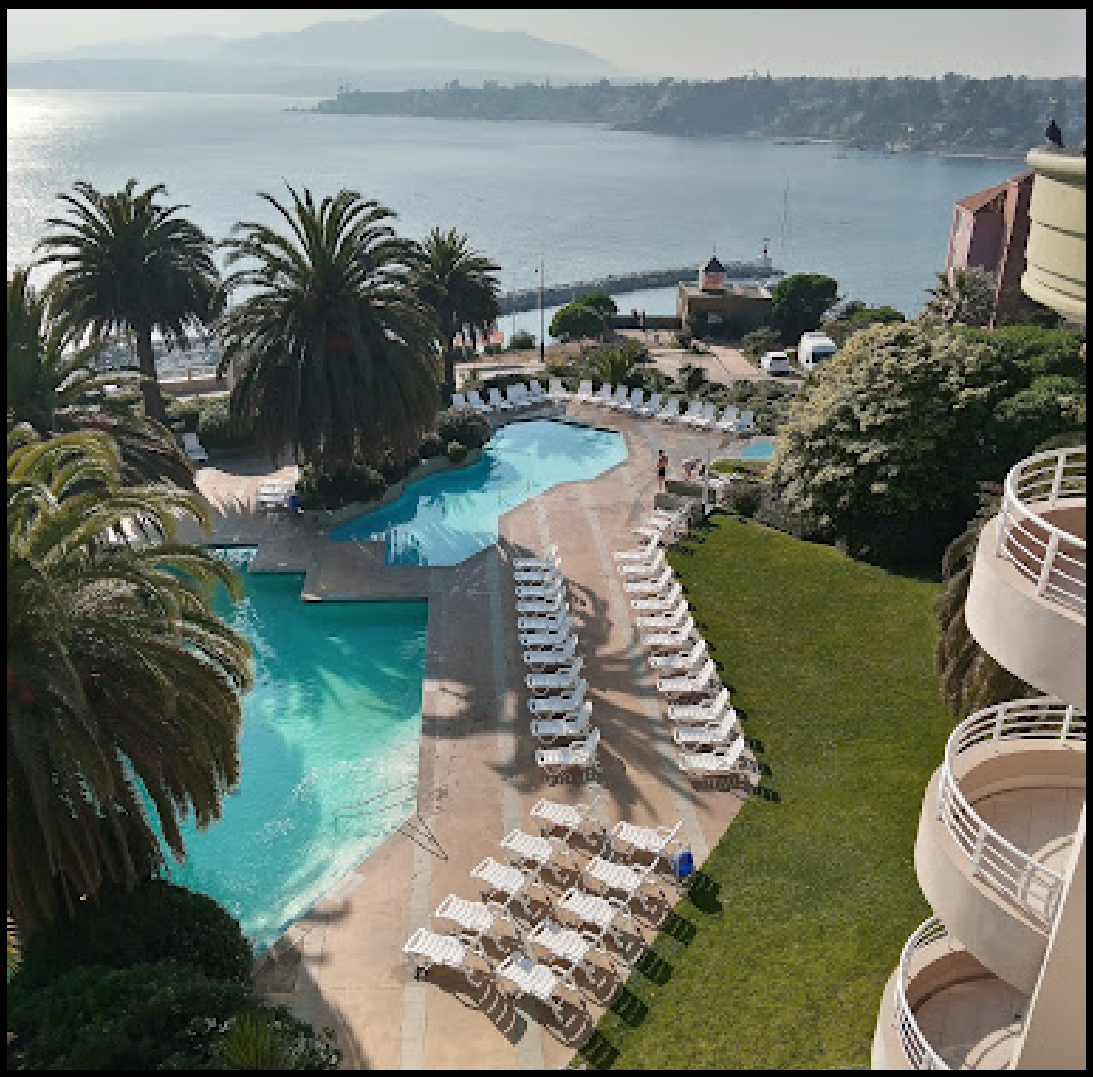
Asociación Chilena de Inmunología

VII Annual Meeting

Abstract Book



27 - 29 November 2024 / Concón - Chile

Asociación Chilena de Inmunología

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Scientific Program

Asociación Chilena de Inmunología

Wednesday 27th

14.00	Check In & poster mounting
15.00-15.15	Welcome words by ASOCHIN President, Karina Pino-Lagos
15.15-16.50	**Symposium "Autoimmunity, Cancer and Cell Therapy"**
	Chair: Patricia Luz-Crawford
15.15-15.50	"Metabolic reprogramming as a checkpoint in the regulation of gene expression: Metabolism meets Epigenetics" Rafael Argüello, University of Marseille, France
15.55-16.20	"EVs derived from glycolytic Mesenchymal stromal cells: New therapeutic strategy for inflammatory and autoimmune mediated disease" Patricia Luz-Crawford, Universidad de los Andes/IMPACT, Chile
16.25-16.50	"Transmitochondria (TRANSMIT): Harnessing Oral Cancer's Mitochondrial transfer for Immune Exhaustion and Stress Induction in T helper cells" Estefania Nova-Lamperti, Universidad de Concepción, Chile
16.55-17.45	Coffee Break
17.45-18.20	**Plenary Talk**
	Chair: Jennifer Alfaro
	"Mechanisms of action of mRNA-based vaccines" Arnaud Didierlaurent, University of Geneva, Switzerland
18.25-18.40	World Courier - sponsor short talk
18.45-19.45	**Poster Session (1-27)**
20.30	Dinner

Scientific Program

Asociación Chilena de Inmunología

Thursday 28th

8.45-9.25	**Plenary Talk** **Chair: Karina Pino-Lagos** "Cellular therapies for Cancer: a view from MINSAL" Julia Palma, MINSAL, Chile
9.30-11.30	**New ASOCHIN members** **Chairs: Carolina Ribeiro and Nicolás Dutzan**
9.30-9.55	"Extracellular vesicles in periodontitis, from immune regulators to systemic inflammation drivers" Carolina Rojas, Universidad de los Andes, Chile
10.00-10.25	"Oral dysbiosis as pathogenic factor and carcinogenesis" Gisela Canedo, Universidad de los Andes, Chile
10.30-10.55	"Deciphering molecular networks of Regulatory T cells: searching for therapeutic innovations in severe autoimmunity" Charlotte Hill, Universidad de los Andes/IMPACT, Chile
11.00-11.25	"Fueling the fight: Mitochondrial supercharged cells enhance the cytotoxicity of NK cells" Pablo Castro, Universidad de los Andes/IMPACT, Chile
11.30-11.45	Genexpress - Sponsor short talk
11.45-12.30	Coffee Break
12.30-14.10	**Short Talks** **Chairs: Lilian Reyes and Octavio Aravena**
12.30-12.39	"Modulation of Natural Killer Cell Phenotype and Function by CD29 Blockage in Murine Melanoma" Dario Donoso, Universidad de los Andes, Chile
12.41-12.50	"Mitochondrial Transfer from Cancer cells to Immune cells: A new cancer immune evasion mechanism?" Camila Fuentes, Universidad de los Andes/IMPACT, Chile
12.52-13.01	"Role of adenosine receptor A2A in the generation of exhausted CD8+ T cells in the tumor microenvironment" Solange Gouet, Universidad de Chile, Chile
13.03-13.12	"Neutrophils Key Role in Acute Local Response Induced by the Melanoma Vaccine TRIMELVax: Implications for Tumor Rejection" Flavio Salazar, Universidad de Chile, Chile
13.14-13.23	"Zika Virus Co-opts the IRE1/XBP1 Axis for Type-I Interferon and Inflammatory Cytokine Responses in Conventional Dendritic Cells" Mónica Guzmán, Universidad de Chile, Chile

Thursday 28th - continuation

13.25-13.34 "Progression of Treg response in severe and mild patients of COVID-19" Camila Kossack, Universidad San Sebastián, Puerto Montt, Chile

13.36-13.45 "Genetic diversity and transcriptional immune response to infectious pathogens in Indigenous South American populations" Lucas Vicuña, The University of Chicago, Chicago, United States

13.47-13.56 "Antioxidant and iron-chelating synthetic peptides as feed additives enhance innate immune markers in atlantic salmon after *Piscirickettsia salmonis* stimuli" Yannick Pombett, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

13.58-14.07 "Gut Immune Responses of Chinook Salmon (*Oncorhynchus tshawytscha*) and how to improve them using probiotics" Brian Dixon, University of Waterloo, Waterloo, Canada

14.10-15.30 Lunch Break/Poster mounting

15.30-17.25 **Symposium "Gut feelings: exploring intestinal immunity and its extraintestinal impact"**
Chairs: Caroll Beltrán and Rodrigo Naves

15.30-15.55 "Regulation of intestinal Th17 responses by the unfolded protein response sensor IRE1 in myeloid cells" Fabiola Osorio, Universidad de Chile, Chile

16.00-16.25 "Microbiota-derived metabolites controls the trafficking of gut immune cells to central nervous system during autoimmunity" Carolina Prado, Universidad San Sebastián/Fundación Ciencia & Vida, Chile

16.30-16.55 "The Brain-Gut-Axis: an integrated approach from neuroscience to immunology" Javier Bravo, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

17.00-17.25 "Microbiota-dependent T-cell response to a-synuclein-derived antigens triggers the development of Parkinson's disease" Rodrigo Pacheco, Universidad San Sebastián/Fundación Ciencia & Vida, Chile

17.30-19.00 Coffee Break + Poster Session (28-54)

20.30 Dinner

Asociación Chilena de Inmunología

Scientific Program

Asociación Chilena de Inmunología

Friday 29th

8.45-9.25 **Plenary Talk**
Chair: Carolina Ribeiro
"One as the sum of many: *Filifactor alocis* requires TLR2 and the oral commensal microbial community to display its full pathogenic potential" Silvia Uriarte, University of Louisville, Louisville, United States

9.35-11.50 **Symposium "Latest advances in mucosal immunology: from oral to gut"**
Chairs: Glauben Landskron and Estefanía Nova-Lamperti

9.35-10.15 "Gut bacteria wrestle with sumo in the intestinal arena" David Ribet, University of Rouen, France

10.20-10.45 "PANX1 hemichannel: A molecular bridge connecting tumor cells and the microenvironment in colon cancer" Marjorie de La Fuente, Universidad Finis Terrae, Chile

10.50-11.15 "Role of STAT3 during oral mucosal immunopathology" Nicolás Dutzan, Universidad de Chile, Chile

11.20-11.45 "Dietary prevention of undernutrition reduced intestinal inflammation in a DSS-induced colitis model" Mathilde Leboutte, University of Rouen, France

11.50-12.05 BD Biosciences - Sponsor short talk

12.10-12.45 Coffee Break

12.45-14.00 **Short Talks**
Chairs: Alejandra Gleisner and Daniela Sauma

12.56-13.05 "Evaluation of GelMA hydrogels immune response as biomaterial for the development of functional implants" Cristina Padilla, Universidad de los Andes, Chile

13.07-13.16 "Role of mitochondrial transfer of Mesenchymal stem/stromal cells on the phenotype of synovial macrophages to restrain the progression of Osteoarthritis" Liliana Yantén-Fuentes, Universidad de los Andes/IMPACT, Chile

13.18-13.27 "Small extracellular vesicles from different subsets of T regulatory cells reveal cell death-related proteins as cargo highlighting cytotoxicity as a suppression mechanism" Javiera de Solminihac, Universidad de los Andes, Chile

Friday 29th - continuation

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13.29-13.38 "Periodontitis and peri-implantitis-associated dysbiotic bacterial communities activate STAT3 in oral epithelial cells" Marion Arce, Universidad de Chile, Chile

13.40-13.49 "HLA class I presented peptides, derived from Rheumatoid Arthritis (RA) synovial tissue differentially activate CD8+ T cells from RA patients" Miqueas Jaime, Universidad de Chile, Chile

13.51-14.00 "Small extracellular vesicles from metabolically reprogrammed mesenchymal stem/stromal cell as a potential immunosuppressive mechanism for inflammatory and autoimmune diseases" Eliana Lara, Universidad de los Andes/IMPACT, Chile

14.10-15.30 Lunch Break

15.30-16.10 **Plenary Talk**
Chair: Karina Pino-Lagos
"Use of highly purified expanded allospecific regulatory T cells as therapeutic tools in kidney transplantation " Gloria Soldevila, Universidad Nacional Autónoma de México, Mexico

16.10-16.30 ASOCHIN Trajectory award 2024

16.30-16.45 Arquimed - Sponsor short talk

16.45-17.15 **Poster and Oral Presentations Awards**

17.15-17.30 Closing words

17.30-18.30 ASOCHIN members meeting

20.00 Dinner

22.00 Party

Asociación Chilena de Inmunología

Short-Talk Presentations

SHORT TALK / MUCOSAL IMMUNOLOGY

Periodontitis and peri-implantitis-associated dysbiotic bacterial communities activate STAT3 in oral epithelial cells

Marion Arce[1,2,3], Matías Morales[1], Joaquin Espinoza[1,2], Mariano Sanz[4], Loreto Abusleme[1,2,5], Nicolás Dutzan[1,2,3]

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(5) University of Chile, Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, Olivos 943, Santiago, Chile

Introduction: Periodontitis and peri-implantitis are diseases associated with a dysbiotic subgingival biofilm, which is highly prevalent in the population. Bacterial dysbiosis generates an immune-inflammatory response from the host, with the consequent loss of supporting tissues; this can lead to the loss of teeth or implants, respectively. STAT3 is a transcription factor associated with inflammatory diseases, and there is evidence of increased STAT3 activation during periodontitis. This study aimed to determine STAT3 activation in oral keratinocytes in response to bacterial communities associated with periodontitis and peri-implantitis.

Methodology: Histology was used to characterize pSTAT3-positive cells in tissues obtained from subjects diagnosed with gingival health, periodontitis, and peri-implantitis. On the other hand, a pool of subgingival biofilm samples was obtained and inactivated by cold/heat. Oral epithelial cells were stimulated with the subgingival biofilm samples. ELISA analyzed the levels of pSTAT3 and STAT3, and the expression of *IL-6, STAT3*, and *SOCS3* was analyzed by qPCR.

Results: A significant increase of pSTAT3-positive epithelial cells was observed in periodontitis and peri-implantitis compared to gingival health. In addition, an increase of pSTAT3 was obtained in oral epithelial cells stimulated with bacterial communities obtained from subgingival biofilm of periodontitis and peri-implantitis when compared with gingival health, and differences were observed in the expression of *IL-6* and *SOCS3*.

Conclusion: In periodontitis and peri-implantitis, there is an increase of STAT3 phosphorylation in oral epithelial cells, which is suggested to be in response to a dysbiosis associated with these conditions compared to gingival health.

Keywords: Periodontitis, Peri-implantitis, pSTAT3, Dysbiosis, Epithelial cells

Funding: Regular FONDECYT Project 1231350 and 1231728 and ANID-Subdirección de Capital Humano/Doctorado Nacional/2022-21221003

Acknowledgments: Regular FONDECYT Project 1231350 and 1231728, ANID-Subdirección de Capital Humano/Doctorado Nacional/2022-21221003, ETEP Research Group and Osteology Foundation.

SHORT TALK / COMPARATIVE IMMUNOLOGY

Gut Immune Responses of Chinook Salmon (Oncorhynchus tshawytscha) and how to improve them using probiotics.

Manuel Soto Davila[1], Luana Langlois Fiorotto[2], Tania Rodríguez-Ramos[3], Emily McKenzie[3], Megan Dyck[3], Abigail G. Dixon[3], Gregor Reid[2], **Brian Dixon**[3]

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(2) The University of Western Ontario, Microbiology and Immunology, Surgery, Medicine, London, Canada
(3) University of Waterloo, Biology, Science, Waterloo, Canada

Chinook salmon (*Oncorhynchus tshawytscha*) are valuable aquaculture species in Canada and Chile that suffer large losses due to disease. The widespread use of antibiotics as a solution has contributed to the development of antibiotic resistance, an issue of concern for the development of resistant strains that threaten the environment as well as fish and human health. Lactic acid bacteria (LAB) are well known for their potential as probiotics, improving fish growth, stress tolerance, and immune responses. Hence, the long-term supplementation of LAB strains in salmonids used for aquaculture has been proven to confer beneficial effects. However, whether these strains could function as immune modulators and enhancers of gut barrier integrity has not yet been investigated in non-traditional cultured salmonids, such as Chinook salmon (*Oncorhynchus tshawytscha*). We first examine baseline levels of immune genes and proteins in the gut, then, we performed a series of experiments in the intestinal epithelial cell line RTgutGC, and Chinook salmon juveniles aimed at establishing the potential of probiotic mixes (Jamieson), Pediococcus strains and lactic acid bacteria as oral immunostimulants. Cytokine gene and protein levels, in addition to tight junction gene expression levels were assessed. Following the experiments conducted in RTgutGC, *in vivo* studies in Chinook salmon juveniles were conducted. Fish were supplemented for four months with probiotic feed, with a regular feed control and a sodium alginate coating control also evaluated. Following probiotic supplementation, fish were challenged with *Vibrio anguillarum* to evaluate the immune effects of probiotic supplementation, and the same parameters were measured, in addition to survival. While changes in gene expression and protein level can be seen, they are modest, differences in survival could be seen in some cases. Overall, our results provide valuable information regarding the immune response of Chinook salmon gut tissues and suggests treatments that can improve fish health without antibiotics in an environmentally sustainable manner.

Keywords: Salmon, antibiotic resistance, probiotic, gut

Funding: NSERC Strategic Grant #119640, Canada Research Chair

Acknowledgments: Thanks to Yellow Island Aquaculture for providing fish net pens and resources

SHORT TALK / TUMOR IMMUNOLOGY

Modulation of Natural Killer Cell Phenotype and Function by CD29 Blockage in Murine Melanoma

Darío Donoso M[1], Javiera de Solminihac[1], Tomás Carrasco[1], Camila Pinto[1], Karina Pino-Lagos[1]

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Scarce information regarding Natural killer cells (NKs) function within lymph nodes (LNs) have been reported in the context of cancer. Despite their low frequency (0.5-1% of leukocytes), active patrolling of these cells is well characterized with cytolytic activity in peripheral LNs. In an immunocompetent syngeneic murine melanoma model, we previously demonstrated that systemic administration of an antibody targeting the integrin CD29 significantly decreased tumor growth. This fact is correlated with increased inflammatory mediators' expression on T-cells of tumor-infiltrating lymphocytes (TILs) and residents in the tumor-draining lymph nodes (TdLNs). Since CD29 interacts with CD49b, an integrin expressed by NKs, in this project, we aimed to evaluate whether the systemic administration of anti-CD29 could also modulate the phenotype and function of NKs in the LNs and intratumoral tissue. For this, intraperitoneal administration of anti-CD29 in both tumor-bearing and naïve mice was performed thrice per week for 10-12 days. Then, NKs were characterized regarding surface receptor expression (CD29, CD49b, CD11b, and CD27), perforin/granzyme B content, and cytokine production (IFN-gamma and TNF-alpha). Our results indicate that NKs display a distinctive phenotype in the LNs with higher CD11b and IFN-gamma expression than NKs from other tissues, exclusively in naïve mice. However, the administration of the anti-CD29 did not modify any of the analyzed parameters on NKs. These results indicate that the anti-tumoral properties of systemic anti-CD29 occur independently of NKs function.

Keywords: Cancer, Natural Killer, Melanoma

Funding: Fondecyt Grant #1210654 and ANID scholarship # 21230162.

SHORT TALK / TUMOR IMMUNOLOGY

Mitochondrial Transfer from Cancer cells to Immune cells: A new cancer immune evasion mechanism?

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Cancer cells use various strategies to evade the immune system and thrive. Recent findings indicate that tumor cells can hijack mitochondria (MT) from neighboring immune cells, inducing metabolic changes that promote cancer progression. While this MT transfer was believed unidirectional, from immune cells to cancer cells, our recent data shows that melanoma cells do release extracellular MT, suggesting these MT might be available to be internalized by tumor infiltrating immune cells.

We assessed the impact of t-MT on tumor-infiltrating immune cells using genetically modified B16-F10 cells expressing GFP-tagged mitochondria ($B16^{mitoGFP}$). C57BL/6 mice were inoculated with $B16^{mitoGFP}$ cells subcutaneously, and after 14-17 days, tumor tissues were analyzed using flow cytometry (FACS) and confocal microscopy to track mitochondrial transfer. In vitro, T cells were co-cultured with $B16^{mitoGFP}$ cells, and t-MT transfer effects were analyzed by FACS.

Tumor-associated macrophages, myeloid-derived cells, and T cells in tumors showed GFP+ events, indicating t-MT transfer. Notably, CD8+ T cells internalizing t-MT in vitro exhibited increased expression of inhibitory receptors Lag-3 and PD-1. *In vivo*, tumor-infiltrating CD8+ T cells with internalized t-MT showed higher levels of PD-1, TIGIT, and Lag-3, suggesting potential T cell exhaustion and enhanced tumor immune evasion.

Despite occurring at low frequencies, these findings represent, to our knowledge, the first evidence of immune cell internalization of t-MT *in vivo*. Future research will further explore the implications of t-MT transfer on tumor-infiltrating immune cells.

Keywords: Cancer, Mitochondria, CD8 T cell, Melanoma

Funding: ANID-BDN#2022-21220451; FONDECYT#1230875; ANID-BASAL#FB210024

SHORT TALK / TUMOR IMMUNOLOGY

Role of adenosine receptor A2A in the generation of exhausted CD8+ T cells in the tumor microenvironment

Solange Gouet[1], Juan Saavedra Almarza[1], Felipe Malgue[1], Valeria Manriquez[2], Christine Sedlik[2], Jordan Denizeau[2], Eliane Piaggio[2], Alberto Martin[3], Álvaro Lladser[3], Mario Rosemblatt[1,3,4], Maria Rosa Bono[1,3], Daniela Sauma[1,3]

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Cytotoxic CD8⁺T cells have the potential to eradicate cancer. However, in the tumor microenvironment, their cytotoxic capacity is decreased in a process known as exhaustion. Exhausted T-cells (Tex) derive from precursor exhausted T-cells (Tpex), which exhibit auto-renewal capacity. These cells are exposed to high concentrations of extracellular adenosine in the tumor niche, which can promote several features associated with Tpex. We aim to study the effect of the adenosine receptor A2A (A2AR) on the differentiation of exhausted cells in the tumor. For this, we generated CD8⁺ conditional A2ARKO mice (A2ARKO), and using this model, we observed an increase in absolute numbers of Tpex populations in A2ARKO compared to WT, suggesting that A2AR may promote terminal exhaustion. Accordingly, the co-transfer of A2ARKO and WT CD8⁺T cells in tumor-bearing mice showed a decrease in Tex and an increase in Tpex in the A2ARKO compared to the WT. Despite this, we observed a reduced number of A2ARKO cells compared to WT, suggesting that A2AR may also promote CD8⁺T cell survival. Also, in an *in vitro* system that mimics exhaustion, A2ARKO cells in the presence of IL2/IL12, showed a decrease of Tex and an increase of Tpex compared to WT cells. Finally, a combined therapy consisting of an A2AR agonist (CGS21680) and anti-PD1 treatment in tumor-bearing mice, showed a decrease in tumoral growth compared with monotherapies. These results suggest that adenosine through A2AR may promote a more terminally exhausted phenotype in CD8⁺T cells but may also be necessary for the anti-tumoral immune response.

Keywords: Exhaustion, A2AR, Adenosine, Precursor Exhausted, Immunotherapies

Funding: PhD scholarship Fundación María Ghilardi Venegas; PhD ANID scholarship 21201553; FONDEQUIP/EQM 220027; FONDEQUIP/EQM140016; ANID/1220196; ANID/BASAL/FB210008.

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SHORT TALK / IMMUNITY AND INFECTION

Zika Virus Co-opts the IRE1/XBP1 Axis for Type-I Interferon and Inflammatory Cytokine Responses in Conventional Dendritic Cells

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Introduction

Conventional dendritic cells type 1 (cDC1s) are essential for inducing long-lasting T cell responses against viral infections, emerging as key immunotherapeutic targets. Upon viral infection, cDC1s detect viral presence directly, by sensing viral components upon infection, or indirectly, through phagocytosis of virally infected dead cells. These recognition modes elicit antiviral responses by cDC1s, which are mediated by type-I interferon (IFN-I) and inflammatory cytokines production and allow initiation of adaptive immunity against a broad variety of viruses. However, in context of infection with Zika virus (ZIKV), a flavivirus transmitted by mosquitoes that can lead to severe inflammatory disease and congenital malformations, there is limited evidence on the role of cDC1s in controlling immune responses to the agent.

In this work, we aimed to elucidate the mechanisms by which cDC1s recognize ZIKV, along with identifying signaling pathways accounting for the process. To this end, we focused on the IRE1/XBP1 axis of the unfolded protein response (UPR), which is an adaptive signaling pathway essential to maintain protein homeostasis of the endoplasmic reticulum (ER) that is also recognized as a regulator of survival and function of cDC1s.

We demonstrate that ZIKV infection activates the IRE1/XBP1s axis in cDCs and induces the expression of IFN-I and inflammatory cytokines such as TNF and IL-6. Notably, XBP1 ablation significantly reduced this cytokine response. This work highlights a potential role for the IRE1/XBP1 axis in the activation of cDCs during the antiviral immune response, which need to be further studied to corroborate these findings and explore it as a potential therapeutic target.

Methods

cDC equivalents were generated from cultures of bone marrow progenitors and OP9/DL1 stromal cells (termed ‘OP9-DL1 cultures’), which generate *bona-fide* cDC1s. OP9-DL1 cDCs were generated from wild type (WT), XBP1 conditional knock-out (Itgax Cre x Xbp1fl/fl) mice and from ER stress activated indicator (ERAI mice, which reports IRE1 activation and activation of its associated transcription factor XBP1 fused to Venus fluorescent RNA).

For the direct infection setting, OP9-DL1-cDC1s were infected with ZIKV isolate BeH819015 at a MOI of 1 for 12/24 hours. As per the indirect infection setting, A549 cells were infected with ZIKV at a MOI of 1 for 24 hours and were then UV-irradiated to inactive the virus. These dead cells were used as stimulus for OP9/DL1-cDCs at a 4:1 ratio, overnight. Then, cells were collected and analyzed through flow cytometry, RT-PCR and qPCR.

Results

Our results indicate that cDC cultures can be directly infected with ZIKV, as evidenced by the presence of viral RNA in cultures (Fig. 1A). Direct ZIKV infection or stimulation with ZIKV-infected irradiated A549 cells led to increased Xbp1 splicing (Fig. 1B), indicating that ZIKV infection elicits activation of IRE1 RNase in cDCs. These findings were recapitulated using OP9-DL1 cDCs derived from ERAI reporter mice, which identified the cDC1 as the main responder cell type to ZIKV (Fig. 1C-D). Direct ZIKV infection also induced additional UPR components including *Bip*, *Erdj4* and *gadd34* in OP9-DL1 cDC cultures (Fig. 1E). These findings indicate that ZIKV directly infects cDCs and induce activation of XBP1s in the cDC1 subset, along with additional UPR components. Conversely, the indirect model of ZIKV sensing induced XBP1 in cDCs

but was not able to induce additional UPR components (Fig. 1F). Thus, we focused our subsequent studies on ZIKV-infected cDCs.

IRE1 RNase activation can also promote Regulated IRE1 dependent decay (or RIDD) to control mRNAs of functional relevance in cDC1s. To assess for RIDD activation after ZIKV infection, we monitored CD11c expression, a surrogate RIDD target. Data in Fig. 1G indicate ZIKV infection decreased CD11c expression significantly at 24 hpi, indicative of RIDD activation in cDCs. Next, we evaluated cDCs activation by measuring the expression of the co-stimulatory molecule CD40. We found that cDC1s increased CD40 expression upon infection at 12 and 24 hpi, without any difference in cDC1s XBP1 cKO (Fig. 2A). Finally, we assessed the production of cytokines and type I interferons upon ZIKV infection and determine the relevance of XBP1s by studying WT cDCs and XBP1 cKO cDCs. ZIKV-infected WT cDCs upregulated mRNAs coding for *Ifna, Ifnb*, *Il6* and *Tnf* expression at 12 hpi (Fig. 2B). XBP1 cKO cDCs also increased cytokine expression upon infection, but it was significantly reduced compared to infected WT cDCs, particularly for *Ifna*, *Ifnb* and *il6,* showing a potential regulatory role for the IRE1/XBP1 axis in the induction of cytokine production upon ZIKV infection.

Discussion

Our data show that ZIKV infects cDCs in culture and therefore, it can be potentially decoded by these cells to initiate immunity. ZIKV-infected cDCs activate the IRE1/XBP1s and additional members of the UPR, particularly in the cDC1 lineage. Interestingly, XBP1 ablation significantly reduced cytokine responses and type I IFN. Overall, this work identifies the IRE1/XBP1 axis as an emerging signaling pathway involved in innate sensing of ZIKV by cDCs. This novel project may provide valuable information on the role of IRE1/XBP1 as a regulatory axis in the activation of cDCs during the antiviral immune response, which could be explored as a new therapeutic target in the management of severe viral infections.

Keywords: Dendritic cells, cDC1s, Unfolded Protein Response, Zika Virus, Type-I interferons

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SHORT TALK / AUTOIMMUNITY AND INFLAMMAGING

HLA class I presented peptides, derived from rheumatoid arthritis (RA) synovial tissue, differentially activate CD8+ T cells from RA patients.

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation of the synovial joints. RA results from a complex immune response initiated and perpetuated by CD4+ T cells that recognize autoantigenic peptides presented by human major histocompatibility complex (HLA) class II molecules on the surface of antigen-presenting cells (APCs) (1). To date, data on the role of CD8+ T cells in RA have been scarce, but recent evidence suggests that their homeostasis may be altered. On one hand, a HLA class I allele (HLA-B*0801) containing an asparagine in position 9 within the peptide binding groove has been found to be associated with a higher risk for anti-citrullinated protein antibodies positive (ACPA+) RA (2). On the other hand, it has been shown that CD8+ T cells from ACPA+ RA patients respond to HLA class I-presented citrullinated peptides with clonal activation and expression of cytotoxic mediators and chemokine receptors. This would indicate that cytotoxic CD8+ T cells that target citrullinated proteins can contribute to synovial inflammation and joint tissue destruction (3). The aim of this study was to define the immunopeptidome of HLA class I molecules from RA synovial tissue (ST) and identify autoantigenic peptides recognized by CD8+ T cells from RA patients.

Methods: A sample of ST was obtained from the knee of a patient with RA by arthroplasty. The tissue was lysed to extract proteins. Peptide-HLA- class I complexes were obtained by immunoprecipitation with a polyclonal antibody against HLA-ABC molecules. The peptides were isolated by high-performance liquid chromatography and then sequenced by high-resolution mass spectrometry. The peptides were filtered according to the following criteria: length greater than 9 and shorter than 12 residues; high or medium theoretical affinity for the HLA class I alleles of the ST donor, or for HLA-A*0201, the most prevalent class I allele in the population, or for HLA-B*0801; and parental proteins described as autoantigenic in autoimmunity or RA. The immunodominance of the selected peptides was estimated by their ability to activate peripheral blood CD8+ T cells from RA patients (n = 10) compared to healthy donors (HD) (n = 18), using flow cytometry measurement of intracellular IFN-γ or surface CD107a (LAMP-1) expression, indicative of pro-inflammatory and cytotoxic functions.

Results: A universe of 3,504 peptides were isolated from ST HLA class I molecules. Among these, 11 peptides were selected from the following parental proteins: Acidic leucine-rich nuclear phosphoprotein 32 family member A (AN32A), Aryl hydrocarbon receptor (AHR), Beta-hexosaminidase subunit beta (HEXB), Cathepsin D (CATD), Exportin-1 (XPO1), Macrophage migration inhibitory factor (MIF), Progressive ankylosis protein homolog (ANKH), Protein C-ets-1 (ETS1), Raftlin-2 (RFTN2), Upstream stimulatory factor 1 (USF1), and Vimentin (VIME). Significantly higher frequencies of IFN-γ-expressing CD8+ T cells in RA patients compared to HD were found for XPO1 ($p<0.01$) and USF1 ($p<0.01$) peptides. For surface CD107a expression, significant differences were observed for XPO1 ($p<0.01$), MIF ($p<0.05$), USF1 ($p<0.01$) and AHR ($p<0.01$) peptides. When comparing the frequencies of CD8+ T cells co-expressing both markers between RA patients and HD, significant differences were observed for XPO1 ($p<0.05$), MIF ($p<0.01$), USF1 ($p<0.001$), VIME ($p<0.01$) and AHR ($p<0.05$) peptides (Figure 1). The magnitude of the responses elicited by the USF1 peptide among RA patients were significantly higher than those elicited the VIME peptide ($p<0.05$), whose parental protein has been widely described as an autoantigen for RA.

Discussion: Stimulation of CD8+ T cells with the USF1 peptide generated a significant and specific response of CD8+ T cells from RA patients in terms of the co-expression of IFN-γ and CD107a. Previously, Granzyme B+/IFN-γ+ CD8+ T cells activated by citrullinated autoantigens have been identified in the blood of RA patients (3). Interestingly, USF1 is an intracellular protein that interacts with RORγT, a transcription factor that regulates the development of Th17 cells and the expression of IL-17, which are deeply involved with RA pathogenesis (4). The identification of autoantigens in RA may open new opportunities for the generation of antigen-specific therapeutic protocols, through the use of tolerogenic dendritic cells pulsed with relevant peptides, that could restore tolerance in patients with this disease.

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Keywords: Rheumatoid arthritis, synovial tissue, autoantigens, CD8+ T cells.

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SHORT TALK / IMMUNITY AND INFECTION

Progression of Treg response in severe and mild patients of COVID-19

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Infection with SARS-CoV-2 can lead to a range of disease severities, with acute respiratory distress syndrome and cytokine storms being significant complications in severe cases. These patients often experience lymphopenia, T-cell exhaustion, and early humoral responses. Regulatory T cells (Tregs) play a critical role in controlling immune responses and inflammation, but their involvement in severe COVID-19 is poorly understood. Conflicting reports exist about their frequency and role in the cytokine storm associated with severe cases. It remains unclear whether Tregs fail to suppress hyperactive immune responses, are directly inhibited by the virus, or interact with host factors such as genotype or latent viral infections to influence disease severity.

To investigate this, we analyzed peripheral blood mononuclear cells (PBMCs) from a cohort of patients with mild and severe COVID-19, tracking them from disease onset to up to one-year post-infection. We assessed Treg phenotype using flow cytometry and gene expression profiles via bulk RNA sequencing.

Our results revealed a reduction in the $CD4^{+}CD25^{+}CD127^{low}$ Treg population in severe COVID-19 patients compared to mild cases during the first 30 days after symptom onset, with normalization occurring after three months. Additionally, key regulatory markers like FoxP3, CTLA-4, and PD-1 were dysregulated in severe cases but normalized by the end of the acute phase. These findings suggest a link between Treg dysregulation and disease severity, providing insights into the immunopathology of severe COVID-19

Keywords: Treg, COVID-19, Inflammatory response

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SHORT TALK / AUTOIMMUNITY AND INFLAMMAGING

Small extracellular vesicles from metabolically reprogrammed mesenchymal stem/stromal cell as a potential immunosuppressive mechanism for inflammatory and autoimmune diseases

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Introduction: Inflammatory and autoimmune diseases impact significantly patients' life and health and their treatment remain challenging. Mesenchymal stem cells (MSCs) offer a promising therapeutic approach due to their immunoregulatory effects, primarily mediated by small extracellular vesicles (sEVs). Our research on human umbilical cord MSCs (UC-MSCs) indicated that metabolic reprogramming to glycolysis enhances their ability to regulate immune responses, particularly by promoting T regulatory cells (Tregs) and inhibiting proinflammatory T cells (Th1, Th17). This study investigates the role of sEVs from glycolytic versus non-glycolytic UC-MSCs in their immunosuppressive effects in vitro and in vivo using a murine model of collagen-induced arthritis (CIA).

Materials and Methods: We isolated sEVs-MSCglyco and sEVs-MSCnon-glyco, characterized them using nanoparticle tracking analysis (NTA) and FACS, and assessed their immunosuppressive activity on peripheral blood mononuclear cells (PBMCs) and proinflammatory T cells. We evaluated sEVs ability to induce Tregs and their internalization in T cells via FACS and confocal microscopy. We also studied the effects on memory T-CD4 cells, including phenotypic changes and IL-10 production, and tested sEVs effects in a CIA mouse model.

Results: sEVs-MSCglyco significantly reduced Th1 cell proliferation and promoted Treg induction in vitro. These sEVs were internalized by memory T-CD4 cells, reducing Th1 and Th17 cell populations while increasing IL-10 production. In vivo, decreased CIA incidence and progression, correlating with reduced Th1 and Th17 cells in lymph nodes and peripheral blood.

Conclusion: sEVs-MSCglyco effectively modulate activated T cells, enhancing immunoregulatory capabilities both in vitro and in vivo, indicating their potential as a therapeutic tool for inflammatory diseases.

Keywords: extracellular vesicles, mesenchymal stem cells, autoimmune diseases

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SHORT TALK / CELLULAR AND MOLECULAR IMMUNOLOGY

Evaluation of GelMA hydrogels immune response as biomaterial for the development of functional implants

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The design of scaffolds/implants to promote immunomodulation in a local and controlled manner is key for tissue engineering and vaccine development. Gelatin methacryloyl (GelMA) stands as a suitable material for development of these scaffolds. Its structure can be tuned by partially promoting collagen native triple-helix configuration prior photocrosslinking. Nevertheless, how this configuration affects the hydrogel biological response has not been extensively studied, nor how these hydrogels affect immunity. Given the important role of dendritic cells (DCs) as antigen presenting cells, our objective is to determine the effect of triple-helix formation on GelMA hydrogels structuring, and its consequence on DCs phenotype. We produced GelMAs with 2 degrees of substitution (DS) from gelatins from 2 different origins. Samples were incubated at 4°C (triple-helix promotion) or 37°C, and photocrosslinked with UV-light. Splenic CD11c+DCs were cultured with hydrogels (previously characterized) and analyzed by flow-cytometry, and ELISA. DCs infiltration on implants and draining-lymph nodes (dLN) were evaluated by intravenous injection (a day before surgery) of CD45.1+DCs into RAG1-KO-mice. Triple-helix formation affected hydrogel structures displaying an increase in stiffness and overall density but did not exert significant changes on DCs phenotype; however, a trend towards greater activation, given by increased expression of CD86 on DCs and IL-6 release *in vitro*, was observed in the presence of hydrogels with lower DS. Additionally, DCs could infiltrate hydrogels and migrate into dLN with no phenotypic differences regardless of triple-helix formation. These results suggest that these hydrogels and their tunable structure, apparently, do not affect DCs phenotype *in vivo*.

Keywords: GelMA, Hydrogels, Implants, Dendritic cells

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SHORT TALK / COMPARATIVE IMMUNOLOGY

ANTIOXIDANT AND IRON-CHELATING SYNTHETIC PEPTIDES AS FEED ADDITIVES ENHANCE INNATE IMMUNE MARKERS IN ATLANTIC SALMON AFTER PISCIRICKETTSIA SALMONIS STIMULI

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Chilean salmonid farming loses over $500 million yearly due to salmonid rickettsial septicemia (SRS) caused by *Piscirickettsia salmonis*. To reduce fish mortality, our study proposes the use of synthetic peptides with bioactive properties, which can improve salmonid immunity without compromising growth performance. Seventeen antioxidant or iron-chelating peptides were synthesized, characterized, and screened based on their respective activity and the ability to modulate innate immune markers in RTS-11 cells. The selected peptides (peptide 4426: antioxidant; peptide 4429: iron-chelating) were microencapsulated and added (150 µg/kg) into a commercial-like diet to produce three experimental diets (AD: antioxidant diet, ID: iron-chelating diet, AID: diet with peptide combination at equal concentration). Then, a 4-week feeding pilot trial using Atlantic salmon was carried out. The relative gene expression of innate immune markers was assessed on the liver and spleen at the end of the trial. The expression of *sod1* (ID group) and *gpx* (AID group) decreased in the liver, while *tnfa* was downregulated in the spleen (AID group). In addition, head-kidney leukocytes (HKLs) from sampled fish were stimulated with total proteins from *P. salmonis* (LF-89 and EM-90 genogroups, and a mix of both), which showed an upregulation of *arg1* (AD group) and *il8* (ID and AID groups), while *sod1* was downregulated (AID group). Moreover, the immune response in HKLs from experimental diet groups was improved against specific *P. salmonis* genogroups. Therefore, peptides with iron-chelating and antioxidant properties could be considered in future research as potential feed additives to enhance the host immune response against *P. salmonis*.

Keywords: Bioactive peptides, In vitro immunomodulation, Novel feeds, Innate immunity, Piscirickettsia salmonis

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SHORT TALK / NEUROIMMUNOLOGY

Periodontal extracellular vesicles activate monocytes and microglial cells triggering potentially neuroinflammatory responses.

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Background. During pathological neuroinflammation -a common feature of neurodegeneration-, brain-resident and infiltrating cells, such as microglial cells (MCs) and monocytes become activated and adopt an activated/reactive phenotype with proinflammatory functions. Additionally, blood-brain barrier breakdown allows monocytes to infiltrate the brain parenchyma prompting tissue damage. Periodontitis (Perio), a chronic inflammatory gum disease, has been linked to neurodegenerative disorders. However, the molecular mechanisms underlying this association remain elusive. Herein, we hypothesize that periodontal extracellular vesicles (PEVs) from the periodontal bacteria *Porphyromonas gingivalis* (*Pg*) and Perio-affected patients (Perio-GCF-EVs) activate monocytes and MCs triggering an inflammatory profile. **Objective.** To assess whether PEVs can activate and induce an inflammatory response in monocytes and MCs. **Methods.** *Pg*-derived outer membrane vesicles (OMVs), were isolated from bacterial cultures supernatant and EVs from gingival crevicular fluid (GCF-EVs) samples of Perio patients or gingivally healthy (GH) subjects. Human THP-1 NF-κB and IRF dual reporter monocytes and HMC3 microglial cells were stimulated with increasing concentrations of *Pg*-OMVs, Perio, or GH GCF-EVs. Monocyte activation was evaluated reported pathway activation, while MCs polarization by morphology, flow cytometry and cytokine secretion assessment. **Results.** *Pg*-OMVs and Perio-GCF-EVs significantly increased NF-κB and IRF monocytic activity at all concentrations, whereas GH-GCF-EVs didn't. Notably, CD11b -a monocyte phenotypic/activation marker- expression, was upregulated accordingly. Similarly, in response to *Pg*-OMVs and Perio-GCF-EVs, MCs acquired an active/reactive morphology and upregulated pro-inflammatory cytokines secretion. **Conclusion.** PEVs activate monocytes and MCs prompting an inflammatory profile. These results suggest a novel mechanism that potentially links periodontitis to neuroinflammation.

Keywords: Extracellular vesicles, monocytes, microglial cells, periodontitis, neuroinflammation

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SHORT TALKS / TUMOR IMMUNOLOGY

Neutrophils Key Role in Acute Local Response Induced by the Melanoma Vaccine TRIMELVax: Implications for Tumor Rejection

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TRIMELVax is an immunotherapeutic vaccine for malignant melanoma, which combines heat shock-conditioned melanoma cell lysates with a mollusk hemocyanin as an adjuvant. Preclinical studies demonstrated significant effectiveness in reducing tumor growth and improving survival in mice with melanoma and colorectal tumors. However, the precise immunological mechanisms behind its anti-tumor effects remain unclear. This study focused on understanding how TRIMELVax induces inflammation at the injection site, recruits innate immune cells, and triggers DC migration to draining lymph nodes. To achieve this, C57BL/6 mice were injected into the footpad with TRIMELVax or controls, and then the skin and the popliteal lymph node were harvested for qPCR or FACS analysis. Results showed that TRIMELVax rapidly induced a specific pattern of proinflammatory cytokines and chemokines, leading to an acute innate immune response in the administration site. Neutrophils, type 1 macrophages, monocytes, cDC1, Langerhans cells, and monocyte derived-DCs were recruited to the footpad, while type 2 macrophages decreased. This early inflammation facilitated a superior migration of cDC1 and a new subtype of antigen-presenting neutrophils to the lymph node compared to controls. We demonstrate that vaccine-induced neutrophils are fundamental for TRIMELVax's mechanism of action. Depleting neutrophils reduced DC migration, especially cDC1, to the draining lymph node and suppressed TRIMELVax's ability to control tumor growth. In summary, TRIMELVax triggers a rapid and potent activation of the innate immune system, which seems crucial for a more effective adaptive immune response against aggressive melanoma tumors. These results underscore the promise of TRIMELVax as a potential immunotherapy for melanoma treatment.

Keywords: Cancer vaccine immunotherapy melanoma colon cancer

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SHORT TALKS / IMMUNITY AND INFECTION

Genetic diversity and transcriptional immune response to infectious pathogens in Indigenous South American populations

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Indigenous peoples from South America have a unique evolutionary history, beginning when East Asians journeyed into the Americas ~ 23,000-25,000 years ago. As a result of complex patterns of genetic exchanges and local adaptations, Indigenous populations are expected to harbor unique phenotypic variation, including immune responses to infection. The *Immune Cell Atlas of Indigenous South American Populations* aims to generate the first comprehensive single-cell resolution atlases of gene expression and epigenetic variation in immune cells from Indigenous South American populations, before and after immune activation.

I will present preliminary results of gene expression from 834,000 whole-transcriptomes of peripheral blood mononuclear cells (PBMCs), before and six hours after stimulation with lipopolysaccharide (LPS), influenza A virus (IAV), and the intracellular parasite *Trypanosoma cruzi*. PBMCs belong to 192 participants from 11 Indigenous populations from the Andes Mountains, Southern Chile, and the Amazon Rainforest. We included a Chilean population with 93% European ancestry on average as control. We found high intra-individual variation in gene expression in response to the three stimuli, both in host genes as well as in IAV and *T. cruzi* genes. In CD14+ monocytes, there is an enrichment of genes associated with IFN-α and IFN-γ pathways in upregulated human genes after stimulation with LPS and IAV. However, we observe the opposite upon *T. cruzi* infection, namely, an enrichment of these pathways in downregulated genes, suggesting that *T. cruzi* shuts down INF signaling in the host. Our findings have medical relevance for Indigenous American populations.

Keywords: immune response, pathogens, Indigenous Americans, single-cell RNA sequencing

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SHORT TALKS / CELLULAR AND MOLECULAR IMMUNOLOGY

Role of mitochondrial transfer of Mesenchymal stem/stromal cells on the phenotype of synovial macrophages to restrain the progression of Osteoarthritis

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Introduction:
Osteoarthritis (OA) causes chronic pain, stiffness, limited mobility, joint enlargement, and synovial inflammation. Current treatments provide palliative relief, necessitating exploration of regenerative therapies like mesenchymal stem/stromal cells (MSCs) and their derivatives, such as mitochondria (mito-MSCs). While synovial macrophages are crucial in OA's symptomology and progression, we hypothesize that mito-MSCs can induce the polarization of pro-inflammatory into anti-inflammatory macrophages restraining OA progression.

Material and Methods:
We obtained synovial macrophages ex vivo from OA patients' synovial membranes. Concurrently, we obtained MSCs from umbilical cord tissue, labeled them with MitoTracker, and employed their mitochondria for synovial macrophage internalization. We visualized this process using confocal microscopy and assessed their phenotype through flow cytometry, measuring CD68, CD163, CD206, CD86, CD11b, and HLA markers

Results:
The isolated mitochondria were confirmed to be intact and functional and confocal images show that synovial macrophages successfully acquired and internalized mitochondria from mesenchymal stem cells (MSCs), as evidenced by the uptake of MitoTracker-stained MSC-derived mitochondria. Furthermore, treatment with mito-MSCs resulted in a significant reduction in the population of M1-like synovial macrophages ($CD68^{+}$, $CD11b^{+}$, HLA^{high}, $CD86^{high}$), suggesting a shift away from the pro-inflammatory phenotype.

Discussion:
Our findings indicate that synovial macrophages successfully acquire mitochondria from MSCs, leading to a reduction in the population of M1-like synovial macrophages following mito-MSC treatment. These results suggest a phenotypic switch in synovial macrophages from a pro-inflammatory state to an anti-inflammatory one, potentially driven by mitochondrial transfer. As a projection of this study, future investigations should focus on exploring the metabolic changes occurring in synovial macrophages post-mitoception.

Keywords: Synovial macrophages, Mitocondrial transfer, Mesenquimal stem/stromal cells, Osteoarthritis, Cell therapy

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SHORT TALKS / CELLULAR AND MOLECULAR IMMUNOLOGY

Small extracellular vesicles from different subsets of T regulatory cells reveal cell death-related proteins as cargo highlighting cytotoxicity as suppression mechanism

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T-regulatory cells (Tregs) act as modulators of immunity where one of their suppressive mechanisms is the release of small-extracellular-vesicles (sEV). Tregs can be classified based on their origin: thymic (nTregs) and induced (iTregs) Tregs. In this study we investigated the composition of nTregs- and iTregs-derived sEV and their suppressive function *in-vitro*. nTregs and naïve CD4+ T-cells were purified using magnetic beads. nTregs were cultured for 72h and iTregs were induced with IL-2 and TGF-b alone or with retinoic-acid (RATregs). sEV were purified using ultracentrifugation and IZON columns, were characterized by size and concentration using the Nanoparticle-tracking-analysis (NTA), and Tandem-mass-spectrometry (MS/MS) was performed to identify their protein content. Suppression assays were performed by polyclonally activating splenocytes for 72h in the presence of sEV obtained from the three types of Tregs, and their phenotype was evaluated by flow cytometry and ELISA. Our results indicate that sEV obtained from the three-types of Tregs share similar characteristics such as particle's number (10^8 part/mL) and size (~150nm). Suppression of T-cell proliferation was sEV-dose-dependent, observing that iTregs-derived sEV are less effective on inhibition that sEV obtained from nTregs or RATregs. sEV from RATregs induced the release of IFN-γ and IL-17. sEV proteomic revealed an enrichment for cell death-related proteins which was confirmed by differential induction of apoptosis and necrosis on activated splenocytes, where sEV from iTregs and RATregs resulted more apoptotic than nTregs, which stimulated necrosis. In conclusion, these results reveal a novel possible suppression mechanism for Tregs-derived sEV, highlighting the role of cell death-related proteins.

Keywords: Treg, sEV, suppression

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Poster Presentations

POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Anti-inflammatory Activity of Quillaic Acid in Modulating the Immune Response

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Introduction: The incidence of inflammatory diseases has been rising in recent years, and the use of alternative therapies has gained popularity. This trend is largely driven by the fact that natural treatments are more biocompatibility. The secondary metabolites such pentacyclic triterpenes (TP), found in a wide variety of herbs, are increasingly being studied for their anti- inflammatory activity. Quillaic Acid (QA), a TP present in *Quillaja Saponaria Molina* (Quillay), has been the focus of increasing research, as it has been shown to exhibit anti-inflammatory effects when applied topically in *in vivo* models. These studies have demonstrated its ability to reduce inflammatory markers and mitigate local inflammatory responses.

Objectives: Evaluate the effect of QA in a model of acute inflammation in murine macrophages.

Methodology: Prophylactic effect of QA was evaluated in murine macrophages using an acute inflammation model induced by LPS. Inflammatory components were evaluated at both protein and transcription levels.

Results and discussion: In acute inflammation due to the administration of LPS, QA has a repressive effect on the nuclear translocation of NF-kB. Coincidentally, results obtained from sequencing (RNAseq) indicate an enrichment in genes associated with the signaling cascade around NF-kB that would be downregulated. Furthermore, in comparison to treatment with QA, certain genes are downregulated even in the absence of an ongoing inflammatory process. This suggests that QA may exert regulatory effects on gene expression independently of inflammation, indicating its potential role in modulating baseline cellular functions. These point to an action for QA beyond its anti-inflammatory properties, potentially affecting various molecular pathways.

Keywords: anti-inflammatory properties, Quillaic Acid, Quillaja saponaria Molina, acute inflammation.

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

The Influence of Maternal Body Mass Index on the Inflammatory Mediators in Breast Milk

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Introduction: Breast milk (BM) is a complex biofluid that contains cells that secrete pro- and anti-inflammatory mediators whose play a crucial role for infant growth and the immune system development. However, little is known about the relationship between these bioactive components in mature BM and postpartum maternal nutritional status. The purpose of this study was to determine whether maternal body mass index (BMI) during the postpartum period influences the levels of several pro- and anti-inflammatory mediators (IL-6, IL-10, IL-1β, IL-17A, IFN-γ, TNF-α, TGF-β1, NGAL, and FOXP3) in plasma and mature breast milk.

Methods: Women among 1-5 months postpartum were recruited from the Family Health Center 1. Clinical information, blood and BM were obtained, and women were classified according to their BMI in normoweight (18.5-24.9 kg/m2), overweight (25- 29.9 kg/m2) and obese (>30 kg/m2; n = 10 per group). BM-macronutrient content (fat, proteins and lactose) was measured by a Milk Analyzer. Plasma levels of IL-6, IL-17A, IL-1β, IFN-γ, and TNF-α were determined by flow cytometry. BM levels of IL-1β, TNF-α and IL-6 were measured by flow cytometry, while NGAL was determined by ELISA. The BM mRNA abundance for IL-10, IL-17A, NGAL, TGF-β and FOXP3 were quantified by RT-qPCR.

Results: No significant differences were found in the maternal clinical parameters, nor the BM macronutrient composition between the different groups. Women with obesity showed decrease plasma protein levels of IL-17A compared to the overweight group ($p < 0.05$), but no significant differences were observed in IL-1β, IFN-γ, TNF-α, and IL-6 between groups ($p > 0.05$). Analysis of BM inflammatory mediators showed no significant differences in IL-1β, TNF-α, IL-6 and NGAL at protein levels between groups ($p > 0.05$). The BM mRNA abundance of IL-10 and FOXP3 were increased in the group of women with obesity compared to women with overweight ($p < 0.05$). No significant differences were observed in the expression of IL-17A, TGFβ-1 and NGAL between groups ($p > 0.05$).

Discussion: Our findings indicate that maternal overweight and obesity differentially influence inflammatory mediators in the postpartum period, as seen in the decreased levels of IL-17A in plasma and the increased expression of IL-10 and FOXP3 in women with obesity. The mRNA abundance levels of characteristic inflammatory markers in BM cells reveal a higher expression of FOXP3, a key regulator of T cells, in the obesity group. These results suggest that maternal BMI may modulate the immune profile of BM, potentially impacting infant immune development. Further research is needed to explore the underlying mechanisms and long-term effects of these changes.

Keywords: Breast milk, inflammation, BMI, postpartum

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Pannexin 1 as a Key Regulator of Antigen Translocation and CD8+ T Cell Activation through Cross-Presentation

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Cross-presentation of antigens is a crucial process for the activation of CD8+ T cells, as it enables the presentation of exogenous antigens through major histocompatibility complex class I (MHC-I), which is essential for adaptive immune responses against viral infections and certain cancers. Although the underlying molecular mechanism is not fully understood, a widely accepted hypothesis suggests that exogenous antigens must be translocated from phagolysosomal compartments to the cytoplasm, following the cytosolic pathway for processing and eventual loading onto MHC-I. In this context, Pannexin 1 (Panx1), a protein from the gap junction family, has emerged as a key candidate due to its ability to form non-selective channels that allow the passage of molecules up to 1.5 kDa, thus facilitating the transport of phagocytosed antigens. In our study, we blocked Panx1 function using the specific inhibitor peptide 10Panx1 in primary bone marrow-derived dendritic cells (BMDCs) and the Mutu1940 cell line. We observed a significant reduction in the translocation of the OVA antigen to the cytoplasm of dendritic cells, which was accompanied by a decrease in cross-presentation, as assessed by the activation of B3Z T hybridomas specific for the SIINFEKL/MHC-I complex. These findings suggest that Panx1 plays a crucial role in regulating antigen trafficking to the cytoplasm and, consequently, in cross-presentation, potentially offering new therapeutic strategies aimed at modulating CD8+ T cell responses.

Keywords: Cross-presentation, Pannexin 1, dendritic cells.

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Immunomodulatory effect of Small Extracellular Vesicles (sEVs) from metabolically reprogrammed Mesenchymal Stem/Stromal Cells (MSCs) on Memory T-CD4+ cells

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An imbalance between proinflammatory and regulatory T-CD4$^+$ subpopulations is a pathogenic feature of chronic autoimmune/inflammatory diseases, thus, balancing the T-CD4$^+$ cells immune response to self–antigens is an ongoing challenge. The use of MSCs as a therapeutic approach have been broadly documented due to their immunomodulatory and differentiation ability. However, clinical outcomes are divergent, and the use of metabolically reprogrammed MSCs towards glycolysis have been proposed to enhance their therapeutic capabilities. One of the mechanisms through which MSCs exert their biological effects relies on secreting sEVs. Therefore, we investigated whether sEVs from metabolically reprogrammed umbilical-cord-derived MSCs (UC-MSC) have an enhanced immunosuppressive effect on T-CD4$^+$ cells.

sEVs where isolated by ultracentrifugation from basal UC-MSCs (sEVs) or reprogrammed towards glycolysis (sEVs-Glyco) and were characterized by nanozide&FACS analysis. Memory T-CD4 from PBMC and culture in the presence or absence of sEVs/sEVs-Glyco. The internalization of sEVs/sEVs-Glyco on memory T-CD4 and the phenotype of proinflammatory Th1&Th17 and anti-inflammatory Treg cells was evaluated by FACS. The immunomodulatory effect of EVs/EVs-Glyco was evaluated in a murine model of DTH by FACS analysis of murine-PBMC.

Both vesicles were able to internalize into memory T-CD4$^+$ cells. Furthermore, we found that sEVs/sEVs-Glyco decrease the percentage of IFNγ and IL17-producing Th1 or Th17 cells, while no effects were observed on the percentage of Treg. Both vesicles conditions shown to reduce the percentage of Th1 and Th17 cells with increased Treg/Th1 and Treg/Th17 ratios in the murine model.

These findings suggest that sEVs/sEVs-Glyco can modulate the immune response by modifying the phenotypes of memory T- CD4$^+$ cells.

Keywords: extracellular vesicles, immunomodulation, memory T cells

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Interleukin-33 promotes the expansion of type-2 innate lymphoid cells to prevent inflammation and organ failure in a mouse model of sepsis

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Sepsis represents an important global challenge, with high incidence and mortality rates, underscoring the urgency of understanding its underlying mechanisms and developing effective treatments. Lipopolysaccharide-induced endotoxemia (LPS, endotoxin) has been an established experimental model to study the pathophysiology of sepsis. IL-33 is known for its repair functions in organs through the expansion and activation of tissue-resident type-2 innate lymphoid cells (ILC2). The present study aims to examine the immunomodulatory effects of interleukin 33 (IL-33) over ILC2 and organ function during endotoxemia.
In this study, we quantified serum biochemical markers as indicators of renal and hepatic function (*i.e.*, blood urea nitrogen [BUN], creatinine, and hepatic transaminases). We observed a significant decrease in circulating BUN and gamma-glutamil transferase of mice treated with IL-33 compared to controls, indicating a protective effect over the kidney and liver. The lung, liver, and kidney content of ILC2, quantified using flow cytometry, was increased in mice treated with IL-33 compared to controls.
Our data shows that IL-33 protects organ function while promoting the tissular expansion of ILC2 during endotoxemia. Because this expansion is at the expense of a regulatory phenotype of ILC2, IL-33 is highlighted as a potentially therapeutic molecule to provide new treatments for sepsis. Future studies should elucidate the molecular mechanisms underlying ILC2-mediated immunoregulation during sepsis.

Keywords: LPS, IL-33, ILC2, Sepsis, Endotoxemia

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Gestational hypothyroxinemia shifts innate lymphoid cell reactivity in gut territories during early EAE

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Background: Maternal thyroxine (T4) is the main token of exchange of thyroid hormones (TH) for the developing fetus. Decreased levels of T4 during pregnancy (gestational hypothyroxinemia, HTX) is asymptomatic for the mother and detrimental in the long-term for the offspring, leading to amplified immune responses. Innate lymphoid cells (ILC) are novel components of the innate immunity with helping functions mirroring T cells. Previous descriptions of ILC content during EAE (a murine model of multiple sclerosis) focus on late onset neuroinflammation, completely overlooking early onset reactivity and ILC content in other peripheral territories. The present work aims to describe ILC content in the CNS, the intestinal tract, mesenteric lymph nodes (mLN), and the spleen, in the HTX offspring and their euthyroid (ETX) counterparts, at baseline and 7 days after EAE immunization.
Methods: Wild type C57BL/6 pregnant dams were administered methimazole (antithyroid drug) or vehicle between days E10 and E15. Their offspring (HTX or ETX) were induced a mild form of EAE at day P55. Brain, spinal cord, small/large intestines, mLN, and spleen, were harvested at day EAE7 and processed for flow cytometry using a panel of antibodies to detect ILC. All procedures were granted institutional bioethical approval (#008/2022). Statistical differences across groups were tested by two- way ANOVA followed by Tukey's *post hoc* test. Data shown as mean ± SEM. n= 4 per group.
Results: A baseline increase in ILC content was present in the colon and mLN of the HTX offspring. A dramatic increase in ILC content was observed in the colon of the HTX offspring at day EAE7, higher than the baseline and its ETX counterpart on the same day.
Conclusions: Decreased levels of T4 during pregnancy disrupted ILC content in adulthood, even in the absence of inflammatory stimuli. Also, HTX modulated EAE inflammation outside of the CNS, increasing ILC reactivity in the colon. The latter is in accordance with previous descriptions of EAE score showing earlier and more severely in the HTX offspring. Further research characterizing the gut/CNS axis during EAE progression is required to properly assess the contribution of HTX to the onset of multiple sclerosis.

Keywords: hypothyroxinemia, innate lymphoid cells, multiple sclerosis, experimental autoimmune encephalomyelitis, thyroid hormones

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Tattoo ink uptake in human macrophages is mediated by actin polymerization but not endocytic pathways

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Tattoos have become increasingly popular in modern society, yet the molecular mechanisms involved in tattoo ink uptake remain poorly understood. Macrophages play a crucial role in tattoo pigment uptake, but their inability to degrade these large particles leads to pigment release upon cell death, allowing new macrophages to engulf them, contributing to tattoo persistence. We hypothesized that macrophages, as phagocytic cells, actively transport tattoo ink into cells. To assess this, human THP-1-derived macrophages were incubated with black tattoo ink or green fluorescent tattoo ink up to three hours. Raman spectroscopy confirmed that black ink was carbon-based, and dynamic light scattering showed that pigment sizes were approximately 300 nm for black ink and 700 nm for green fluorescent ink. After three hours, 80% of macrophages had incorporated the green pigment. We observed significantly lower uptake of green fluorescent ink when cells were incubated at 4°C compared to 37°C, indicating the need for membrane transport. Although dynasore, an endocytosis inhibitor, did not alter green fluorescent ink uptake, actin polymerization inhibitor cytochalasin D significantly reduced it, which was confirmed by flow cytometry and confocal microscopy. Black tattoo ink uptake was also inhibited by cytochalasin D, assessed by optic and transmission electron microscopy, suggesting a non-specific uptake mechanism in macrophages. By contrast, human skin keratinocytes HaCat incorporated low levels of green pigment, supporting the idea that phagocytic cells play a key role in tattoo persistence. Further research is needed to clarify the contribution of micropinocytosis and phagocytosis in tattoo ink uptake in macrophages.

Keywords: Macrophages, tattoo ink, phagocytosis, endocytosis

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Functional Analysis of P2X7 Receptor Variants.

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Cross-dressing is a non-conventional form of antigen presentation, where MHC-peptide complexes are transferred from the membrane of an antigen donor cell to antigen-presenting cells. Previous research in our laboratory has shown that the P2X7 receptor is essential for this process in bone marrow-derived dendritic cells and J774 macrophages, using apoptotic bodies as membrane donors. However, how P2X7 mediates this function is still not understood.
P2X7 is an ion channel activated by ATP, which also forms a macropore and activates signaling pathways such as ERK1/2. To understand which of these functions is critical for cross-dressing, we first need to isolate them. In order to achieve this, we evaluated the functions of different P2X7 variants (reported to lose one of their functions) in HEK293 cells: P2X7A (wild type), P2X7B (without macropore activity), P2X7 ΔN (without signaling transduction capacity), SNP T283M (without channel activity), and a dominant-negative variant (W167A; C168A; dn).
After stimulating the cells with 1 mM ATP, preliminary results indicated that only P2X7A retains ion channel activity. For macropore formation and signal transduction assays, cells were stimulated with 60 µM BzATP. In macropore formation, P2X7A showed a 15-fold increase in BrEt uptake, while the P2X7 T283M and dn variants only doubled the basal fluorescence. In signal transduction, P2X7A induced greater ERK phosphorylation compared to the other variants, which showed reduced activity.
Although these results provide useful information, it will be necessary to study more variants that specifically affect individual functions to identify the mechanism by which P2X7 participates in cross-dressing.

Keywords: P2X7, Cross-presentation, Ionic channel, Cell signaling, Macropore

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Intercellular Interactions of P2X7: Bioinformatic Analysis of P2X7-P2X7 Complex Formation

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P2X7 is an ATP-activated ionotropic receptor, characterized, like other P2X receptors, by its canonical function as a non- selective cation channel. However, various non-canonical functions of P2X7 have been described, including its involvement in cell fusion processes, where it is essential for the receptor to be present on both cells to allow cell-cell contact. In our laboratory, we identified that P2X7 plays a crucial role in Cross-Dressing, an unconventional antigen presentation mechanism that involves the transfer of peptide/MHC complexes from the membrane of donor cells to antigen-presenting cells (APCs). For this process to occur, the presence of P2X7 is required in both the donor and recipient cells, and it occurs independently of ATP, suggesting the existence of alternative mechanisms that trigger these non-conventional functions of the receptor. In this work, we analyzed the possibility of an interaction between P2X7 receptors from different cells to identify whether a P2X7-P2X7 complex is established, which could be related to the activation of the receptor's non-canonical functions. Using bioinformatic analyses, we performed docking and molecular dynamics to evaluate interaction energies and characterize the key residues involved in the formation of the P2X7-P2X7 complex. Our models revealed that the determinant amino acids for this possible interaction coincide with important residues in the formation of complexes between the described agonists for P2X7 and the receptor. These results suggest that the extracellular domain of the P2X7 receptor could interact with P2X7 intercellularly, at residues relevant for receptor activation.

Keywords: P2X7, Cross-Dressing, Intercellular interaction, P2X7-P2X7 complex, Bioinformatic analysis

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Long-term effect of gestational chronodisruption in Sprague-Dawley rats on the immune system of male progeny.

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In modern societies, exposure to artificial light at night disrupts the natural cycles of light and darkness resulting in negative effects on human health. These negative effects have been associated with an alteration in circadian rhythms and are denominated as chronodisruption. On the other hand, several evidence indicate that conditions during the intrauterine life plays a critical role on the homeostasis and development of immune system, therefore maternal environment is an important factor that can influence in the susceptibility to immune-mediated disorders of the offspring at adult stage. The present study aims to determine in animal model of gestational chronodisruption the effects on the immune system of the offspring. Pregnant females were exposed to constant lighting throughout the second half of their gestation and the immune compartment circadian organization and humoral response was compared with that observed in progeny gestated in control photoperiod. Our results showed that at 90 days of postnatal development the level of transcription of the clock genes in the spleen is altered with respect to that of the gestated in control photoperiod. Also, in this progeny we determined a loss of the circadian oscillation in the abundance of B cells in the spleen. Correlated with these results, we determined a greater response to the challenge of the immune system with protein antigen in the progeny gestated under chronodisruption. Our results reveal a long-term effect of gestational chronodisruption on the immune system of male progeny, even though the postnatal development of this progeny was in normal photoperiod.

Keywords: circadian system, light pollution, chrono-immunology, chronodisruption, allergy

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POSTERS / CELLULAR AND MOLECULAR IMMUNOLOGY

Role of transcription factors associated with the response to misfolded proteins ATF6 and XBP1 in the immunometabolism of dendritic cells.

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P. Lisboa and J. López are co-first authors.

Dendritic cells (DCs) are leukocytes of the innate immune system that, upon recognition of inflammatory stimuli, initiate an activation program supported by substantial changes in their cellular metabolism and organelle capacity. As highly secretory cells, activated DCs exhibit high protein synthesis demand, which can lead to endoplasmic reticulum stress and activation of the UPR (Unfolded Protein Response). Interestingly, the UPR has emerged as a crucial regulator of the immunogenic functions of DCs. However, the metabolic control of these cells by the UPR and the contribution of particular UPR transducers remain poorly understood.

In this project, we explored the contribution of two main UPR-associated transcription factors; XBP1 and ATF6 in DC immunometabolism. Using cultured DCs (GM-CSF DC cultures) from transgenic mice selectively lacking XBP1 and/or ATF6 in DCs, we assessed DC metabolic capacity following activation with microbial agonists. The double knockout model was validated through qPCR and flow cytometry. Utilizing the novel 'SCENITH' method for single-cell metabolic profiling, we determined that the loss of XBP1 causes an increased glycolytic capacity in DCs, reducing their mitochondrial dependence in both steady and activated states. Pharmacological induction of XBP1s has the opposite metabolic effect, suggesting that XBP1 transcriptional activity promotes oxidative phosphorylation for cellular energy production. Additionally, the joint absence of ATF6 and XBP1 results in increased protein synthesis under immune stimulation.

Although conclusive results are pending, data show that these UPR axes modulate DC immunometabolism. The next steps of the research will explore how these metabolic changes impact DC immune functions, such as cytokine production.

Keywords: DCs, Immunometabolism, Unfolded Protein Response, ATF6, XBP1.

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POSTERS / AUTOIMMUNITY AND INFLAMMAGING

Characterization of Tissue-Resident Memory B cells in the kidney of a mouse model of Systemic Lupus Erythematosus

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Systemic autoimmune diseases, such as Systemic lupus erythematosus (SLE), are characterized by the activation of self- reactive B cells, leading to the generation of self-reactive memory B cells (Bmem) and plasma cells. SLE patients exhibit increased circulating memory B cells, often correlating with disease activity. While Bmem can acquire tissue residency during infections, whether tissue-resident Bmem can also arise in autoimmune disease and contribute to tissue damage remains unclear.

In this work, we used the BWF1 mouse model, where 7-month-old female mice spontaneously develop lupus-like disease with severe glomerulonephritis and autoantibody accumulation. We distinguished circulating vs non-circulating lymphoid cells by combining intravascular labeling and immune phenotyping. BWF1 mice with glomerulonephritis exhibit a significant accumulation of non-circulating B cells in the kidneys. A substantial fraction of these non-circulating kidney B cells express the memory markers CD73 and PD-L2, and the residency markers CXCR3 and CD69. Furthermore, lupus-prone mice harbor many self-reactive plasma cells in their kidneys. Remarkably, these tissue-resident B cells are resistant to B cell-depletion therapy with Rituximab, with memory phenotype cells remaining in the kidney, unlike their circulating counterpart, highlighting a relevant limitation to the current use of Rituximab in SLE patients.

In conclusion, our findings demonstrate that in the kidney of mice with spontaneous lupus, there is an accumulation of tissue- resident memory B cells that are resistant to elimination. This population, previously observed only in the lungs of infected mice, may play a crucial role in the pathogenesis of this disease, shedding new light on the mechanisms of autoimmune diseases.

Keywords: Autoimmunity, Lupus, Resident-memory B cells

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POSTERS / AUTOIMMUNITY AND INFLAMMAGING

Incorporation of circulating cell-free circulating mitochondria from older donor cells into CD4+ T cells from young donors modulates the senescent phenotype

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During aging, one of the features that has been described as a key factor for its progression is mitochondrial dysfunction in different cell types, such as immune cells. In immune cells, mitochondrial dysfunction is one of the features that can lead to the senescent phenotype causing immuno-senescence, which is related to chronic low-grade inflammation, known as inflammaging. The immuno-senescence state is characterized by dysfunction of the immune response and is related to susceptibility to infections, low efficacy of vaccination, occurrence of age-related diseases and others. On the other hand, a couple of years ago, was demonstrated the presence of circulating cell-free (ccf)-mitochondria in plasma in healthy donors, and we know from in vitro studies that they can be incorporated into T cells. However, they have not been characterized in elderly donors and whether they contribute to immuno-senescence has not been investigated. Consequently, we propose that ccf- mitochondria (ccf-MT) from old donors (>60 years) can induce the senescent phenotype in CD4+ T cells from healthy adult donors (<35 years) in vitro. For this purpose, after incorporation of ccf-MT from old donors into CD4+ T cells from adult donors. We evaluated the levels of co-stimulatory surface markers and intracellular proteins related to the senescent phenotype in T cells, in addition to assessing mitochondrial parameters since mitochondrial dysfunction is one of the hallmarks of lymphocyte senescence.

Keywords: Mitochondria, Immunosenescence, Aging

Funding: Fondecyt Iniciación 11221017

POSTERS / AUTOIMMUNITY AND INFLAMMAGING

Circulating cell-free mitochondria treatment in the memory CD4+ T cells reduces proliferation and differentiation.

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Introduction: Mitochondria are organelles that regulate cellular processes such as apoptosis, calcium homeostasis, energy production, and control reactive species of oxygen. It has been reported that mitochondria can be transferred between cells, improving cell survival. Recently, it has been discovered the existence of circulating cell-free mitochondria (ccf-Mito) in human plasma of healthy donors; oxygen consumption analysis and electron microscopy images revealed that ccf-Mito are morphologically intact and respiratory competent; however, its effect on immune cells has not been studied. On the other hand, memory CD4+ T cells play an important role in the immune system; they express helper molecules, release effector cytokines and develop a quicker and enhanced response than effector cells, that's why our group has focused on studying the effect of ccf-Mito on memory CD4+ T cells.

Methodology: To evaluate this, the memory CD4+ T cells were sorted from peripheral blood mononuclear cells of healthy donors and cultured for 4 days in activating conditions in the presence or absence of ccf-Mito. On day 4, the cells were stained with different antibodies and dyes to evaluate their proliferation, activation, exhaustion and Treg differentiation by flow cytometry.

Results: We observed that the treatment with ccf-Mito in activating conditions inhibits proliferation, activation, and exhausted phenotype, while the effect of the ccf-Mito in the differentiation to Treg remains uncertain.

Conclusion: The ccf-Mito significantly impacts memory CD4+ T cells, reducing proliferation and differentiation. This can be relevant in the autoimmunity context where it could regulate the memory CD4+ T cells functions.

Keywords: mitochondria, memory T cells

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POSTERS / AUTOIMMUNITY AND INFLAMMAGING

Automated analysis of lymphatic vasculature from immunofluorescence images of different tissues

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Lymphatic vasculature plays a critical role in fluid balance, immunity and metabolic regulation of different tissues. Accordingly, defects in lymphatic vasculature leads to inflammation and are associated with different pathologies. Thus, the interest in measuring lymphatic vasculature changes in different diseases by image-based analysis of specific lymphatic markers, has been growing exponentially. However, most of these analyzes are made manually, which can be inaccurate, due to human subjectivity and are time-consuming. The purpose of this study was to develop an automated analysis of lymphatic vasculature, quantifying parameters like area coverage, length and branching from immunofluorescence images of colon and meninges.
Through the use of this protocol, zones of interest (ROIs) can be identified faster and more precisely than manually selecting ROIs, making it possible to analyze changes in lymphatic vasculature coverage during inflammation more efficiently. Intestinal sections and whole-mount meninges from mice under different genetic background or different treatment were stained using the lymphatic vasculature marker 1 (Lyve-1) and lymphatic coverage was analyzed manually by using the manual ROI selection protocol and by our automatized protocol using the 'Threshold' tool included in the Fiji program. By using this automatized protocol, we get similar results than the manually selected protocol, but our automatic protocol selects ROIs with much more specific selections than those made manually and significatively faster, helping us to refine the quantification of lymphatic vasculature, avoiding human bias analysis, reducing the complexity of the analysis and saving time.

Keywords: Morphometric analysis, meningeal Lymphatic Vessel, Quantification, Lymphatic Vessel

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POSTERS / TUMOR IMMUNOLOGY

Tumoral mitochondrial transfer like a potential mechanism to induce exhausted phenotype and a dysfunctional CD4+T-cell in Oral Cancer.

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The tumor microenvironment in oral squamous cell carcinoma (OSCC) promotes an exhausted phenotype in CD4⁺T-cells, which has been associated with changes in cell metabolism. The main organelle of cell metabolism is the mitochondria and in recent years it has been indicated that several cells have the capacity to transfer mitochondria. However, it has not been evaluated whether mitochondria transfer from cancer cells to CD4⁺T-cells induces an exhausted phenotype. The aim of this study was evaluated the functional and metabolic effect of artificial mitochondria transfer from the oral cancer cells HSC-3 into activated CD4+ T cells. Mitochondria from HSC-3 cells were labelled with MitoTracker dye, isolated and transfer into activated CD4⁺ T cells mediated by artificial mitochondrial transfer protocol. Then, surface molecule expression, proliferation, cytokine secretion, mitochondrial oxidative stress and glucose metabolism after mitochondria transfer were analyzed by flow cytometry. In addition, the metabolome and proteome in CD4+ T cells were analyzed after mitochondrial transfer by mass spectrometry and the enrichment proteomic pathways were visualized by platform FragPipe.

Our results showed that mitocepted CD4⁺ T cells significantly decrease proliferation and increased expression of two inhibitory proteins (TIGIT; CTLA4), and proteins associated with exhausted phenotype (PD-1; PDL-1; LAG3). Regarding cytokine secretion, a significant decrease was observed in the mitocepted group for IFN-gamma, TNF-alpha, IL-10, and IL-4 production in comparison with the control group. Metabolomic assay show a reduction in the pyruvate dehydrogenase cofactor Vitamin B1and the enrichment proteomic pathways revealed that T cells that acquired malignant mitochondrial showed a hypoxic state with more reactive oxidative species (ROS) production and more glycolysis. Finally, we confirmed that mitocepted CD4+ T cells increased glucose uptake, glucose consumption and lactate and ROS production. In summary, the acquisition of isolated mitochondria from HSC-3 cancer cells by CD4⁺ T lymphocyte induces recipient mitochondrial oxidative stress and possible reduction of the Krebs cycle mediated by insufficient Vitamin B1, forcing the cell to use glycolysis as a salvage pathway. This effect impairs the antitumor response by promoting exhausted and dysfunctional CD4⁺ T cells.

Keywords: Oral cancer, Mitochondrial transfer, MitoCeption, Exhausted phenotype

POSTERS / TUMOR IMMUNOLOGY

Adenosine together with IL-2 and IL-12 promote terminal exhaustion of CD8 T cells in vitro.

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Exhaustion is a state of reduced functionality that CD8+ T lymphocytes experience when undergoing repeated stimulation of their T cell receptor and is a relevant phenomenon in cancer. Exhaustion develops progressively, ranging from stem-like cells called precursor exhausted cells (Tpex) to terminally exhausted cells (Tex). These populations differ in their effector capacity, proliferation, and therapeutic potential. It has been proposed that, in addition to chronic TCR stimulation, cytokines such as interleukin-2 (IL-2) and IL-12 may promote terminal exhaustion. Likewise, adenosine, a nucleoside found in high concentrations within the tumor niche, is a molecule that restricts the cytotoxic activity of effector CD8+ T cells mainly through its A2A receptor (A2AR). Despite this, few studies address its involvement in the exhaustion phenomenon. Thus, using a standardized in vitro exhaustion protocol, we characterize exhausted CD8+ T cells generated in the presence of IL-2/12 cytokines. Using a pharmacological approach, in these cultures we analyzed the role of adenosine in the establishment of the exhausted populations. These cytokines led to the appearance of mainly Tex cells, with a limited capacity to produce Tpex. Upon addition of the A2AR agonist, CGS21680, as well as the non-hydrolyzable adenosine analog, NECA, the cells maintained their Tex phenotype, while adding the A2AR antagonist, SCH58261, Tex populations decreased. These results suggest that IL-2/12 cytokines lead to the differentiation of terminally exhausted populations and adenosine promotes this state through the A2AR, so using blocking drugs of this receptor may be useful to achieve longer-lasting immune response against the tumor.

Keywords: Exhaustion., A2AR, Adenosine, Cytokines, Immunotherapies

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POSTERS / TUMOR IMMUNOLOGY

Challenges in recapitulating Human Exhausted T Cell phenotypes in vitro using PBMC-derived models.

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Exhaustion of cytotoxic T cells (CD8+) is a key mechanism of immune evasion and tumor progression. In the tumor microenvironment, cancer cells directly and indirectly interact with immune cells, promoting an exhausted phenotype characterized by the expression of inhibitory molecules such as PD-1, TIM-3, and LAG-3, among others. This state prevents T cells from effectively eliminating tumor cells, contributing to cancer progression.

This study compared two in vitro protocols to induce exhaustion on human CD8+ T cells. 1) cells were activated with a commercial activator containing soluble anti-CD3/CD28 antibodies. 2) an activation protocol using plate-bound anti-CD3 antibodies was performed

After 14 days of culture, the expression of inhibitory markers was assessed using spectral flow cytometry. The results showed that plate-bound antibodies induced a higher expression of inhibitory molecules compared to soluble antibodies. However, neither protocol fully induced the exhausted phenotype typically seen in the tumor microenvironment.

These findings suggest that activation with plate-bound antibodies may be more effective than soluble antibodies in inducing an exhausted phenotype. Nevertheless, new activation strategies are needed to develop more robust in vitro models that allow for the study of immune exhaustion mechanisms in cancer.

Keywords: Tumor Immune Evasion, PD1, In Vitro Activation protocols, Cytotoxic T Lymphocytes

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POSTERS / TUMOR IMMUNOLOGY

Peripheral and tumor-infiltrating NKG2D-positive invariant NKT cells do not improve clinical outcomes for patients with gastric cancer after therapeutic stomach resection

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Gastric cancer (GC) is the fourth most common cause of cancer-related death worldwide and the leading cause of cancer- related mortality in Chile. Human invariant Natural Killer T (iNKT) cells are innate-like T lymphocytes that recognize CD1d- presented glycolipid antigens through their invariant TCR α chain, which contains the Vα24Jα18 rearrangement, and Vβ11 β chain. Activation of iNKT cells can also be triggered by NKG2D, a cytotoxicity activation receptor that controls tumor development upon binding to MICA and other ligands on target cells. Although iNKT cells participate in the immune response against tumors, their definite role in GC remains uncovered. Here, we investigated whether the frequencies of circulating and tumor-infiltrating iNKT cells, as well as their levels of membrane NKG2D, all detected by flow cytometry at gastrectomy, correlate with disease outcome. Contrary to the protective role of tumor-infiltrating CD3-positive T cells, a large iNKT cell fraction in the blood or tumor did not improve patient survival rate after 100 months of surgery. In addition, high expression of NKG2D receptor on tumor-infiltrating iNKT cells, but not on CD3-positive T lymphocytes, was associated with the presence of poorly differentiated tumor cells and increased tumor size. Our results suggest that NKG2D receptor expression on iNKT cells may paradoxically facilitate tumor progression in GC.

Keywords: Gastric Cancer, iNKT cells, NKG2D, immunosurveillance

POSTERS / TUMOR IMMUNOLOGY

Activation of PRR-Mediated Signaling Pathways by Novel iDAMPs in Human Immature Primary Dendritic Cells and THP-1 Cells.

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Dendritic cells (DCs) play a critical role in initiating immune responses, particularly in cancer immunotherapy. Immature dendritic cells (iDCs) can be activated by damage-associated molecular patterns (DAMPs) via pattern recognition receptors (PRRs), promoting their maturation and enhancing antigen presentation. Recently, we identified the inducible DAMPs (iDAMPs) haptoglobin, filamin-C, histone H2A, and TDP-43 as potential PRR ligands with therapeutic relevance for DC-based therapies.

We investigated the activation of PRR-associated signaling pathways in iDCs and THP-1 cells following stimulation with these putative iDAMPs. Western blot analysis was used to assess the expression and phosphorylation of key proteins, including p65, p38 MAPK, and Akt. Protein lysates were analyzed via SDS-PAGE and immunoblotting, with GAPDH as a control. Additionally, cell surface markers and intracellular signaling were examined by flow cytometry, providing a comprehensive evaluation of the phenotypic effects of iDAMPs.

Our results demonstrate that iDAMPs activate signaling pathways in both iDCs and THP-1 cells, as indicated by enhanced phosphorylation of p65, p38 MAPK, and Akt. The iDAMPs—haptoglobin, filamin-C, histone H2A, and TDP-43—significantly influenced iDC maturation, evidenced by changes in surface markers and signaling pathways.

This study highlights the potential of these iDAMPs as novel PRR ligands capable of modulating key signaling pathways involved in iDC maturation. These findings suggest that iDAMPs may enhance the efficacy of DC-based immunotherapies by promoting iDC maturation and antigen presentation, offering promising implications for cancer treatment.

Keywords: Dendritic Cells (DCs), Pattern Recognition Receptors (PRRs), Inducible DAMPs (iDAMPs), Signaling Pathways, Cancer Immunotherapy

Funding: Proyecto Fondecyt Regular 1231853

POSTERS / TUMOR IMMUNOLOGY

Co-localization of Putative Inducible Damage-Associated Molecular Patterns (PiDAMPs) with Different PRRs in ex vivo Generated Human Dendritic Cells and THP-1 Cells.

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Malignant melanoma (MM) is a relatively immunogenic type of cancer, expressing melanoma associated antigens (MAA) that are primarily recognized by dendritic cells (DCs). DCs capture and present these antigens to T cells, generating strong and specific antitumor immune responses in both murine and human models. Additionally, damage-associated molecular patterns (DAMPs), molecules released by stressed or dying cells, serve as key danger signals that help trigger adaptive antitumoral immune responses by activating immune cells such as DCs. We have previously identified specific putative inducible protein DAMPs (PiDAMPs) from a heat-conditioned human melanoma cell lysate (HS-MCL). In this context, we aim to establish the physical interaction between PiDAMPs present in the lysates and different pattern recognition receptors (PRRs) on DCs and THP-1 cells. The target proteins considered for this study include: Haptoglobin (HP), Filamin-C (FLNC), histone cluster 2H2A family member a3 (HIST2H2AA3), histone cluster 2H2A family member c (HIST2H2AC), and TAR DNA-binding protein 43 (TDP-43).

Primary ex vivo generated human DCs from healthy donors' buffy coats and THP-1 cells were stimulated with different PiDAMPs. Using confocal immunofluorescence microscopy on cells previously fixed on coverslips, we characterized the potential interactions of the studied ligands with PRRs expressed by these cells. The identification of previously undescribed specific protein iDAMPs/PRRs interactions will help to better understand the role of iDAMPs in the maturation and function of DCs during antitumor immunity and contribute to the development of improved DC-based therapies.

Keywords: DCs, Immunofluorescence, PRRs, iDAMPs, Malignant Melanoma

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POSTERS / TUMOR IMMUNOLOGY

A2AR promotes differentiation of exhausted progenitor T cells into exhausted T cells in vitro.

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Chronic stimulation, such as cancer, can lead to T cell exhaustion, reducing their function. Exhausted T cells (Tex) arise from precursors (Tpex), which are more self-renewing and responsive to therapies. Tpex differ from Tex in marker expression (PD-1, TIM-3).
Adenosine, a metabolite produced from the breakdown of adenine nucleotides, plays a critical role in suppressing the antitumor immune response. Pharmacological blockade of A2AR has been demonstrated to enhance the antitumor immune response, making it a promising therapeutic strategy for cancer.

In our study, we employed standardized protocols to induce the differentiation of virgin OT-I CD8+ T lymphocytes into either Tpex or Tex cells. Chronic stimulation with the OVA peptide in the presence of IL-7 and IL-15 favored the generation of Tpex, while the presence of IL-2 and IL-12 primarily induced differentiation into Tex.

Our results indicated Tpex generated *in vitro* retained their ability to differentiate into Tex cells in vivo. Furthermore, with bioinformatic studio we observed that Tpex expressed higher levels of the A2Ar receptor compared to Tex. Pharmacological blockade of the A2Ar receptor in Tpex cultured in the presence of IL-2 and IL-12 reduced the generation of exhausted cells, suggesting that signaling through A2Ar promotes T cell exhaustion. Intriguingly, Tpex with a genetic deletion of the A2Ar receptor (KO A2Ar) generated fewer Tex, indicating a potential compensatory mechanism.

Our findings demonstrate that Tpex cells are precursors of exhausted T cells and that signaling through the A2Ar receptor plays a fundamental role in the development of T cell exhaustion.

Keywords: exhaustion, adenosine, precursor exhausted, A2AR

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POSTERS / TUMOR IMMUNOLOGY

Characterizing TRIMEL-Induced-Neutrophils as Antigen-Presenting Cells in the Antitumor Immune Response

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Neutrophils, essential phagocytic cells of the innate immune system, play a fundamental role in the immune response against pathogens and regulating inflammation. Traditionally, they have been considered short-lived effector cells whose primary function is the phagocytosis and destruction of microorganisms. However, recent research has revealed the functional diversity of neutrophils, showing that they not only participate in eliminating pathogens but also play crucial roles in modulating adaptive immune responses and presenting antigens.

The potential of neutrophils to function as antigen-presenting cells (APCs) has sparked significant interest in immunological research. Recent studies have shown that neutrophils can adopt an APC-like phenotype, expressing costimulatory molecules and presenting antigens to T lymphocytes. Furthermore, a lysate-based vaccine called TRIMELVax has been found to induce acute local inflammation in neutrophils, which is associated with inhibiting tumor growth.

TAPCells is an autologous vaccine comprising activated monocytes loaded with a heat-shock-conditioned melanoma lysate (TRIMEL®) combined with mollusk hemocyanin as an adjuvant. TAPCells are capable of inducing tumor-specific immune responses in melanoma and prostate cancer patients, associated with prolonged survival.

Here, we explore the influence of TRIMEL on the maturation and function of neutrophils, with a specific focus on their potential to transform into APCs. We will measure their ability to activate T lymphocytes and induce cytotoxicity on tumor cells. Through a series of carefully designed experiments to assess the response of neutrophils to TRIMEL stimulation, our aim is to gain a more comprehensive understanding of the interaction between neutrophils and DC in the context of the antitumor immune response.

Intracellular cholesterol accumulation is related to inflammasome activation in IBD patients

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation and an aberrant interaction between the immune response and the intestinal microbiota. Recent studies suggest that intracellular cholesterol is related to the immune response in IBD, a possible activator of the NLRP3 inflammasome, a complex inducing the secretion of IL-1β and IL-18. It is still unclear whether cholesterol acts as a DAMP to activate this inflammasome in IBD. Additionally, gene expression related to cholesterol efflux, as *ABCG1*, is decreased, and cholesterol influx-related genes, *LDLR*, and intracellular cholesterol transporters, such as *STARD3* and *NPC1*, are increased in RNA microarray analysis of inflamed mucosa compared to non-inflamed UC patient´s mucosa, suggesting cholesterol accumulation in IBD tissues. The main objective of this study was to evaluate the relationship between cholesterol and NLRP3 inflammasome activation in IBD patients. Colonic biopsies were collected from healthy individuals, UC and CD patients and fixed in 4% paraformaldehyde and embedded in paraffin to determine ABCA1 and NLRP3 expression by immunofluorescence. *Ex-vivo* cultures of colonic biopsies from UC, CD patients, and healthy individuals were treated with beta-methyl-cyclodextrin (βMCD) to explore the effects on cholesterol, IL-1β, and IL-18 levels. Decreased ABCA1 and increased expression of NLRP3 in UC biopsies was shown. Acute cholesterol depletion with βMCD reduced IL-18 levels in UC biopsies. These results suggest that intracellular cholesterol accumulation may activate the inflammasome, aggravating inflammation in UC. We believe that targeting cholesterol modulation could offer a potential therapeutic strategy for UC patients.

Keywords: inflammatory bowel disease, cholesterol, inflammasome, interleukin 1β, interleukin 18.

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POSTERS / MUCOSAL IMMUNOLOGY

The microbiota metabolites of cholesterol: 4- colesten-3-ona and coprostanol reduce the inflammatory response in macrophages THP1- MØ

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Inflammatory bowel diseases (IBD) show increased infiltrating and resident macrophages (MØ) in the gut mucosa, secreting inflammatory cytokines mostly via Toll like receptors (TLRs) and the NFκB signaling pathway. In MØ, cholesterol potentiates the inflammatory response increasing TLR4 recruitment to the "lipid rafts" (LR) microdomains. The dietary cholesterol is partially metabolized by intestinal microbiota, its metabolites 4-cholesten-3-one and coprostanol reduce activation of NFκB or phagocytosis in MØ. We propose the cholesterol metabolites 4-cholesten-3-one and coprostanol reduce the inflammatory response of THP1-MØ.

THP1 cells were polarized to MØ with PMA and later incubated with 100 ng/ml LPS and 12.5 mM 4-cholesten-3-one or coprostanol 24 hrs. Cytokines were measured in culture medium using "cytometric bead array" (Becton-Dickinson) and quantified using a Flow Cytometer (Becton-Dickinson). Proteins were determined using Bicinchoninic assay, whist cholesterol content was measured using Amplex Red assay kit (Thermo Fisher).

Coprostanol reduced TNF and IL-6 secretion whilst 4-cholesten-3-one only reduced IL-6. The levels of IL-1β and IL-8 were not affected. Moreover, coprostanol increased two-fold the cellular cholesterol content in MØ under inflammatory stimulus, whilst the 4-cholesten-3-one has no effect.

In our results coprostanol increased MØ cholesterol content and reduced cytokine secretion, possibly via a mechanism involving LR homeostasis and intracellular cholesterol redistribution. These findings can contribute to understanding the role of microbiota cholesterol metabolism in the inflammatory response of IBD.

Keywords: cholesterol, cholesterol metabolites, Lipid Rafts

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POSTERS / MUCOSAL IMMUNOLOGY

Role of the Gut Microbiota in the Modulation of Meningeal and Colonic Lymphatic Vasculature in Homeostasis

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Lymphatic vasculature (LV) is a complex network of vessels capable of transporting lymph and immune cells back into the blood. LV is found in different tissues such as in the meninges surrounding the brain and spinal cord parenchyma, known as meningeal lymphatic vasculature (mLV) or in the colon, playing key roles in immune cell trafficking in homeostasis, since LV alterations in the meninges lead to neuroinflammation and in the colon participates in inflammatory bowel disease. Interestingly, although the maintaining of healthy LV is crucial to maintaining tissue stability, the mechanisms modulating LV morphology and functions remain largely unknown. This study aims to assess the role of the gut microbiota in regulating mLV and colon LV function during homeostasis. Using C57BL/6 (WT) mice treated with an antibiotic cocktail (Abx), we observed that microbiota depletion leads to a decrease in LV coverage, as well as functional alterations in both colon and meninges through immunofluorescence analysis. Furthermore, microbiota depleted animals showed a drastic reduction of macrophages in the mesentery, together with a reduction of macrophages and T cells in the meninges and increased levels of B cells in the meninges. In fact, reduced exploratory abilities were observed in Abx-treated mice compared to control animals. These findings suggest that the gut microbiota may serve as a key regulator of mLV and colon LV function in homeostasis, influencing lymphatic structure and modulating immune cell infiltration in the CNS and intestine, impacting behavioral patterns.

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POSTERS / MUCOSAL IMMUNOLOGY

Evaluation of the activation of the IRE1/XBP1 axis in ILC3 cells and its role in intestinal immunity.

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Intestinal immunity is crucial for maintaining homeostasis and defending against pathogens. Group 3 innate lymphoid cells (ILC3) in the intestine play a vital role in immune regulation and epithelial barrier protection through cytokines IL-22 and IL-17. However, the molecular mechanisms controlling ILC3 activation, particularly the role of the IRE1/XBP1 axis in the unfolded protein response (UPR), remain unclear. Additionally, the interaction between this axis and ILC3 function in Th17 cell differentiation is still unknown.

This study aims to characterize the ILC3 function in the IRE1/XBP1 axis and its role in mouse intestinal immunity. We used ERAI reporter mice to visualize IRE1 RNase activity via XBP1s fluorescence. Preliminary data indicate that T cells, B cells, and dendritic cells were successfully identified in the intestinal lamina propria through cytometry, providing a solid basis for further ILC3 analysis.

Flow cytometry will be used to identify ILC3, employing markers such as CD127, CD90.2, and KLRG1, while cytokine production (IL-22 and IL-17) will be measured using ELISA and Cytometric Bead Array. This study expects to elucidate ILC3 populations and activation of the IRE1/XBP1 axis, providing insights into ILC3 regulation and their influence on Th17 differentiation. These findings could contribute to new therapeutic approaches for inflammatory bowel diseases.

Keywords: Intestinal immunity, group 3 innate lymphoid cells (ILC3), IRE1/XBP1 axis.

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POSTERS / NEUROIMMUNOLOGY

Short-chain fatty acids signalling in T-cells promotes the development of Parkinson's disease in a mouse model.

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Parkinson's disease (PD) involves the development of a T-cell response to α-synuclein (αSyn) in human and animal models, which promotes neuroinflammation and neurodegeneration. Moreover, the composition of the gut microbiota has been strongly associated to PD. The evidence indicates that short-chain fatty acids (SCFA) produced by some bacteria of the gut microbiota are the responsible for triggering the development of parkinsonism in genetically susceptible individuals. Here, we studied whether the detrimental effects exerted by bacterial SCFA are mediated by T-cells.

We used a transgenic mouse model that overexpress the human aSyn (SNCA mice), which develop parkinsonism spontaneously with age in a manner dependent on the microbiota.

Our results show that T-cells express SCFA receptors, especially in those located in the colonic mucosa. Moreover, SNCA mice developed an inflammatory response mediated by $CD4^+$ and CD8+ T-cells specific to aSyn-derived antigens in lymphoid tissues draining the colonic mucosa and the central nervous system. Importantly, the genetic deficiency of the SCFA receptor specifically in T-cells abrogated the development of motor impairment. Furthermore, the systemic pharmacological antagonism of the SCFA receptor dampened the motor decline manifestation and the inflammatory T-cell response to aSyn-derived antigens in SNCA mice.

Our results suggest that SCFA-signalling promotes the triggering of an inflammatory T-cell response in the colonic mucosa at early age, which would favour neuroinflammation and the subsequent development of motor decline in later stages in a pre- clinical model of PD.

Keywords: Parkinson's disease, Microbiota, T-cells, Short-chain fatty acids

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POSTERS / NEUROIMMUNOLOGY

Enhanced therapeutic delivery of Astaxanthin by polyarginine nanocapsules for neuroinflammatory pathologies

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Astaxanthin (AST) is a natural carotenoid with a high antioxidant, anti-inflammatory, and neuroprotective activity. However, its therapeutic application is hampered by low aqueous solubility and stability. We investigated the therapeutic role of AST in experimental autoimmune encephalomyelitis (EAE), a preclinical model of multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS). Our results show that two different doses (4 µg and 2.5 mg) of AST administered daily via intraperitoneal induced a significant amelioration of EAE. However, intranasal administration of the lower dose had significantly better effect than both intraperitoneal doses. To enhance AST stability, bioavailability, and its potential application in neuroinflammation, we developed polyarginine nanocapsules loaded with AST (AST-PARG-NCs) for intranasal delivery.
Polyarginine, a cationic polymer promoting cell interaction and transport, was tested at different concentrations (0.06-0.25 mg/mL). Cytotoxicity was assessed by exposing rat astrocytes to AST-PARG-NCs for 48 hours. Cell viability was determined by flow cytometry. Anti-inflammatory activity was analyzed in IFN-gamma-activated astrocytes determining GFAP expression, an astroglial activation marker, by flow cytometry. We obtained stable PARG-NCs in the range of 157-204 nm, PDI values (0.1- 0.2) and a positive zeta potential of 33.8-57.5 mV. AST-PARG-NCs were not cytotoxic and significantly inhibited astrocyte reactivity. Intranasal AST-PARG-NCs administration at disease onset delayed EAE progression and reduced disease severity significantly better than intranasal and intraperitoneal free AST. Intranasal AST-PARG-NCs administered at peak of disease significantly suppressed clinical symptoms and reduced CNS-immune cell infiltration and demyelination. Therefore, encapsulating AST enhances its therapeutic activity and suggests its potential application for MS

Keywords: Nanoparticle, multiple sclerosis, anti-inflammatory, intranasal administration

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Nanoencapsulated Resolvin E1 inhibits astrocyte reactivity

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During neuroinflammation, astrocytes, the most abundant glial cells in the central nervous system (CNS), respond to damage through a process called “astrogliosis”. This state is characterized by elevated glial fibrillary acidic protein (GFAP) expression and can exacerbate the neuroinflammatory process by releasing proinflammatory cytokines. The resolution of neuroinflammation is an active process regulated by specialized pro-resolving lipid molecules (SPMs). Resolvin E1 (RvE1) is a rapidly degrading SPM derived from Omega-3 that exhibits potential as therapeutic agent for inflammatory and neurodegenerative diseases. However, whether RvE1 exerts protective effects on the CNS by inhibiting astrocyte reactivity and whether its nanoencapsulation prevents its degradation and increases its delivery efficacy in a neuroinflammatory context is unknown. In the present study, we analyzed the pro-resolving activity of free and nanoencapsulated RvE1 on astrocyte reactivity *in vitro*. Polyarginine-coated lipid nanocapsules loaded with RvE1 (RvE1-NCs) were synthesized by a modified solvent displacement method. GFAP expression and astrogliosis-associated morphology were analyzed using flow cytometry and confocal microscopy, respectively. The activation status of the proinflammatory NF-κB transcriptional pathway was determined by fluorescence microscopy. RvE1-NCs, with a size of 158±5 nm, polydispersity index of 0,101±0,002, and zeta potential of 49,4±1,8 mV, were not cytotoxic. Interestingly, RvE1-NCs significantly reduced GFAP expression in astrocytes stimulated with IFN-gand LPS and delayed astrogliosis-associated cellular hypertrophy, compared to free RvE1. Preliminary results show that RvE1-NCs inhibit NF-κB pathway activation. Therefore, RvE1 encapsulation could offer CNS protection by reducing astrocyte reactivity, proposing it as a potential new treatment for neurodegenerative diseases.

Keywords: Neuroinflammation, Resolvin E1, Astrogliosis, Lipid Nanocapsules

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THE ROLE SUBSTANCE P-INDUCED MAST CELL (MC) ACTIVATION IN LIVER FIBROSIS IN THE METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD).

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Introduction: MASLD progression is associated to psychosocial stress (PS). Despite hepatic stellate cells(HSCs) activation by MC mediators has a role in liver fibrosis, MC activation by stress related neuropeptide Substance P (SP) remains unexplored.
Objective: To evaluate PS in MASLD patients and the role of SP-dependent MC activation in HSCs transdifferentiation (HSCT).
Methods: PS was assessed in 13 MASLD patients recruited from HCUCH by self-report questionnaires. Liver damage, by blood test and imaging. *In vitro*, Human MC line (HMC-1), were stimulated with SP and 48/80 compound(30min), and HSCs line(LX-2) with SP-MC activated supernatants by 24h. Alfa-smooth muscle actine (α-SMA) and GADPH expression was assessed by Immunoblot; and α-SMA immunostaining in HSCs directly stimulated with tryptase by 24h by IFI. Statistics: t-Test, X^2; and ANOVA-test. Significance $p<0.05$.
Results: Moderate-stress was observed in 53.8% of participants, with higher stress in moderate-severe steatosis vs mild-steatosis (p=0.047). Patients with significant-fibrosis reported poorer mental QoL vs without-fibrosis (p=0.022). Despite not statistically differences in α-SMA/GADPH fold-change among MC-stimulated conditions, increased α-SMA expression (p=0,002) and morphological changes associated to HSCT were observed after tryptase stimulation.
Conclusion: Our results suggest that PS have a pathogenic role in MASLD progression. Despite MC tryptase can induce HSCT, a long term of SP-MC stimulation needs to be explored to evaluate its impact in HSCT.

Keywords: Mast Cell, Psychological stress, MASLD, Hepatic stellate cells, Substance P

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POSTERS / NEUROIMMUNOLOGY

Contribution of Pneumoviridae viruses to the expression of neurotrophins and their receptors on lung epithelial cells

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Background: Among the family of *Pneumoviridae* viruses, we can find human metapneumovirus (hMPV) and human respiratory syncytial virus (hRSV). Both viruses are responsible for severe respiratory tract infections, such as bronchiolitis and pneumonia in children, immunocompromised individuals and the elderly. The main target of these viruses is lung epithelial cells, which are capable of secreting neurotrophins that can modulate the immune response against these pathogens. Studies in hRSV-infected patients have shown that nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) play an important role in the pathogenesis of the virus. However, the contribution of other neurotrophins or hMPV to neurotrophins secretion has not been evaluated.

Methods: Lung epithelial type 1 cells (LET-1) were infected with hMPV or hRSV at MOI 5 or MOI 10. The expression of neurotrophins NGF, BDNF, NT3, NT4 and NT5 and their receptors was evaluated by ELISA, RT- qPCR and WB analysis at 24, 48 and 72h post infection (h.p.i).

Results: hMPV infection promoted an increase mostly of NGF and BDNF at 24 h.p.i. Interestingly, NT3, 4 and 5 showed higher levels at 48 and 72 h.p.i. However, hRSV shows different expression kinetics than hMPV.

Conclusion: hMPV and hRSV play a role in the modulation of neurotrophins expression in LET-1 cells, possibly contributing to the imbalanced immune response.

Keywords: human metapneumovirus, human respiratory syncytial virus, BDNF, NGF, neurotrophins

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POSTERS / NEUROIMMUNOLOGY

Therapeutic and Immunomodulatory Activity of Nanoencapsulated Resolvin E1 in Experimental Autoimmune Encephalomyelitis

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Multiple Sclerosis (MS) is a neurodegenerative disease characterized by chronic inflammation of the central nervous system (CNS). The resolution of neuroinflammation is an active process involving specialized pro-resolutive lipids molecules (SPMs). Resolvin E1 (RvE1) is an Omega-3-derived SPM proposed as a potential therapeutic agent for treating neuroinflammatory diseases. Nevertheless, poor water solubility and short half-life of RvE1 hamper its biomedical application. The use of particle- based drug carriers can overcome these limitations and facilitate inflammation resolution and disease treatment. Here, we evaluated the therapeutic potential and immunomodulatory activity of nanoencapsulated RvE1 (RvE1-NC) administered via intranasal in mice induced with experimental autoimmune encephalomyelitis (EAE), a preclinical model of MS. Polyarginine covered nanocapsules loaded with 25 or 50 ng RvE1 (RvE1-NC) were synthesized by a modified solvent displacement method. EAE mice were treated daily with RvE1-NC, free RvE1, blank NC, or saline (vehicle) over 10 days at the peak of the disease. Myeloid cell populations were analyzed in the brain, spinal cord, lymph node, and spleen by multiparametric flow cytometry.
Our results show that 25 or 50 ng RvE1-NC significantly suppressed disease progression compared with control groups. RvE1- NC-treated EAE mice exhibited a significantly reduced brain infiltration of neutrophils, dendritic cells, and M1-macrophages and a significant increase of resting microglia and M2-macrophages compared with vehicle-treated group. On the other hand, M1- Macrophages and dendritic cells were increased in the spleen. No differences were found in the spinal cord and lymph nodes. Therefore, RvE1-NC treatment exerts promising therapeutic activity in EAE and MS.

Keywords: Multiple Sclerosis, Resolvine E1, Neuroinflammation, Nanomedicine, Drug Delivery

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POSTERS / NEUROIMMUNOLOGY

Interferon-gamma-induced dendritic cells exhibit an anti-inflammatory profile, inhibit CD4+ T cell proliferation, and promote regulatory T cell induction in a neuroantigen-specific manner

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Our previous results have shown that low doses of interferon-gamma (IFN-g) induce murine dendritic cell differentiation (IFN-g- DC) with therapeutic activity in experimental autoimmune encephalomyelitis, a preclinical model of Multiple Sclerosis. Here, we investigated the capability of IFN-g-DC in exerting tolerogenic function in a neuroantigen-specific manner. Murine bone marrow- derived dendritic cell (BMDC) precursors were differentiated into dendritic cells (DC) with GM-CSF for 7 days in the absence (iDC) or presence of IFN-g (IFN-g-DC). Lipopolysaccharide (LPS) was added during the last 24 h to obtain mature DC (mDC) and to evaluate phenotypic and functional stability of IFN-g-DC (LPS-IFN-g-DC). Phenotypic profile was determined by flow cytometry. The concentration of cytokines in supernatants from BMDC culture was determined by multiplex assay. Proliferation of $CD4^+$ T cells and conversion of regulatory T cells (Tregs) in response to myelin oligodendrocyte glycoprotein (MOG) peptide stimulation were evaluated in cell co-cultures between BMDC and total $CD4^+$ T cells from 2D2 transgenic mice expressing a MOG-specific T cell receptor. We found that IFN-g-DC exhibited a tolerogenic phenotype characterized by significantly lower levels of CD80, CD86, and MHC-II, and significantly higher levels of PD-L1 and LAP (TGF-beta precursor protein) than mDC. LPS-IFN-g-DC showed a stable phenotype. Furthermore, IFN-y-DC secreted significantly lower levels of pro-inflammatory cytokines than mDC and LPS-IFN-y-DC, including TNF, IL-1β, IL-17A, IL-12p70, IL-23, and IL-6. IFN-g-DC and LPS-IFN-g-DC inhibited MOG-specific $CD4^+$ T cell proliferation and induced a significantly higher MOG-specific Tregs conversion compared to mDC. Therefore, IFN-g induces tolerogenic activity in murine DC in a neuroantigen-specific manner.

Keywords: Interferon-gamma, tolerogenic dendritic cells, multiple sclerosis

Funding: FONDECYT/ANID 1231672

POSTERS / IMMUNITY AND INFECTION

Interaction between the SARS-CoV-2 ORF8 protein and MHC-I and its implication in immune evasion.

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COVID-19, caused by SARS-CoV-2 infection, has resulted in the death of over 7 million people worldwide. The viral genome encodes several proteins, including accessory proteins involved in immune evasion, such as ORF8, which has been implicated in the reduction of antigen presentation mediated by MHC-I. While previous bioinformatic studies have suggested potential interaction sites between ORF8 and MHC-I, these findings have not yet been confirmed by experimental data. This study aims to identify the critical residues involved in this binding interaction to elucidate the mechanism of MHC-I downregulation resulting from the direct interaction with ORF8. Using bioinformatic tools such as molecular docking and molecular dynamics analyses, we identified that ORF8 may interact either with MHC-I at the peptide-binding region or at the β2m-binding region. We identified key residues involved in the ORF8-MHC-I interaction and analyzed alanine substitutions to assess their contribution to stability of the complex. To evaluate the impact on MHC-I, ORF8 was expressed in HEK 293T cells, and surface levels of MHC-I were measured via flow cytometry, revealing a reduction in expression consistent with previous studies. Expression of alanine mutants of interacting-residues in ORF8 is expected to suppress or dampen the downregulation effect on MHC-I. This study will contribute to advancing our understanding of SARS-CoV-2 immune evasion mechanisms, helping to better comprehend the pathogenesis of COVID-19 and potentially identify therapeutic targets to combat the disease.

Keywords: Immune evasion, SARS-CoV-2, ORF8, MHC-I

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POSTERS / IMMUNITY AND INFECTION

Antibiotic-induced dysbiosis affects innate immunity against human metapneumovirus infection in a murine model

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The intestinal microbiota plays a crucial role in preventing gastrointestinal infections and modulating systemic immunity.
Emerging evidence suggests that antibiotic-induced dysbiosis affects immune responses to respiratory viruses, increasing susceptibility to infection. Human metapneumovirus (HMPV) is a common respiratory virus in Chile and worldwide, primarily affecting young children and older adults. Despite similarities with other respiratory viruses, the mechanisms regulating immune responses to HMPV are not fully understood. We hypothesized that antibiotic-induced dysbiosis might impair innate immune cell function, delaying viral clearance and worsening lung pathology during HMPV infection. To address this, we treated mice with antibiotics (ampicillin, kanamycin, vancomycin, metronidazole) in drinking water for three weeks before HMPV challenge. We confirmed decreased bacterial load culturing feces in Luria Bertani agar plates compared to a control group, without antibiotic treatment. Mice were intranasally infected with 10^6 PFU of HMPV and euthanized at day 3 post-infection. We observed increased weight loss at day 2 post-infection in antibiotic-treated mice infected with HPMV but no differences in viral load assessed by qPCR or lung pathology using hematoxylin and eosin staining compared to control groups. Similarly, no significant differences were found in lung and bronchoalveolar lavage (BAL) innate cell frequency assessed by flow cytometry. However, we detected a reduction in antiviral interferon transcription in lung tissue and decreased levels of the anti- inflammatory cytokine IL-10 in BAL assessed by ELISA. These findings suggest that antibiotic-induced dysbiosis may affect innate immune function during HMPV infection, warranting further investigation into the role of gut microbiota and microbiota- derived signals.

Keywords: HMPV, Lung immunity, innate immunity, gut microbiota, viral infection

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POSTERS / IMMUNITY AND INFECTION

Human metapneumovirus infection alters the intrinsic apoptotic pathway in alveolar macrophages.

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Human metapneumovirus (hMPV) is an RNA virus belonging to the *Paramyxoviridae* family that interacts with host cells through its structural proteins. This interaction triggers immune responses, including apoptosis, which play a critical role in the progression of hMPV infection. Apoptosis facilitates the release of new viral particles and contributes to lung tissue damage, particularly in vulnerable populations such as young children, the elderly, and immunocompromised patients. Alveolar macrophages, resident immune cells in the lung lumen, express various proteins, including pattern recognition receptors (PRRs) and MHC (class I and II). These proteins enable antigen detection, processing, and presentation, resulting in an amplified immune response, positioning alveolar macrophages as a critical first line of defense in the respiratory tract. Given their pivotal role in defending against respiratory viruses, it would be expected that infected macrophages would undergo apoptosis due to cellular stress. The intrinsic apoptosis pathway, mediated by pro-apoptotic proteins of the Bcl-2 family, such as Bax, and cellular stress sensors like PUMA, typically leads to the release of cytochrome c and activation of caspase-9.
However, this study demonstrates an opposite effect in alveolar macrophages infected with hMPV *in vitro*. Using qPCR, we observed a downregulation in the relative expression of key pro-apoptotic factors of the intrinsic pathway, including Bax, PUMA, cytochrome c, and caspase-9, alongside an upregulation in the relative expression of anti-apoptotic proteins Bcl-2 and Mcl-1.
These findings suggest a potential mechanism by which hMPV evades host defenses by inhibiting apoptosis, providing new insights into viral pathogenesis.

Keywords: Human metapneumovirus, Alveolar macrophages, Intrinsic apoptotic pathway, Viral infection

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POSTERS / IMMUNITY AND INFECTION

Non-neutralizing IgG Response against Choclo Orthohantavirus glycoproteins in acutely ill HCPS patients in Panama.

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Background: Hantavirus cardiopulmonary syndrome (HCPS) is an emerging disease in the Americas. Choclo Orthohantavirus (CHOV) is the sole pathogenic hantavirus identified in Panama that causes HCPS, with a cumulative fatality rate of 12.4% and a seroprevalence ranging from 16% to 62%. The pathogenesis of HCPS remains poorly understood and is influenced by multiple factors, with evidence suggesting a predominant role of the immune system.
Methods: We characterize the response of non-neutralizing specific IgG antibodies against CHOV glycoproteins (CHOV-GP) to explore their potential contribution to HCPS pathogenesis. We analyzed the IgG antibody profile and its subclasses using ELISA and assessed the capacity of these antibodies to induce antibody-dependent cellular cytotoxicity (ADCC). Additionally, cytokine profiles were evaluated via multiplex immunoassay using sera from 15 acute HCPS patients and 10 healthy donors. **Results:** Our findings demonstrate that CHOV-infected individuals predominantly produce IgG1 antibodies, which are capable of mediating ADCC in vitro. Furthermore, elevated levels of serum cytokines, including IFNγ, TNFα, IL-6, and IL-10, were detected in acute patients.
Conclusion: This study supports existing clinical evidence regarding orthohantavirus infection and expands our understanding of the immunological response in patients with varying clinical presentations of HCPS in Panama.

Keywords: CHOV, HCPS, IgG, ADCC, Cytokines

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POSTERS / IMMUNITY AND INFECTION

Evaluating the direct interaction of ORF9b with CARD domains of RIG-I, MDA5, and MAVS and its impact on type I interferon response

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The SARS-CoV-2 virus, responsible for the global COVID-19 pandemic, employs several mechanisms to evade host immune responses, enhancing its pathogenicity. Among its accessory proteins, ORF9b has been identified as a key modulator of the host's type I interferon (IFN-I) response, a crucial antiviral pathway. This study explores the hypothesis that ORF9b interferes with IFN-I signaling by binding to the CARD domains of RIG-I, MDA5, and MAVS, thereby disrupting the RIG-I-like receptor pathway. We used sequence and structural alignment to identify similarities between ORF9b and CARD domains, followed by molecular docking and dynamics simulations to explore their interactions. Experimental validation was performed in HEK293 cells expressing wild-type ORF9b and mutant variants with alterations in key interacting residues. To assess IFN-I pathway activation, poly(I:C) stimulation will be used following the expression of these ORF9b variants. Our bioinformatic analysis suggests that ORF9b preferentially binds to CARD domains, and the introduction of specific ORF9b variants may restore IFN-I pathway activation in response to poly(I:C) stimulation. These findings suggest that interaction of ORF9b with CARD domains is a strategic viral mechanism to inhibit IFN-I signaling, facilitating immune evasion. Further exploration of these interactions will provide insights into SARS-CoV-2 pathogenesis and may lead to development of novel therapeutic strategies targeting ORF9b to restore effective IFN-I signaling in infected cells.

Keywords: SARS-CoV-2, ORF9b, immune evasion, Type I IFN pathway

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POSTERS / IMMUNITY AND INFECTION

Metaproteomics of human gingival crevicular fluid reveals shifts in bacterial functional diversity but stable taxonomic composition across the dynamics of periodontitis progression.

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Introduction: Periodontitis progresses through alternating phases of rapid tissue destruction and relative quiescent periods, driven by a dysbiotic microbiome and a disrupted immune response. However, metaproteomics in the dynamics of periodontitis progression has not been described.
Methods: Sixteen periodontitis-diagnosed patients were clinically monitored through weekly clinical evaluations for disease progression. Gingival crevicular fluid (GCF) samples were collected for further analysis. According to the clinical status of periodontitis, we studied three groups: P (Progressive pockets, n = 22), N (Non-progressive pockets, n = 22), and C (Pre- progressive pockets, n = 22), resulting in a total of 66 GCF samples individually assessed by HPLC-MS/MS. Bioinformatic enrichment analysis, functional distribution, and phylogenetics were performed.
Results: Bacterial proteins constituted 22.4% of the proteome diversity but only 0.2% of the total relative abundance. α- diversity and richness showed consistent taxonomic composition across all samples. However, functional shifts were observed, with the N group showing enrichment compatible with antioxidant defense mechanisms. In contrast, the P group exhibited an increased representation of processes such as ferric ion binding and arginine-deaminase activity. Notably, features and hub bacterial interactions differed between groups, with Actinomycetota dominating in N and Bacteroidota in P.
Conclusion: This study provides new insights into the functional adaptations of the oral microbiota linked to periodontitis progression, suggesting that ecological and functional shifts play a crucial role in disease progression. These findings underscore the importance of considering both taxonomic and functional aspects of the oral microbiota in understanding the pathogenesis of periodontitis and may guide the development of targeted therapeutic strategies.

Keywords: Metaproteomics, Gingival crevicular fluid, Periodontitis, disease progression, Proteomics

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POSTERS / IMMUNITY AND INFECTION

New Therapeutic Strategies: Synergy of Hypochlorous Acid and Essential Oils Against multidrug-resistant Bacteria

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Introduction: As rates of multidrug-resistant (MDR) pathogens continue increasing, particularly in strains such as *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Staphylococcus aureus* [1, 7-18], new antimicrobial development struggles to keep up. This resistance is a growing public health issue, and novel approaches to tackle MDR bacteria are urgently needed. One promising strategy is a combination therapy, where two or more drugs work together to treat infections. When the combined effect is greater than the sum of individual effects, it is considered synergistic. In vitro evaluation of drug synergy is a key step in exploring these treatment strategies [3]. Among potential treatments, essential oils have shown antimicrobial activity against MDR bacteria[5, 9, 13]. Thyme [10, 15]and oregano[17]essential oils, combined with hypochlorous acid (HCLO)[8], show potential. HCLO, widely used for infection control and wound healing, is synthesized by immune cells to fight pathogens[4,16]. Laboratory stabilization of HCLO has opened new therapeutic possibilities. Its antimicrobial characteristics position it as an excellent candidate for further research.

Methods: The checkerboard array method was used to assess synergy between HCLO and essential oils. This method is a variation of the minimum inhibitory concentration (MIC) test, where bacteria are exposed to different concentration combinations of antibiotics and essential oils. Bacterial growth was evaluated after overnight incubation, and MIC was determined as the lowest concentration inhibiting growth [6,3]. Thyme and oregano essential oils were combined with HCLO to assess bactericidal and inhibitory effects.

Results: We could observe a minimum bactericidal concentration of 250 ppm for HCLO. Essential oils of *Thymus vulgare* and *Origanum vulgare* showed inhibitory effects, with concentrations ranging from 1% to 0.06% as the MIC across various bacterial strains. These findings indicate a potent antimicrobial activity from the combination of essential oils and HCLO.

Discussion: The combination of essential oils and HCLO showed strong synergy. Similar studies have found that combining thyme essential oil with ciprofloxacin reduced biofilm formation by 80.1–98% in MDR *Klebsiella pneumoniae* strains at 10 µL/mL[10]. Another study showed that 0.041% thyme essential oil reduced biofilm formation by 98.4–99.6% in two *Pseudomonas aeruginosa* strains, one MDR and one reference strain[15]. Our results align with these studies, showing significant growth inhibition at low concentrations.

Essential oils act by disrupting bacterial membranes, damaging cell walls, and causing intracellular leakage. They also generate reactive oxygen species (ROS), which damage nucleic acids, proteins, and ribosomes, interfering with ATP generation [2]. This supports the observed synergy with HCLO, suggesting that these combinations could enhance antimicrobial effects while reducing the required doses, potentially minimizing toxicity and resistance risks.

In conclusion, the combination of HCLO and essential oils, such as thyme and oregano, shows promise for treating MDR bacterial infections. Further studies are needed to validate these findings in clinical settings[18].

Keywords: Hypochlorous acid, Multidrug-resistant, Essential oils

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Survival advantage of native and engineered T cells is acquired by mitochondrial transfer from mesenchymal stem cells

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Background: Apoptosis is crucial for immune system development and homeostasis. Chimeric antigen receptor T (CAR-T) cell therapy, though effective for hematologic cancers, faces limitations, including CAR-T cell susceptibility to apoptosis. Strategies to enhance T-cell survival are needed. Mesenchymal stem/stromal cells (MSCs) exhibit immunoregulatory properties. We previously demonstrated that mitochondrial transfer (MitoT) from umbilical cord MSCs (UC-MSCs) reprograms CD3+ T cells towards a Treg lineage. Enhancing T-cell metabolic fitness is vital for improving immunotherapy. This study evaluates the impact of MitoT on apoptosis in native lymphocytes and CAR-T cells.

Methods: MitoT from UC-MSCs was transferred to peripheral blood mononuclear cells (PBMCs) via a cell-free Mitoception approach. RNA-seq was performed on MitoT-positive (MitoTpos) and MitoT-negative (MitoTneg) CD3+ T cells. Apoptosis was induced with staurosporine (STS), and viability was assessed using Annexin V/7AAD and TUNEL assays. Changes in apoptotic regulators were analyzed by flow cytometry, western blot, and qRT-PCR. The effect of MitoT on 19BBz CAR-T cells post- electroporation was evaluated with Annexin V/7AAD.

Results: MitoTpos CD3+ T cells showed resistance to STS-induced apoptosis, with reduced apoptotic markers and preserved mitochondrial membrane potential. MitoT decreased caspase-3 cleavage, increased BCL2 levels, and reduced apoptosis in CAR-T cells after electroporation, enhancing cytotoxic activity.

Conclusions: MitoT protects human CD3+ T cells from STS-induced apoptosis by modulating the caspase pathway. MitoT also enhances CAR-T cell resistance to apoptosis, suggesting improved metabolic fitness and potential to enhance CAR-T therapy outcomes.

Keywords: Mesenchymal stromal/stem cells, chimeric antigen receptor T (CAR-T) cells, mitochondria transfer, induced- apoptosis

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POSTERS / APPLIED IMMUNOLOGY AND VACCINES

ROLE OF NATURAL KILLER T CELLS IN GERMINAL CENTER FORMATION AND MEMORY DEVELOPMENT IN RESPONSE TO T-INDEPENDENT ANTIGENS

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Humoral immune response against T-independent antigens is characterized by lack of germinal center formation and absence of long-lasting memory development, as both processes rely heavily on T-cell involvement. Natural Killer T (NKT) cells rapidly secrete vast quantities of cytokines when recognizing glycolipid ligands presented by the CD1d molecule. α-GalCer, a glycolipid ligand, has demonstrated to strongly stimulate NKT cells inducing the production of IFN-γ and IL-4. Different α- GalCer analogs like AH10-7 or OCH polarize the secretion of cytokines to IFN-γ or IL-4, respectively. NKT cells have previously been reported to promote the development of memory and germinal center formation in a T-dependent manner, however it is yet unknown if α-GalCer or its analogs, can promote these processes in a T-independent manner. In this study we use
mice immunized with the T-independent type antigen NP-Ficoll, co-administered with α-GalCer analogs and evaluate their effects on the development of class-switch recombination, germinal center formation, and memory development. Class switch recombination was evaluated via isotype specific ELISA, and germinal center formation will be analyzed by immunofluorescence and flow cytometry detecting FAS+/GL7+ B cells. Finally, memory will be evaluated by detecting CD80+PDL2+ B cells using flow cytometry and antibody-secreting cells by ELISPOT. AH10-7 induced the production of NP specific IgG1 antibodies, in line with the presence of B cells and plasma cells expressing high levels of IgG1 and IgG3. We also found that α-GalCer promoted the generation of GL7+ B cells. We hypothesize that the use of analogs as adjuvants improve responses against T-independent antigens.

Keywords: Immunotherapy, NKT cells, T-independent antigens

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POSTERS / APPLIED IMMUNOLOGY AND VACCINES

Adaptation of tumor cell lines to three-dimensional culture for immunogenic lysates production for melanoma vaccines

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Malignant melanoma is the most aggressive skin cancer, responsible for 65% of skin cancer-related deaths despite accounting for less than 5% of diagnoses. We have developed an immunotherapy for melanoma using lysates from three melanoma cell lines: MEL-1, MEL-2, and MEL-3. The complexity of the lysate generation process limits the production of this vaccine, so we are focusing on strategies to enhance production.

Our ongoing work focuses on implementing strategies to improve the production of tumor cell lines. The approach includes culture characterization, implementation of a suspension culture process to enhance scalability, optimization of media composition and feeding to increase productivity and laying the foundation for a biomanufacturing process design.

Batches of adherent cell cultures were carried out for each melanoma cell line, monitoring cell density, nutrient profiles, and viability. These results provide insight into their metabolic requirements and growth conditions. Additionally, repeated cell cultures in suspension were conducted to promote the formation of self-assembled structures or spheroids. MEL-1 and MEL-3 reached cell densities similar to the batch adherent culture, while MEL-2 maintained a consistent cell density.

For media optimization, MEL-1 and MEL-2 were cultured in a fed-batch suspension with the addition of a concentrated medium containing primary carbon and nitrogen sources. MEL-1 achieved a lower cell density than the batch culture, whereas MEL-2 exceeded it.

Ongoing experiments aim to determine the specific consumption rates of carbon and nitrogen sources in fed-batch conditions to refine feeding strategies. Additionally, DAMPs will be evaluated in three-dimensional cultures to assess their immunogenicity.

Keywords: immunotherapy, melanoma cell lines, mammalian cell culture, suspension culture, malignant melanoma

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POSTERS / APPLIED IMMUNOLOGY AND VACCINES

Mitochondrial augmentation regulates the redox state in natural killer cells in an oxidative environment.

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Natural killer (NK) cells are immune effectors capable of killing cancer cells by recognizing surface ligands without prior antigen presentation. NK-based therapies, including allogeneic NK and CAR-NK cells, are promising options for treating hematological malignancies, as clinical trials have shown their safety profile. These therapies do not induce graft-versus-host disease and have a lower risk of severe side effects like cytokine storms. While NK-based therapies show promise for hematological malignancies, their efficacy is hampered by elevated reactive oxygen species (ROS) leading to cellular exhaustion.
Mitochondrial metabolism critically regulates NK cytotoxicity, suggesting a potential target.

Mitochondrial transfer from mesenchymal stem cells (MSCs) has been shown to regulate ROS levels in fibroblast cell lines with mitochondrial disorders. In this study, we assessed whether NK cells could acquire mitochondria from MSCs and if this transfer could regulate the redox state of peripheral blood NK cells under an oxidative environment.

The NK cells were isolated from peripheral blood and then received mitochondrial transfer. Subsequently, they were challenged with Menadione, an oxidative stress-inducing agent.

We successfully demonstrated the mitochondrial transfer from MSCs to NK cells by Flow cytometry. Importantly, this transfer significantly reduced ROS levels in NK cells exposed to oxidative stress.

Mitochondrial augmentation in NK cells through MSC-mediated transfer represents a novel approach to enhance their function under oxidative conditions. This strategy holds promise for optimizing NK-based immunotherapies, potentially improving their efficacy in cancer treatment.

Keywords: Natural killer, Mitochondrial transfer, Mesenchymal Stem Cell, Reactive Oxygen Species

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