheraphy

VM-094

SEXUAL DIMORPHISM IN TISSUE DAMAGE, CARDIAC FUNCTION, AND CYTOKINE RESPONSE DURING CHRONIC T. CRUZI INFECTION: PROTECTIVE EFFECTS OF A TRANS-SIALIDASE-BASED VACCINE

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Sex influences immune and pathological responses during chronic Trypanosoma cruzi infection, affecting disease progression and potentially vaccine outcomes. We evaluated the protective effects of a mucosal vaccine based on recombinant Trans-sialidase (TS) combined with c-di-AMP on tissue damage, cardiac function, and cytokine profiles in male and female BALB/c mice during

the chronic phase. Mice received three intranasal doses (two weeks apart) of saline as vehicle (V-group), TS (TS-group), c-di-AMP (A-group), or the vaccine TS+A (TS+A-group), followed by oral challenge with 3,000 Tulahuen trypomastigotes. At 110 days post-infection, cardiac electrical activity was recorded under ketamine/xylazine anesthesia, analyzing QRS duration, corrected QT interval (QTc), and arrhythmias. Heart, skeletal muscle, and adipose tissue were collected, fixed, and histologically assessed with Hematoxylin/Eosin and Picrosirius red staining; inflammation and fibrosis were scored blindly. Plasma cytokines (IL-2, IL-4, IL-6, IFN-γ, TNF-α, IL-17A) were measured by Cytometric Bead Array, and myocardial cytokine mRNA expression by RT-qPCR. TS+A vaccination significantly reduced inflammation and fibrosis across tissues. In V-infected females, cardiac lesions were moderate (33%) and mild (66%), while in vaccinated females, moderate lesions disappeared, and a significant proportion of the tissue remained uninjured (60%). Similar histological changes were observed in males. Fibrosis was less evident in vaccinated animals, irrespective of sex. Cardiac tissue showed reduced profibrotic TGF-β in both sexes and decreased TNF-α and IL-1β mRNAs in males ($p<0.05$ vs. respective V-groups). ECG analysis revealed decreased arrhythmias, prevention of QRS prolongation, and maintenance of normal QTc values, mainly in both sexes, being more evident in females. Plasma cytokines increased in all infected groups, with higher IL-4 in TS+A vaccinated females ($p<0.05$ vs V-group). The chronic phase in vaccinated animals reflected subtle sex-dependent differences in cytokine responses and inflammation. However, TS+A vaccination confers robust protection against chronic tissue damage and cardiac dysfunction in both sexes. Overall, TS+A represents a promising strategy to mitigate chronic Chagas pathology, highlighting the need to consider sex as a factor in vaccine design and evaluation.

Key words: Sexual Dimorphism; Chronic *T. cruzi*; Trans-Sialidase

VM-096

Homologous and heterologous COVID-19 vaccines imprint distinct innate immune signatures consistent with trained immunity

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Previous studies from our group integrating data from phenotypic analyses of $CD4^+$ and $CD8^+$ T cells, SARS-CoV-2 Spike-specific memory B cell responses, antibodies (IgG and IgA), and spike-specific IFN-γ production revealed distinct activation patterns across 16 different COVID-19 vaccine combinations. These data defined specific adaptive immune profiles for each regimen, illustrating how vaccine type and combinations shape immune landscapes and potentially influence protective outcomes. Building on this background, we aimed to investigate whether COVID-19 vaccines also reprogram innate immunity, a process described as trained immunity, which has been shown to enhance host defense and modulate responses to subsequent infection. To this end, we focused on the two most immunogenic vaccine regimens from our previous study: homologous mRNA (mRNA-1273/mRNA-1273) and a heterologous combination (BBIBP/mRNA-1273), with homologous inactivated virus (BBIBP/BBIBP) serving as a reference. Using multiparametric flow cytometry (36 markers), we characterized the activation profiles of innate immune cells in these groups. Principal component analysis revealed clear segregation among the 3 regimens. The homologous mRNA group exhibited the highest PD-1 expression ($p < 0.05$) and a trend towards elevated TBET expression across various innate populations. To further explore functional features of trained immunity, we measured plasma cytokines four weeks after the second vaccine dose, a time point selected to capture stable innate immune imprinting. LEGENDplex analysis of 13 cytokines associated with COVID-19 hyperinflammation revealed significant increases ($p < 0.05$) in CCL3, IL-10, and IL-1RA in the homologous mRNA group, suggesting an innate pro-inflammatory response accompanied by regulatory signals. Integration with publicly available CITE-seq datasets from individuals vaccinated with a homologous mRNA regimen confirmed that these cytokines were predominantly produced by innate cell clusters, particularly monocytes producing CCL3 and,

to a lesser extent, IL-1RA. Together, these findings demonstrate that distinct COVID-19 vaccine regimens not only shape adaptive immunity but also leave durable imprints on innate immune responses, consistent with vaccine-induced trained immunity. Recognizing and harnessing these signatures may be crucial for optimizing next-generation vaccine strategies aimed at enhancing both adaptive and innate arms of immunity.

Key words: vaccine; trained immunity; COVID-19

VM-115

Nasal Vaccination Against Trypanosoma cruzi: A Dual Approach for Prevention and Treatment of Chronic Chagas Cardiomyopathy

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Chagas disease, caused by the parasite *Trypanosoma cruzi*, is a neglected life-threatening disease. Given that available pharmacologic treatments are effective only in the acute phase, and that diagnosis typically occurs during the chronic phase when cardiac damage is already present, current efforts should aim to mitigate cardiac pathology during chronic infection.This study evaluates the effectiveness of a nasal vaccine based on trans-sialidase (TS) plus c-di-AMP in both prophylactic and therapeutic settings against chronic Chagas cardiomyopathy (CCC) in a mouse model of *T. cruzi* oral infection. Prophylactic and therapeutic vaccination significantly reduced cardiac inflammation, fibrosis, and parasite load. Histological analysis confirmed less cardiac damage in vaccinated groups compared to infected, unvaccinated controls. While electrocardiographic abnormalities were fully prevented in the prophylactic group, therapeutic vaccination still halved arrhythmia incidence, indicating functional benefits despite late administration. Immunologically, both vaccine regimens promoted a Th17-skewed response, with increased IL-17 expression in cardiac tissue. However, distinct immune signatures were observed: prophylactic vaccination reduced TGF-β and T-bet expression, correlating with less fibrosis and inflammation; therapeutic vaccination elevated Foxp3, suggesting regulatory T cell involvement in controlling chronic inflammation. Both strategies enhanced TS-specific antibodies and reduced non-protective, parasite-wide antibody responses, shifting the humoral profile toward functional protection. Importantly, vaccinated animals also showed a marked reduction in heart auto-reactive antibodies. The findings suggest that early intervention yields greater benefits, but even post-infection, immunization can also significantly mitigate cardiac damage. These results underscore the potential of nasal TS-based vaccines as a non-invasive, dual-action strategy to both prevent and treat CCC.

Key words: Chagas disease; Trypanosoma cruzi; nasal vaccine; trans-sialidase; Th17 response

VM-126

PD-L2^{+} Lung Tissue-Resident Memory B Cells Drive Klebsiella pneumoniae-Specific IgA Responses Following Intranasal Immunization

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Memory B cells can establish residency in non-lymphoid tissues, becoming tissue-resident

memory B cells (BRM) that play a key role in protective immunity. However, their role in bacterial infections or immunizations remains poorly understood. We previously demonstrated that intranasal immunization with Klebsiella pneumoniae (Kp) induces lung-infiltrating B cells at day 14 (effector phase) and a subset persisting until day 60 (memory phase). Phenotypic analysis at day 35 postimmunization revealed expression of tissue residency and memory markers, including CD38, CD69, and PD-L2. In this study, we aimed to evaluate the ability of BRM to differentiate into antibody secreting cells (ASC). C57BL/6 mice were intranasally immunized with heat-killed Kp on days 0 and 7. To distinguish resident from circulating cells, mice received intravenous anti-CD45 labeling before sacrifice, combined with in-vitro anti-B220 staining of lung cells on day 35 post-immunization. Using differential expression of PD-L2, a marker associated with B cells with higher potential to become ASC, we sorted BRM PD-L2$^+$ and PD-L2$^-$ from lungs, as well as memory B cells PD-L2$^+$ and PD-L2$^-$ from spleen. These populations were cultured for 96 hours with various stimuli, with or without Kp antigen, and immunoglobulin (Ig) secretion in the culture supernatants was subsequently measured. Total Ig levels in culture supernatants were measured by LEGENDplex. Lung BRM PD-L2$^+$ cells produced both IgM and IgA under different stimuli, whereas lung BRM PD-L2$^-$ cells mainly produced IgM. In the spleen, both PD-L2$^+$ and PD-L2$^-$ memory B cells predominantly produced IgM. Using indirect ELISA to assess antigen specificity, only lung BRM PD-L2$^+$ cells secreted IgA specific for Kp. These results indicate that lung BRM PD-L2$^+$ cells are strategically positioned and primed to produce Kp-specific IgA, whereas lung BRM PD-L2$^-$ cells and splenic memory B cells, regardless of PD-L2 expression, predominantly produce IgM but do not generate antibodies specific for Kp. Our findings highlight the potential of intranasal immunization with Kp to elicit protective, antigen-specific IgA in the lung, supporting its consideration in vaccine development against antibiotic-resistant Kp.

Key words: Tissue Residents B Cells; Bacterial Immunization; Antibody Response

VM-164

OMV-Based Strategies to Overcome Current Pertussis Vaccine Limitations

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Protein-based acellular pertussis vaccines (aP) restored confidence in vaccination and enabled booster schedules, but long experience has revealed key limitations: short-lived immunity, failure to block transmission, and reduced effectiveness against emerging Bordetella pertussis isolates. To overcome these limitations, our group developed an outer-membrane-vesicle (OMV) vaccine derived from Bordetella pertussis and established preclinical proof-of-concept for its safety, immunogenicity and protection. Building on that, here we evaluate a combined formulation (OMV+aP), co-formulation of B. pertussis OMVs with licensed aP, to streamline clinical translation through non-inferiority comparisons based on antibody titers against aP immunogens shared by both vaccines. In a murine 2-dose model, we compared OMV+aP with aP alone for safety, immunogenicity and protection. OMV+aP was as safe as aP, showing normal weight gain and low IL-6 induction. It elicited higher serum antibody titers than aP for total IgG ($p<0.05$) and IgG2a ($p<0.01$), indicating a Th1-skewed response which is particularly advantageous due to its association with protective capacity. Splenocyte restimulation assayed showed significant higher levels of IFN-γ, IL-17 and IL-22 in combined vaccine vs aP. Moreover, OMV+aP conferred superior protection in both lower (Δ log CFU in lungs: OMV+aP vs. aP = 1.6) and upper airways (Δ log CFU in lungs: OMV+aP vs. aP = 0.7), and reduced lung colonization by aP immune resistant bacterial isolates (Δ log CFU in lungs: OMV+aP vs. aP = 2.9). Flow cytometry showed CD4$^+$ tissue-resident memory (TRM) cells in lungs and nasal mucosa after OMV+aP, but not after aP. Passive-transfer studies supported roles for humoral and cellular immunity: sera from OMV+aP donors eliminated lung colonization, whereas aP sera left detectable loads; splenocytes from OMV+aP donors yielded a 3.1-log reduction vs controls ($p<0.0001$), compared with 1.3-log after aP ($p<0.01$).

Together with the prior proof of concept for OMV alone, these data demonstrate that OMV+aP synergistically enhances the magnitude and quality of immunity, supporting its potential advancement toward clinical evaluation as a next-generation pertussis vaccine strategy.

Key words: Pertussis; OMV; combined vaccines; aP

VM-168

Bordetella pertussis OMV composition shapes Th1-skewing adjuvant responses

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We recently demonstrated that outer membrane vesicles (OMVs) derived from Bordetella pertussis exhibit strong adjuvant activity when formulated with heterologous immunogens, inducing immune responses comparable to those triggered by licensed aluminum salt-based adjuvants. Unlike the latter, OMVs drive a predominantly Th1-type response. While similar adjuvant properties have been described for OMVs from other bacteria, their Th1 polarization is less pronounced, suggesting that the molecular composition of B. pertussis OMVs may underlie this effect. To identify the components responsible for immune modulation and their potential role in memory induction, we compared OMVs from wild-type strains expressing all major immunogenic virulence factors (OMVvir+) with those from mutant strains lacking these factors (OMVvir−). Both OMVs were co-formulated with three heterologous antigens: tetanus and diphtheria toxoids, and the SARS-CoV-2 Spike protein. Immunization experiments in mice confirmed the capacity of OMVvir+ to drive a Th1-skewed response, as shown by significantly elevated IgG2a levels, particularly against tetanus ($p<0.0001$). In contrast, OMVvir− promoted a Th2-biased profile with significantly increased IgG1 levels ($p<0.05$), reducing the magnitude of Th1 profile detected for the OMVvir+. This effect was consistent across all studied heterologous antigens, although the magnitude of IgG1 and IgG2a induction varied depending on the immunogen. When experiments using OMVs derived from B. pertussis mutant strain lacking the expression of a single virulence factor, pertussis toxin (OMVPT−) or filamentous hemagglutinin (OMVFHA−), similar results were obtained. That is, both OMVPT− and OMVFHA− favored Th2-skewed responses ($p<0.01$), confirming that these virulence factors contribute to Th1 polarization. Splenocyte transfer assays demonstrated that neither the loss of all virulence factors nor the absence of individual ones, affected the capacity to elicit long-lasting, antigen-specific memory responses.

Overall, these findings demonstrate that B. pertussis OMVs possess potent adjuvant properties and that their molecular composition critically shapes the immune response profile. This knowledge enables the rational design of OMV-based vaccine platforms aimed at selectively modulating immune responses.

Key words: Bordetella pertussis; OMV; Th1; adjuvant

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