The 3rd EFIS Forum on innate immunity in Sterile Inflammation, Autoimmunity, and their Resolution (iiSIAR)

Abstract book

May 11-13, 2026 | Hilton Garden Inn, Vilnius, Lithuania

FOREWORD

Innate immune cells and mediators are essential effectors in long-lasting pathologies, such as autoimmunity, metabolic disorders, vascular diseases and cancer, as well as normal physiological processes like wound repair and resolution of inflammation. In recent years, it has become evident that innate myeloid and lymphoid cells also play key roles in maintaining homeostasis of the host and that their development and tissue-specific properties are more intricate than initially appreciated.

The EFIS Study Group on Innate Immunity in Sterile Inflammation, Autoimmunity and their Resolution (iiSIAR) underscores its activities with a Forum meeting in Vilnius (May 11-13, 2026). This gathering aims to expand and develop the European network of innate immunologists working on sterile inflammation, autoimmunity and cancer to maximize the use of resources, exchange of research ideas, training of young scientists as experts in innate inflammation and its resolution collaborative research, translatability of innovative science, and establish large research groups.

Prof. Amiram Ariel
Spokesperson and chair of the board
EFIS iiSIAR Study group

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PARTNERS AND SPONSORS

SCIENTIFIC PROGRAM

DAY 1 (Monday, May 11)

11:00-13:00 ***Registration, welcome coffee***

13:00-14:00 **Plenary session 1**
Key Effectors in the Resolution of Inflammation
Chairs - Amiram Ariel and Adriano Rossi

13:00-13:30 Annexin A1: from the control of neutrophil reactivity to a pro-resolving therapy in Phase II
Mauro Perretti (London, UK)

13:30-14:00 Role of MS4A tetraspans in macrophage activation.
Massimo Locati (Milan, Italy)

14:00-15:55 **Session 1**
Innate Immunity in Sterile Inflammation
Chairs - Amiram Ariel and Adriano Rossi

14:00-14:20 The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis
Amiram Ariel (Haifa, Israel)

14:20-14:40 Eyes that tell stories: visualising inflammation in the zebrafish eye
Adriano Rossi (Edinburg, UK)

14:40-14:55 Extracellular succinate as a novel DAMP-like signal in the central nervous system that regulates microglial inflammatory and mitochondrial responses
Andis Klegeris (UBC, Canada)

14:55-15:25 ***Coffee break***

15:25-15:40 Macrophages rescue cells from ferroptotic death
Jacob Rachmilewitz (Jerusalem, Israel)

15:40-15:55 Uncoupling Mitochondria and Bacteria-Induced NETosis by selective FPR1 agonism
Emil Becka (Bratislava, Slovakia)

15:55-17:45 **Plenary session 2**
Key Effectors in Immunity of Cancer
Chairs - Jo Van Gindreachter and Sven Brandau

15:55-16:25 Metabolic and epigenetic control of cancer immunity
Massimiliano Mazzone (Leuven, Belgium)

16:25-16:45 Depleting IL1R2+ tumor-infiltrating regulatory T cells with an ADCC-prone nanobody construct boosts the efficacy of anti-PD-1 immunotherapy.
Jo Van Ginderachter (Brussels, Belgium)

16:45-17:00 TAM reprogramming with CD5L blockade overcomes chemotherapy resistance
Rodney Macedo Gonzales (Badalona, Spain)

17:00-17:15 The innate immune receptor Formyl Peptide Receptor 1 exploits the cholesterol biosynthetic machinery to sustain resolution of inflammation in gastric cancer cells
Nella Prevete (Naples, Italy)

17:15-17:30 A combined experimental–computational platform to characterize the dynamics of tumor-macrophage cell interaction using 3D cultures
Qiaoling Ye (Badalona, Spain)

17:30-17:45 Soluble PD-1/PD-L1 as biomarkers in prostate cancer: innate immune landscape, potential cellular contributors, and clinical utility
Margarita Žvirblė (Vilnius, Lithuania)

17:45-18:30 **Flash talks (3 min each)**

18:30-18:45 **The update on iiSIAR activities.**
Amiram Ariel (Haifa, Israel)

19:00-22:00 ***Welcome reception***
(restaurant Neringa, Gedimino av. 23)

DAY 2 (Tuesday, May 12)

9:00-11:00 **Session 2**
Innate Immunity in Autoimmunity
Chairs - Zeinab Abdullah and Marzena Garley

9:00-9:30	Type 1 IFN autoantibodies in autoimmunity *Pärt Peterson (Tartu, Estonia)*
9:30-9:50	Intranasal immunotherapy with Fc-fused antigens for the treatment of type 1 diabetes and allergy *Sylvaine You (Paris, France)*
9:50-10:10	It takes two TLRs to drive autoimmunity in HIV *Zeinab Abdullah (Bonn, Germany)*
10:10-10:30	From defense to dysregulation: The pleiotropic role of neutrophils in autoimmunity *Marzena Garley (Bialystok, Poland)*
10:30-10:45	Spatially-resolved functional maps of synovial macrophages and structural cells in remission and arthritis *Janine Lueckgen (Ljubljana, Slovenia)*
10:45-11:00	Targeting extracellular traps with CIT-013: Linking ET inhibition, immune cell reprogramming, and therapeutic potential in rheumatoid arthritis *Annemarie Kip (Cytril, Netherlands)*

11:00-11:30 ***Coffee break***

11:30-13:00 **SBL Mini-symposium 1**
Neutrophils in Innate Immunity
Chairs - Elodie Segura and Tal Burstyn-Cohen

11:30-12:00	Advancing our knowledge on human immunosuppressive neutrophils *Marco Cassatella (Verona, Italy)*
12:00-12:30	Targeting the cytosolic scaffold of PCNA (Proliferating Cell Nuclear Antigen) in neutrophils to promote the resolution of inflammation *Veronique Witko-Sarsat (Paris, France)*

12:30-13:00 Intratumoral biology of neutrophils
Sven Brandau (Essen, Germany)

13:00-14:00 ***Lunch (Hilton Garden Inn)***

14:00-15:30 **IRA Mini-symposium**
Therapeutic Targets in Acute and Chronic Inflammatory Memory
Chairs – Veronique Witko-Sarsat and Marco Casatella

14:00-14:30 Innate immune exhaustion and rejuvenation in the treatment of disease
Liwu Li (Virginia Tech, VA, USA)

14:30-15:00 Targeting eNAMPT/TLR4 signaling to modulate persistent innate immunity dysregulation in the lung
Joe "Skip" Garcia (UF Scripps, FL, USA)

15:00-15:30 The Galectin-3 Dichotomy in Macrophages: From Secreted Pro-atherogenic Mediator to Intracellular Guardian of Lysosomal Function
Babak Razani (Pittsburg, PA, USA)

15:30-16:00 ***Coffee break***

16:00-17:05 **Session 3**
Innate Immunity and metabolism
Chairs - Annabel Valledor-Fernandez and Sylvaine You

16:00-16:20 The pathway in immune regulation: novel insights from the LXR-CD38 axis
Annabel Valledor-Fernandez (Barcelona, Spain)

16:20-16:35 Immune stimulation can change the phenotype and function of hematopoietic stem cells
Roi Gazit (Be'er Sheva, Israel)

16:35-16:50 Neutrophils and NETs induce a pro-inflammatory and matrix-degrading phenotype in cardiac fibroblasts
Razvan Macarie (Bucharest, Romania)

16:50-17:05 CD5L inhibition drives metabolic and immune reprogramming of human macrophages
Jana Vázquez (Badalona, Spain)

17:05-17:35 **Plenary session 3**
Innate immune development
Chairs - Annabel Valledor-Fernandez and Sylvaine You

17:05-17:35 Transcriptional control of the macrophage repair program
Elodie Segura (Paris, France)

17:35-18:00 **Flash talks (3 min each)**

18:00-20:00 ***Guided tour to Vilnius University courtyards, the bell tower of St.John's church and the Old-town***

20:00-22:00 ***Meeting dinner (Hilton Garden Inn)***

DAY 3 (Wednesday, May 13)

9:00-10:00 **Plenary Session 4**
Innate Immunity in Precision Medicine
Chairs - Maciej Kurpisz and Janusz Marcinkiewicz

9:00-9:30 *Eicosanoid Research Foundation speaker- Valerio Chiurchiu (Rome, Italy)*

9:30-10:00 Innate Immune Checkpoints Revisited: Alternative Splicing, Novel Targets and the Glycan Dimension
Angel Porgador (Be'er Sheva, Israel)

10:00-11:25 **Session 4**
Innate Immunity in the Clinic
Chairs - Maciej Kurpisz and Janusz Marcinkiewicz

10:00-10:20 Local cytokine network and its implication in male reproductive failure
Maciej Kurpisz (Poznan, Poland)

10:20-10:40 Macrophages: sentinels, guardians, soldiers, and saboteurs of the immune system
Janusz Marcinkiewicz (Krakow, Poland)

10:40-11:00 Dysregulated immunometabolism in SLE neutrophils is reversible with anifrolumab therapy
Gina Leisching (Dublin, Ireland)

10:00-11:25 Neutrophils and monocytes research parameters as initial evaluation of innate immune response in common variable immunodeficiency (CVID) and hypogammaglobulinemia.
Elżbieta Rutkowska (Warsaw, Poland)

11:25-11:55 ***Coffee break***

11:55-14:40 **Session 5**
The Future of Innate Immunity (short talks)
Chairs – Angel Porgador and Valerio Chiurchiu

11:55-12:10 Toll-like receptor 9 activation contributes to Alzheimer`s disease pathology
Bénédicte Manoury (Paris, France)

12:10-12:25 Reframing neurodegeneration: Protein aggregation as a maladaptive antimicrobial response
Esther Silberberg (Jerusalem, Israel)

12:25-12:40 CRISPR-Cas9 screening in human iPSC-derived microglia identifies novel drivers of microglial health and CNS disease susceptibility
Larisa Janzic (Ljubljana, Slovenia)

12:40-12:55 Targeted delivery of specialized pro-resolving mediators reduces atherosclerosis and induces durable innate immune reprogramming
Maria Anghelache (Bucharest, Romania)

12:55-13:10 Impaired resolution of LPS-induced endothelial dysfunction: role of GPR18 receptor and systemic inflammatory response
Barbara Sitek (Krakow, Poland)

13:10-13:25 Neutrophil extracellular traps promote a pro-resolving macrophage phenotype characterized by inflammasome suppression and enhanced phagocytosis
Miruna Larisa Naie (Bucharest, Romania)

	13:25-13:40	Can computational flow cytometry reveal specific immune resolution signatures during the resolution of intestinal inflammation? *Javier Conde Aranda (Santiago de Compostela, Spain)*
	13:40-13:55	Tumor antigen presentation defects: Impact on immunotherapy response and strategies for pharmacologic modulation in preclinical models *Karolina Suveizdė (Vilnius, Lithuania)*
	13:55-14:10	Fibronectin-targeted nanoparticles mediate accumulation of HSP90 inhibitors in the fibrotic heart with possible neutrophil contribution *Geanina Voicu (Bucharest, Romania)*
	14:10-14:25	An image is worth a thousand words: Machine learning-assisted quantification of lung fibrosis *Shahar Levy (Haifa, Israel)*
	14:25-14:40	Bioethical aspects of the development process of innate immunity *Serghei Sprincean (Chisinau, Moldova)*
14:40-16:15	***ySG - National coordinators lunch (Hilton Garden Inn)*** ***Lunch on your own for others***	
16:15-16:55	**Joint iiSIAR-EU-RESOLVE presentation** PROS1-TAM Signalling in Microglia Regulate Microglia Development, Homeostasis and Sterile Inflammation *Tal Burstyn-Cohen (Jerusalem, Israel)*	
16:55-17:10	**Closing remarks**	
17:10	***Meeting departure***	

FLASH TALKS (3 min each)

DAY 1 (Monday, May 11)
17:45-18:30

Chairs – Aurelija Žvirblienė and Martynas Simanavičius

SYK-mediated NLRP3 inflammasome activation induced by immune complexes of virus-like particles in macrophages	*Kristina Mašalaitė*
Effects of plant-derived antioxidants and PUFAs on functional recovery following spinal cord injury in zebrafish	*Viktorija Povilionytė*
miR-210 modulates human macrophage innate immune response in sterile inflammation following myocardial infarction	*Cristina-Andreea Mihaila*
Nucleolin-targeted liposomes improve tumor delivery of dexamethasone and promote selective immunomodulation	*Delia Boteanu*
Donor baseline transcriptomic state shapes dendritic cell reprogramming across ovarian tumor immune subtypes	*Emiljia Paberalė*
Dietary prebiotic intervention modulates high-fat diet-associated microglial morphological alterations in aged mice	*Urtė Minkevičiūtė*
Innate Immune Responses to Acinetobacter baumannii: Modulation of Neutrophil Migration and NETosis	*Danas Ivanauskas*
Immune Signatures in Unexplained Infertility: A Precision Medicine Approach Using Peripheral Blood Cytometry	*Gabija Didžiokaitė*
miR-146a-5p Links Innate Immune Signaling to Mitochondrial and Metabolic Regulation in Neural Cells	*Greta Bertašiūtė*
Nonclassical Monocyte Depletion as an Indicator of Disturbed Innate Immune Response in Sarcoidosis and COVID-19	*Agata Raniszewska-Borys*

IFN-β and ACKR2 Modulate Tissue Fibrosis and Corresponding Macrophage Subsets in Early CCl_4-Induced Liver Injury	*Priya Goswami*
Accumulation of small peritoneal macrophages and dendritic cells during tumor rejection in a concomitant tumor immunity model	*Milda Vanagaitė-Žičkienė*
Dissecting Platelet-Activating Factor Signalling in Microbial Keratitis Using a Zebrafish Corneal Injury Model	*Kelvin KW Cheng*

DAY 2 (Tuesday, May 12)
17:35-18:00

Chairs – Aurelija Žvirblienė and Martynas Simanavičius

Flow Cytometric Comparison of Different Human Myeloid Cell Enrichment Strategies	*Simona Malmigė*
Association between the IFN-γ/IL-4 ratio in saliva and self-reported health status and factors affecting it in patients with Sjogren's disease	*Vilius Kontenis*
Characterization and application of monoclonal antibodies to human Calnexin	*Meda Pakėnaitė*
Molecular mechanisms of endometriosis: The involvement of endoplasmic reticulum aminopeptidases 1/2 (ERAP1/ERAP2) and cysteinyl aminopeptidase (LNPEP) in disease susceptibility and severity	*Patrycja Bochen*
Development and validation of sensitive immunoassays to quantify human serum C-reactive protein	*Ghodke Tanhaji Sandu*
Clinical case of Nijmegen breakage syndrome: diagnostic challenges in early infancy	*Nataliia Ipatii*
Investigation of Potential Biomarkers Predicting Acute or Chronic Progression of Inflammatory Diseases	*Mantė Butkevičiūtė*

ABSTRACTS

DAY 1 (Monday, May 11, 2026)

Plenary session 1
Key effectors in the Resolution of Inflammation

Session 1
Innate Immunity in Sterile Inflammation

Plenary session 2
Key Effectors in Immunity of Cancer

Flash talks

Annexin A1: from the control of neutrophil reactivity to a pro-resolving therapy in Phase II

Mauro Perretti
The William Harvey Research Institute, Queen Mary University of London, United Kingdom

The resolution of acute inflammation is a physiological, life-saving process. Annexin A1 (AnxA1) is a pro-resolving mediators that guarantee the time and spatial control of the inflammatory response. Blocking AnxA1 in human neutrophils augments their migration, delays spontaneous apoptosis and impedes efficient efferocytosis. At low concentrations AnxA1 (~10-30 nM) provokes opposite regulatory effects. These biological effects are mediated by activation of the pro-resolving formyl-peptide receptor type 2 or FPR2. The non-redundant role of AnxA1 and Fpr2 is confirmed by the strong phenotype of mice and murine cells lacking these proteins, with heightened inflammatory reactions, larger and longer-lasting organ damage as shown in disease models for the gut, heart, kidney or joint.
Resolution pharmacology is a branch of therapeutic development based on the fundamental biology of the resolution of inflammation, as an innovative approach to the pharmacological control of chronic diseases (1). It dictates the potential for agonists that mimic the actions of pro-resolving mediators. In the case of AnxA1, the focus has been its N-terminal region, long known to represent its pharmacophore. A synthetic peptide modelled on this sequence of AnxA1, modified to resist to protease cleavage, exerts pro-resolving properties on immune cells and significant protection against infarct and heart failure associated with sepsis (2). This compound is owned by ResoTher Pharma Aps and termed RTP-026 of
In the ischemic infarction in the rat, RTP-206 given at reperfusion, protects against damage quantified at 2h and 24h post-reperfusion. Modulation, not abrogation, of immune cell activation in the circulation and recruitment to the injured heart is the mode of action (3). In a longer study, delivery of RTP-026 three times during the first 24h post-reperfusion, ameliorated cardiac functionality at day 14. These data, together with the large body of work on the pro-resolving properties of AnxA1 and Fpr2 agonists, prompted phase 1 studies (dose-response and multiple dose-ascending designs) which showed RTP-026 to be devoid of any toxic effects. This outcome justified testing RTP-026 in an exploratory, randomised, double-blind, multicentre, placebo-controlled study with three 30-minute intravenous infusions of RTP-026 or placebo to be given as add on to standard therapy in patients with STEMI undergoing primary Percutaneous Coronary

Intervention. This phase 2a study is still on-going, data are blinded, and will be discussed at the meeting.

(1) Perretti M and Montero-Melendez T. Resolution Pharmacology: State-of-the-art and therapeutic landscape. *Pharmacol Rev*. 2025 Nov;77(6):100097.
(2) Dalli J, et al. Proresolving and tissue-protective actions of annexin A1-based cleavage-resistant peptides are mediated by formyl peptide receptor 2/lipoxin A4 receptor. *J Immunol*. 2013 Jun 15;190(12):6478-87.
(3) Chen J, Oggero S, et al. The Annexin-A1 mimetic RTP-026 promotes acute cardioprotection through modulation of immune cell activation. *Pharmacol Res.* 2023 Dec;198:107005.

The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis

***Amiram Ariel*, Nofar Ben Jashar, Uzma Saqib, Anat Ablin, & Sagie Schif-Zuck**
University of Haifa, Department of Human Biology, Israel

Stimulator of IFN Genes (STING) is a cytosolic DNA sensor that plays a central role in host protection against pathogens upon binding DNA-derived ligands. STING primarily controls the transcription of type I interferons (IFNs) and pro-inflammatory cytokines. Notably, STING can be tuned pharmacologically to mitigate human immune pathologies. DMXAA is a pharmacological activator of murine STING that induces IFN-β and its affected genes. Here, we report that macrophages from DMXAA-treated mice engulfed significantly higher numbers of apoptotic cells ex vivo, and exhibited enhanced reprogramming reflected by an increased IL-10 and reduced inflammatory cytokine secretion upon LPS exposure. Macrophage reprogramming was significantly hampered in STING and IFN-β -deficient macrophages. Furthermore, we used virtual docking and batch screening to identify biased STING agonists (BiSTs) that enhanced IL-10 and IFN-β production by splenocytes while inhibiting TNFα. One of these compounds, termed BiST 2.1, also induced the murine STING pathway in vivo and in human macrophages. Finally, we found BiST 2.1 to enhance wound repair by mouse embryonic fibroblasts and promote the resolution of liver fibrosis induced by CCl_4. Thus, our findings indicate that STING can be harnessed to drive IFN-β-mediated IL-10 secretion by resolution phase macrophages and consequently shape macrophage and fibroblast function to enhance the resolution of inflammation and treat fibrotic disorders.

Eyes that tell stories: visualising inflammation in the zebrafish eye

Kelvin KW Cheng, Bethany Mills and Adriano G Rossi
Centre for Inflammation Research, Institute for Regeneration and Repair, University of Edinburgh, United Kingdom

Real-time, high-resolution intravital microscopy enables visualization of dynamic cellular and molecular processes in living organisms, significantly advancing our understanding of cell function and behaviour. However, its application to the eye, a complex and delicate organ, remains limited. Microbial keratitis (MK), a leading cause of vision loss, is an infection of the cornea caused by pathogens such as bacteria and fungi. Here, we describe a newly developed larval zebrafish (Danio rerio) intravital model of MK, incorporating fluorescently labelled neutrophils, macrophages, and basal epithelial cells in genetically modified transgenic lines *(Cheng et al., Commun Biol. 2026 Apr 14; doi:10.1038/s42003-026-09985-1).*
Mechanically-induced corneal injury triggered a rapid, robust, dynamic, and reversible recruitment of neutrophils and macrophages. This response was further amplified by key pro-inflammatory mediators and live bacterial challenge. Collectively, this model provides a powerful platform for advancing our understanding of immune processes in the eye, as well as inflammation more broadly.

Extracellular succinate as a novel DAMP-like signal in the central nervous system that regulates microglial inflammatory and mitochondrial responses

Andis Klegeris
UBC, Vancouver, Canada

Neuroinflammation mediated by reactive microglia, the resident immune cells of the brain, contributes to many neuropathologies, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, and ischemic stroke. Damage-associated molecular patterns (DAMPs), which are released from stressed or injured cells, play important roles in neuroinflammation and neurodegeneration. Succinate, a key intermediate of the tricarboxylic acid cycle, can accumulate inside cells and may also be released into the extracellular space, where it could function as a DAMP-like signaling molecule. However, its specific roles in central nervous system (CNS) neuroimmune responses, particularly in the extracellular environment, remain insufficiently characterized.

In this study, we used cell-impermeable disodium succinate to model extracellular effects and cell-permeable diethyl succinate to evaluate intracellular actions in cell culture systems, including BV-2 murine microglia, human peripheral blood mononuclear cells, and NSC-34 neuronal cells. Extracellular disodium succinate significantly decreased the secretion of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF) and interleukin-6 (IL-6), and reduced both neurotoxic and phagocytic activities in immune-stimulated BV-2 microglia. It also restored lipopolysaccharide-induced impairments in mitochondrial respiration, an effect that was associated with reduced phagocytic activity. The effects of extracellular succinate on BV-2 microglia are likely mediated, at least in part, by activation of the succinate receptor SUCNR1, which we demonstrate is expressed in this cell line.

In contrast, intracellular diethyl succinate lowered TNF and IL-6 secretion but did not prevent microglia-mediated neurotoxicity, highlighting distinct, compartment-specific actions of succinate. Collectively, these findings identify extracellular succinate as a novel DAMP in the CNS that exerts predominantly anti-inflammatory effects on microglia, with potential implications for neuroimmune regulation and the pathogenesis of neurological disorders.

Macrophages rescue cells from ferroptotic death

Jacob Rachmilewitz
Hadassah Medical Center, Hebrew University of Jerusalem, Israel

Ferroptosis, a non-apoptotic form of cell death marked by iron-dependent lipid peroxidation, has a key role in organ injury, degenerative disease, and vulnerability of therapy-resistant cancers. Although substantial progress has been made in understanding the molecular processes relevant to ferroptosis, additional cell-extrinsic processes that determine cell sensitivity toward ferroptosis remain unknown. Here we demonstrate that macrophages co-cultured with ferroptotic cancer cells from various types effectively mitigate cell death induced by GPX4 inhibitors (RSL3 and ML162), GPX4 silencing via shRNA, or the Xc- system inhibitor IKE. Furthermore, macrophages effectively reduced lipid peroxidation in ferroptotic cells. Importantly, macrophage function relies on direct cell-to-cell contact and is affected by their differentiation. Specifically, polarization into M1 macrophages, but not M2, greatly hinders their protective capabilities. Interestingly, unlike apoptotic cells, ferroptotic cells retain elevated levels of the ‘don’t eat me’ signal, CD47, and conversely, fail to present the "eat me" signal phosphatidylserine (PS) on the outer layer of the plasma membrane, providing an opportunity for their rescue. Furthermore, in placental villi explants, macrophages protect trophoblasts from ferroptotic death. These results underscore the intricate interplay between ferroptotic cells and their microenvironment and provide compelling evidence of a yet-unrecognized anti-ferroptotic activity of macrophages as a cell-extrinsic mechanism that could be exploited by cancer cells to escape ferroptosis.

Uncoupling Mitochondria and Bacteria-Induced NETosis by selective FPR1 agonism

Emil Becka
Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Background: Trauma and major surgery release mitochondria-derived damage-associated molecular patterns (mtDAMPs), engaging neutrophils. This can divert neutrophil activity away from microbes, increasing susceptibility to secondary infection. Additionally, mtDAMPs may induce unwanted NETosis that further promotes tissue injury. We aimed to define mechanism by which mitochondria drive NETosis, focusing on FPR1 modulation to selectively limit mtDAMP-induced NETosis without suppressing antibacterial responses.
Methods: Sterile injury and mtDAMP exposure in vivo were used to assess neutrophil recruitment and NET formation. Human neutrophils were stimulated with intact mitochondria and individual mtDAMPs and NET formation was quantified by live-cell imaging and MPO–DNA ELISA. Mechanisms were tested using receptor/enzyme modulation (including FPR1 antagonism and agonism) and kinase phosphorylation profiling. In vitro results were supported using healthy neutrophils co-incubated with trauma patient plasma.
Results: Sterile trauma and mtDAMPs induced neutrophil swarming and NET formation in vivo. NETosis required intact mitochondria, not individual mtDAMPs, and was reduced by blocking TLR9-, PAD4-, and NOX2-linked pathways. Concurrent engagement of FPR1 and TLR9 was sufficient to drive NETosis while pre-engagement of FPR1 selectively attenuated NETosis to intact mitochondria and trauma patient plasma. Phosphorylation profiling showed a selective mitochondria-driven signature (including p38α/STAT-associated signaling) that was dampened by FPR1 agonist pre-treatment. Functionally, FPR1 agonism reduced responses to sterile mitochondrial cues while preserving antibacterial NETosis and bacterial clearance and partially restoring migration toward bacteria after mitochondrial exposure.
Conclusion: FPR1 agonism can selectively suppress mitochondria-induced NETosis while preserving neutrophil responses to bacteria, offering a strategy to reduce post-traumatic infection risk.

Metabolic and epigenetic control of cancer immunity

Massimiliano Mazzone
VIB Center for Cancer Biology, KU Leuven Department of Oncology, Leuven, Belgium

Anti-cancer immunotherapy has provided patients with a promising treatment. Yet, it has also unveiled that the immunosuppressive tumor microenvironment (TME) hampers the efficiency of this therapeutic option and limits its success. The concept that metabolism is able to shape the immune response has gained general acceptance. Nonetheless, little is known on how the metabolic crosstalk between different tumor compartments contributes to the harsh TME and ultimately impairs T cell fitness within the tumor. This lecture will decipher some of the metabolic changes in the TME impeding proper anti-tumor immunity. Starting from the meta-analysis of public human datasets, corroborated by metabolomics and transcriptomics data from several mouse tumors, we ranked clinically relevant and altered metabolic pathways that correlate with resistance to immunotherapy. Using a CRISPR/Cas9 platform for their functional *in vivo* selection, we have identified cancer cell intrinsic metabolic mediators and, indirectly, distinguished those belonging specifically to the stroma. By means of genetic tools and small molecules, we have targeted promising metabolic pathways in cancer cells and stromal cells (particularly in tumor-associated macrophages) to harness tumor immunosuppression. Finally, we went back to patient samples to assess the relevance of these metabolic networks in humans. By analyzing the metabolic crosstalk within the TME, this lecture would like to shed some light on how metabolism contributes to the immunosuppressive TME and T cell maladaptation.

Depleting IL1R2+ Tumor-Infiltrating Regulatory T Cells with an ADCC-Prone Nanobody Construct Boosts the Efficacy of Anti-PD-1 Immunotherapy

Jo Van Ginderachter
Lab of Cellular and Molecular Immunology, *Vrije Universiteit Brussel, Belgium*

Eliminating immunosuppressive cells, such as regulatory T cells (Tregs), is a promising approach to boost immunotherapy success. However, this approach may suffer from systemic auto-immune adverse events, highlighting the need to specifically target tumor-infiltrating (ti)Tregs. Based on CITE-seq and single-cell RNA-seq data from mouse models of triple-negative breast cancer (TNBC) and colorectal carcinoma (CRC), as well as a meta-analysis of human TNBC and CRC datasets, we obtained a comprehensive overview of the tiTreg heterogeneity and IL1R2 expression. Several IL1R2-expressing tiTreg clusters were identified in mouse and human TNBC and CRC tumors, with some level of conservation. IL1R2 was identified as a surface marker that was most highly expressed by activated and strongly T-cell suppressive tiTregs in the tumor microenvironment but not by peripheral Tregs. IL1R2 upregulation resulted from TCR-mediated Treg triggering in a Rel-dependent fashion, but the receptor itself was dispensable for tiTreg abundance and activation and did not influence tumor growth. Accordingly, the blockade of IL1R2, by using an antibody-dependent cell-mediated cytotoxicity (ADCC)-dead anti-IL1R2 nanobody (Nb)-Fc construct, had no impact on tumor growth. Conversely, anti-IL1R2 Nb-Fc constructs with an optimized ADCC functionality, mediated by the SDALIE mutation, resulted in the specific depletion of IL1R2+ tiTregs, elicited antitumor immunity and reduced tumor growth, in synergy with anti-PD-1 therapy. Collectively, these findings identify IL1R2 as a marker for highly activated and suppressive tiTregs that is suitable as a target for ADCC-dependent tiTreg depletion, which can synergize with immune checkpoint blockade.

TAM reprogramming with CD5L blockade overcomes chemotherapy resistance

Rodney Macedo Gonzales
IGTP, Badalona, Spain

Chemotherapy resistance remains a critical obstacle to achieving effective treatment outcomes. Tumor-associated macrophages (TAMs) are implicated in this resistance across numerous types of cancer. To improve the efficacy of chemotherapy, a better understanding of how these treatments affect macrophage activity and novel strategies are needed. In our previous works, we observed that the macrophage checkpoint “CD5L” promotes an immunosuppressive phenotype in TAMs. Increased numbers of CD5L-TAMs in human lung adenocarcinoma are associated with poor prognosis. We generated a first-in class CD5L-blocking monoclonal antibody (RImAb), that, when administered in a mouse model of lung cancer, reduced tumor growth, reprogrammed TAMs, and shifted the pro-tumorigenic tumor microenvironment (TME) towards a less tumor-permissive environment. We hypothesized that targeting CD5L with RImAb may overcome chemotherapy resistance.

To address this, we performed in silico analysis of public bulk and single cell RNAseq data (TCGA and GEO) from patients with lung cancer under different treatment regimens to better characterize CD5L expression in these samples. We found that CD5L is highly expressed in TAM subsets and its expression correlates with worse survival. Moreover, using a three-dimensional model combining human THP1 macrophages, MRC-5 fibroblasts and lung cancer (H23) cells, we found that RImAb and chemotherapy treatment reduced tumor cell viability as assessed by flow cytometry, suggesting a putative synergy between these treatments. More importantly, using a chemotherapy-resistant mouse model of lung cancer (3LL-R), we found that RImAb treatment significantly reduced tumor growth and increased survival in comparison to combination therapy with cisplatin/ gemcitabine.

Overall, these results support TAM reprogramming with CD5L blockade as a promising strategy to combine with chemotherapy and overcome tumor-resistance.

The innate immune receptor Formyl Peptide Receptor 1 exploits the cholesterol biosynthetic machinery to sustain resolution of inflammation in gastric cancer cells

Nella Prevete
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Formyl Peptide Receptor 1 (FPR1) is an innate immune receptor activating both inflammatory and pro-resolving responses. In gastric cancer (GC), FPR1 displays tumor-suppressive activity by promoting inflammation resolution. Accordingly, FPR1-depletion causes defective inflammation resolution and tumor progression.
A transcriptomic analysis of GC cells in which FPR1 expression was genetically modulated highlighted that FPR1 levels directly correlate with cholesterol biosynthetic genes. We used Filipin III to measure intracellular cholesterol and a fluorescent probe to detect cholesterol uptake. FPR1 depletion downregulated cholesterol biosynthesis in delipidated serum. In complete serum, however, total cholesterol remained unchanged due to increased uptake. Thus, we hypothesized that intermediates of cholesterol synthesis, rather than cholesterol itself, could affect FPR1-mediated pro-resolving responses. GC cells were stimulated with the FPR1 agonist fMLF in the presence or absence of Lovastatin (Lova), Zoledronic acid (ZA), or Naftifine, that inhibit cholesterol biosynthesis at distinct levels. Each inhibitor significant reduced FPR1 ability to sustain pro-resolving responses.
Lova and ZA inhibit protein prenylation dampening the production of farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). This last intermediate is required for the activation of Rac1, necessary to FPR1-induced inflammation resolution. Accordingly, FPR1 stimulation increased geranylgeranylated Rac1. Furthermore, adding-back geraniol to Lova- and ZA-treated GC cells recovers the ability of fMLF to sustain a pro-resolving response. Naftifine instead blocks low cholesterol pathway causing the reduction of various metabolites including desmosterol, agonist of the nuclear transcription factors Liver X receptors (LXRs), known to suppress inflammation. We confirmed the involvement of LXRs in FPR1-mediated resolution: antagonists to LXRs blocked the ability of FPR1 to activate a pro-resolving program.
Thus, in GC cells FPR1 sustains the biosynthesis of cholesterol, allowing the production of specific intermediates required for FPR1 pro-resolving effects.

A combined experimental–computational platform to characterize the dynamics of tumor-macrophage cell interaction using 3D cultures

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Tumor-associated macrophages (TAMs) can constitute up to 30–50% of infiltrating immune cells and are linked to poor clinical outcomes. However, understanding how TAMs influence tumor growth dynamics and treatment response remains a major challenge. Three-dimensional (3D) tumor spheroid models recapitulate key features of the tumor microenvironment, including cell–cell interactions and metabolic gradients, and are particularly valuable for quantitatively capturing TAM dynamics in cancer. Here, we present a combined experimental–computational framework that combines 3D multicellular tumor spheroids (MCTS) with mathematical modelling to quantitatively dissect tumor–macrophage interactions.

Spheroids were generated from human liver (HepG2, Huh7, SNU398) and lung (A549, H23, EBC1) cancer cell lines co-cultured with fibroblasts (MRC-5) and macrophages (THP-1). These models were used to investigate the role of resident vs infiltrating macrophages on the temporal evolution of tumor growth, and responses to chemotherapy (sorafenib, cisplatin) and macrophage-directed therapies. We adapted a Gompertz-based growth model-a sigmoid function for modelling biological systems- to analyse experimental imaging data.

The model accurately captured spheroid growth dynamics across conditions and revealed differences between resident and infiltrating macrophages, while significantly shaping tumor growth kinetics in a quantitative manner. Furthermore, the model successfully described therapeutic responses in a cell line–dependent manner.

Overall, this hybrid model provides a valuable tool to better understand the dynamics of macrophage responses in cancer, offering a novel combined experimental–computational platform to evaluate macrophage -targeting therapies.

Soluble PD-1/PD-L1 as Biomarkers in Prostate Cancer: Innate Immune Landscape, Potential Cellular Contributors, and Clinical Utility

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Introduction: Prostate cancer (PCa) is shaped by immunosuppressive innate–adaptive crosstalk, where myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) promote immune escape. Soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) are promising liquid-biopsy candidates that may reflect real-time PD-1/PD-L1 axis and may be influenced by circulating immune cells; however, their relationships with innate immune compartments and their cellular sources in PCa remain insufficiently defined.
Methods: Peripheral blood from 88 patients with pT2–pT3 PCa was analyzed. Multiparametric flow cytometry quantified circulating innate and immunoregulatory subsets, including NK cells and MDSC populations (granulocytic/PMN [neutrophils/eosinophils/basophils]-MDSC and monocytic/M-MDSC), alongside Tregs. Plasma sPD-1 and sPD-L1 were measured by ELISA. Associations with immune subsets and clinical outcomes (biochemical recurrence and progression-free survival) were assessed, and diagnostic performance was evaluated by ROC analyses for single and combined markers.
Results: sPD-1 levels positively associated with NK cell frequencies, suggesting that NK cells may contribute to circulating sPD-1 in PCa. sPD-L1 aligned with immunosuppressive profiles: in patients with biochemical recurrence, preoperative sPD-L1 correlated strongly with Tregs ($r = 0.73$, $p < 0.05$) and inversely with M-MDSC% ($r = -0.72$, $p < 0.05$), whereas no meaningful correlations were observed in patients with favorable postoperative courses. After prostatectomy, sPD-L1 displayed two distinct postoperative patterns, suggesting heterogeneous sPD-L1 sources. Importantly, combining sPD-L1 with granulocytic/PMN-MDSCs markedly improved discrimination of clinically significant PCa (AUC = 0.93; sensitivity = 100%), outperforming combinations with M-MDSCs or Tregs.
Conclusion: Innate immune cells may help shape the soluble PD-1/PD-L1 profile in PCa, consistent with a potential role in disease evolution; when integrated with granulocytic/PMN-MDSC measures, these biomarkers may improve risk stratification and support immunomonitoring.

SYK-mediated NLRP3 inflammasome activation induced by immune complexes of virus-like particles in macrophages

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The inflammasome is a vital component of innate immunity, with NLRP3 as the best-described inflammasome. NLRP3 inflammasome activation triggers the release of proinflammatory cytokines, such as IL-1β, and induces pyroptosis. Spleen tyrosine kinase (SYK), a non-receptor kinase involved in immunoreceptor signalling, has been implicated in regulating NLRP3 inflammasome activation. Our previous research demonstrated that viral antigens and their immune complexes (IC) trigger NLRP3 inflammasome activation in macrophages. However, limited data are available on IC-induced signalling related to the NLRP3 inflammasome and its relationship to macrophage functions, such as phagocytosis and antigen presentation. Therefore, this study aimed to determine SYK role in the IC-induced NLRP3 inflammasome activation pathway and macrophage effector functions.

Primary murine microglia were treated with spherical virus-like particles (VLPs) of human WU polyomavirus and their IC formed with different murine IgG subtypes. NLRP3 inflammasome activation was assessed by measuring IL-1β and TNF-α cytokine release and ASC speck formation. Specific inhibitor R406 was used to inhibit SYK activity and define its role. Protein expression and activation were analysed by Western blot, and phagocytosis with antigen presentation were measured by flow cytometry.

It was found that VLPs and their ICs activate SYK, while R406 blocks SYK activation, NLRP3 expression, cytokine secretion, and ASC speck formation in microglia, indicating inhibition of NLRP3 inflammasome activation. IC mediated a higher cellular response than VLPs alone. The results also revealed that SYK signalling is required for antigen presentation induced by ICs. In conclusion, our study demonstrates that IC can enhance the inflammatory response in microglia via SYK dependent pathway.

Effects of plant-derived antioxidants and PUFAs on functional recovery following spinal cord injury in zebrafish

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Spinal cord injury (SCI) induces inflammatory and tissue repair responses that critically influence regenerative outcomes. While zebrafish possess a remarkable capacity for spinal cord regeneration, identifying interventions that enhance functional recovery remains an important objective. Natural bioactive compounds, including flavonoids and omega-3 polyunsaturated fatty acids (PUFAs) have been reported to modulate inflammatory and tissue repair pathways. This study investigated whether selected natural plant-derived antioxidants and PUFAs improve recovery following SCI in zebrafish.

Larval zebrafish underwent a standardized spinal cord transection. Lesion integrity was confirmed using a neuronal fluorescent reporter line to verify disruption at the injury site. Following injury, larvae were treated with plant antioxidants, PUFAs, or a combination of both. Regeneration was initially assessed by imaging of the lesion site, and functional recovery was evaluated using a locomotor assay measuring total distance moved during 40-minute recording period with controlled tapping stimuli. Data was analyzed using repeated measures two-way ANOVA.

Treated groups exhibited increased tissue regrowth at the lesion site compared with untreated controls. Locomotor analysis demonstrated an improvement in distance moved in injured fish receiving compound treatment, with the combination group showing the strongest effect. No significant changes were observed in uninjured fish, indicating that baseline locomotor activity was not altered.

These findings suggest that treatment with plant antioxidants and PUFAs, particularly in combination, shows positive trend on functional recovery following spinal cord injury in zebrafish. The results support further investigation into the potential role of natural bioactive compounds in modulating regenerative process and promoting neural repair.

miR-210 modulates human macrophage innate immune response in sterile inflammation following myocardial infarction

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Background: Myocardial infarction (MI) induces a sterile inflammatory response driven by innate immune activation. Macrophage polarization toward pro-inflammatory/reparative phenotypes critically determines post-infarction remodeling. miR-210, a hypoxia-responsive microRNA, regulates metabolic adaptation and immune signaling, yet its role in modulating macrophage innate immunity post-MI remains unclear. Therefore, we investigated the effects of miR-210 overexpression on inflammatory/pro-fibrotic macrophages. *Methods:* Macrophages derived from THP-1 monocytes and monocytes of MI patients (≤24 h post-MI) or healthy donors were transfected with miR-210 mimic. Inflammatory and remodeling markers were assessed by qPCR, Western blot, and ELISA. Metabolic function was analyzed using Seahorse assays. Inflammatory and fibrotic markers were evaluated by immunostaining in human cardiac tissue from ischemic/non-ischemic dilated cardiomyopathy. *Results:* In THP1-derived macrophages, miR-210 overexpression enhanced innate immune activation, increasing TNF-α, CCL3, IL-1β, iNOS, and MMPs. In contrast, macrophages from MI patients, exhibiting elevated basal miR-210, didn't further upregulate M1 markers but showed increased expression of resolution-associated and pro-fibrotic markers. miR-210 overexpression in MI-derived macrophages reduced OCR and maximal respiration, indicating impaired oxidative phosphorylation. Consistently, fibrotic markers α-SMA and periostin were increased in ischemic cardiomyopathy as compared to non-ischemic hearts.
Conclusion: miR-210 exerts context-dependent effects on macrophage innate immunity in post-MI sterile inflammation. It promotes pro-inflammatory and matrix-degrading responses in naive macrophages, while driving resolution-associated and pro-remodeling programs in MI-derived macrophages. The increased fibrotic signatures observed in ischemic human hearts support a potential link between miR-210-driven macrophage reprogramming and adverse cardiac remodeling.
Acknowledgment: This work was supported by PNRR-III-C9-2022-I8-186, FibroThera project, Contract no. 760062/23.05.2023, and Romanian Academy.

Nucleolin-targeted liposomes improve tumor delivery of dexamethasone and promote selective immunomodulation

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Nucleolin (NCL), overexpressed on the surface of cancer cells, can serve as a receptor, enabling selective delivery of dexamethasone (Dexa) via NCL-targeted liposomes (NCL-LP) to modulate tumor-associated immune responses. This study aimed to evaluate whether Dexa-loaded liposomes targeted to NCL affect immune cell populations and enhance tumor-specific delivery. C57BL/6 mice were subcutaneously injected with NK/Ly-RB cells derived from the Nemeth-Kellner ascites to develop solid tumors and were treated with free Dexa or Dexa incorporated in non-targeted or NCL-LP over two weeks. Liposomes were prepared using lipid film hydration and functionalized with a peptide that specifically recognizes NCL. Targeted and non-targeted liposomes were fluorescently labeled and injected intravenously for biodistribution studies, while blood and tumor immune cell populations, nanoparticle uptake, and NCL expression were analyzed by flow cytometry. Tumor-bearing mice were also treated over the course of two weeks with free Dexa and Dexa-loaded in non-targeted or NCL-targeted liposomes. Flow cytometry revealed that ~30% of NK-Ly-RB cells expressed NCL. NCL-LP showed greater tumor uptake, while uptake by circulating and tumor-infiltrating $CD11b^+$ cells was similar to that of non-targeted liposomes. Importantly, free Dexa and non-targeted Dexa-loaded liposomes increased tumor Ly6Clow monocytes, whereas Dexa-loaded NCL-LP significantly reduced this monocyte population, which is pro-angiogenic and immunosuppressive, indicating a less tumor-supportive microenvironment. These results demonstrate that NCL-targeted liposomes enhance tumor-specific delivery of Dexa and selectively modulate tumor-associated monocytes, highlighting their potential as a strategy for targeted immunomodulatory therapy in cancer.
This work was funded by CNCS/CCCDI–UEFISCDI, project COFUND-ERA4HEALTH-NANOTECMEC-SAIL (contract no. 92/2025).

Donor baseline transcriptomic state shapes dendritic cell reprogramming across ovarian tumor immune subtypes

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Background: Dendritic cells (DCs) integrate environmental signals to initiate adaptive immunity and provide a controlled system to study responses to tumor-derived factors. Ovarian tumors are transcriptionally classified into immune-desert (D), immune-excluded (E), and immune-inflamed (I) subtypes, reflecting distinct microenvironmental states. Although inter-individual variability in dendritic cell responses is well recognized, the relative impact of donor variability and tumor immune subtype in human DC–tumor lysate models remains insufficiently quantified.
Methods: Human monocyte-derived DCs were generated from PBMCs of 9 healthy female donors and stimulated for 32 hours with ovarian tumor lysates representing D, E, and I subtypes. Surface maturation was assessed by flow cytometry, and transcriptomic profiling was performed using BRB-seq with downstream differential and pathway-level analyses. Donor-specific response magnitude was quantified using an L2-based metric derived from pathway activity changes, enabling stratification into low-, intermediate-, and high-programmability groups.
Results: All tumor lysates induced a mature dendritic cell phenotype with high CD80, CD86, and CD83 expression compared to immature controls (ANOVA $p \leq 0.03$). Transcriptomic profiling showed consistent activation of inflammatory pathways across desert, excluded, and inflamed conditions, including TNFA_SIGNALING_VIA_NFKB, interferon responses, and IL6_JAK_STAT3_SIGNALING (adj. $p<0.001$). Subtype-specific differences were modest, and the overall inflammatory activation trajectory remained conserved. In contrast, donor identity explained most transcriptomic variance ($R^2=0.58$, $p=0.001$), whereas lysate subtype accounted for only 4% ($R^2=0.04$, not significant). Baseline pathway activity correlated with response magnitude, linking higher programmability to immune and metabolic pathway enrichment.
Conclusions: Across ovarian tumor immune subtypes, dendritic cell activation was largely conserved, whereas response magnitude was mainly associated with donor baseline transcriptomic states, indicating contributions from both tumor signals and donor-specific factors.

Dietary Prebiotic Intervention Modulates High-Fat Diet-Associated Microglial Morphological Alterations in Aged Mice

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Consumption of a chronic high-fat diet (HFD) and ageing are both associated with metabolic dysfunction and increased neuroinflammation. It contributes to the loss of gut microbiota diversity and the development of neurodegenerative diseases. Microglia are highly sensitive to inflammatory signals and their morphology is closely linked to its functional state. These molecular signals can derive from gut microbiota and influence microglia activation and disease progression. Gut microbiota-focused interventions have the potential to reduce the chronic inflammation and have a protective effect on microglia.

This study investigated how prebiotic supplementation with galacto-oligosaccharides and fructooligosaccharides (GOS+FOS) modulates microglial morphology in aged mice exposed to long-term HFD. C57BL/6J mice were assigned to four dietary conditions for 18 months: control diet (CD), CD - GOS+FOS, HFD, and HFD - GOS+FOS. We utilized immunohistochemical staining to quantify three-dimensional branching architecture and two-dimensional shape descriptors.

Compared with CD, microglia in HFD-fed conditions exhibited quantifiable morphological changes consistent with a metabolically primed microglia phenotype. HFD supplementation with GOS+FOS reduced those HFD-induced structural alterations: average branch length decreased toward CD levels, projected soma area was significantly smaller than in HFD, indicating partial structural recovery. In the CD - GOS+FOS group, microglial morphology were similar to CD group, indicating that the effects of GOS+FOS are specific to reducing HFD-associated microglial alterations rather than modifying baseline microglial structure.

These findings suggest that prebiotic supplementation with GOS+FOS can modulate microglial structural remodeling in the aged brain under metabolic challenge, highlighting a protective role of gut microbiota-targeted interventions in diet-induced neuroinflammatory conditions.

Innate Immune Responses to Acinetobacter baumannii: Modulation of Neutrophil Migration and NETosis

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Acinetobacter baumannii is a healthcare-associated opportunistic pathogen of increasing concern due to multidrug resistance. Carbapenem-resistant strains are classified as critical priority pathogens by the WHO, highlighting the need for improved understanding of host-pathogen interactions. Neutrophils are among the first innate immune cells recruited to infection sites, where their activation is essential for antimicrobial defence. In addition to phagocytosis and degranulation, neutrophils release neutrophil extracellular traps (NETs) that immobilize pathogens. In this study, we investigated how surface structures of *A. baumannii*, including OmpA, lipooligosaccharides, capsular polysaccharides and outer membrane vesicles (OMVs), influence neutrophil migration and NETosis.

Human neutrophils were isolated by density gradient centrifugation and infected with a clinical isolate and mutant strains lacking key virulence-associated surface components. OMVs from these strains were isolated by ultracentrifugation and used to induce NET formation. Confocal fluorescence microscopy and image analysis were used to quantify NETosis and neutrophil viability, while migration assays assessed neutrophil recruitment.

Results demonstrated that *A. baumannii* induces NETosis, with strain-dependent differences in NET formation and neutrophil viability. Neutrophil migration was enhanced toward capsule-producing strains compared to capsule-deficient mutants.

These findings indicate that *A. baumannii* surface-associated virulence factors regulate neutrophil migration and NETosis, highlighting their role in shaping innate immune responses.

Immune Signatures in Unexplained Infertility: A Precision Medicine Approach Using Peripheral Blood Cytometry

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Introduction: Unexplained infertility remains a major clinical challenge, and accumulating evidence suggests that systemic immune imbalance—particularly within innate immune compartments and their interaction with adaptive immunity—may contribute to impaired reproductive success. In a precision-medicine context, standardized peripheral blood immunophenotyping may enable immune endotyping and patient stratification through clinically translatable immune signatures.

Methods: Peripheral blood was collected from 42 women with unexplained infertility and 10 fertile controls. Multiparametric flow cytometry was used to quantify key innate and adaptive immune populations, with emphasis on innate immunity–relevant compartments: $CD14^+$ monocytes and NK cells, alongside $CD19^+$ B cells and $CD3^+$ T cells ($CD8^+$ cytotoxic and $CD4^+$ helper subsets). Immune parameters were compared between groups to assess candidate stratification signatures.

Results: The workflow enabled robust enumeration of circulating leukocyte lineages across innate and adaptive immunity, including $CD4^+$ and $CD8^+$ T cells, B cells, $CD14^+$ monocytes, NK/NKT-like subsets, and granulocyte (neutrophil-/eosinophil-enriched) fractions.

Conclusion: Multiparametric flow cytometry–based peripheral blood immunophenotyping provides a scalable, clinically translatable approach to identify immune signatures in unexplained infertility, with particular focus on innate immune compartments (monocytes and NK/NKT-like, and granulocyte) integrated with adaptive immune polarization. These findings motivate validation in larger cohorts and linkage to clinical and reproductive outcomes to evaluate utility for precision diagnostics, risk stratification, and treatment monitoring.

miR-146a-5p Links Innate Immune Signaling to Mitochondrial and Metabolic Regulation in Neural Cells

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MicroRNA miR-146a plays a key role in regulating innate immunity by targeting adaptors IRAK1 and TRAF6, and thereby reducing the production of pro-inflammatory cytokines (Gilyazova et al., 2023). Although its role in immune cells has been thoroughly validated, its functional impact in neural cells remains poorly understood.

In this study, we investigated the molecular and metabolic/ bioenergetic changes in SH-SY5Y neuro-differentiated cells following the suppression of microRNA miR-146a-5p. For miR-146a-5p inhibition, transfection of mirVana inhibitor with Lipofectamine RNAi/MAX was used. The efficacy of inhibition was confirmed using RT-qPCR, which demonstrated the upregulation of miR-146a-5p target genes: IRAK1, NOTCH2, SMAD2, and TRAF6.

To further evaluate the impact of miR-146a-5p inhibition, we conducted mRNA-seq analysis. It showed significant downregulation of 86 mRNAs and upregulation of 42 mRNAs. Notably, many of the downregulated genes were associated with the modulation of mitochondrial protein translation, such as MT-TC, MT-TH, and MRPL23. Additionally, we observed a significant reduction—approximately 2.3-fold—in the expression of the hypoxia-inducible factor 3 alpha (HIF3A) gene. Seahorse Mito Stress test also demonstrated the role of miR-146a-5p in cellular metabolic regulation. These data suggest that miR-146a-5p may influence mitochondrial dynamics and metabolic pathways in neural cells, supporting a broader, context-dependent role outside classical innate immune regulation.

Reference: *Gilyazova I, Asadullina D, Kagirova E, Sikka R, Mustafin A, Ivanova E, Bakhtiyarova K, Gilyazova G, Gupta S, Khusnutdinova E, Gupta H, Pavlov V. MiRNA-146a-A Key Player in Immunity and Diseases. Int J Mol Sci. 2023 Aug 14;24(16):12767. doi: 10.3390/ijms241612767*.

Nonclassical Monocyte Depletion as an Indicator of Disturbed Innate Immune Response in Sarcoidosis and COVID-19

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Nonclassical monocytes ($CD14^{+}CD16^{++}$) are key regulators of vascular homeostasis and the repair phase of inflammation. Their depletion may indicate dysregulated innate immune activation, particularly when the transition from inflammation to repair phase is impaired. Comparative data on this subset in chronic granulomatous inflammation, acute SARS-CoV-2 infection, and the post-infectious period remain limited.
To evaluate and compare the proportion of nonclassical monocytes in healthy individuals, patients with sarcoidosis (SA), individuals with acute COVID-19, and convalescents, with emphasis on disturbances in resolution-phase immunity.
Peripheral blood from 153 participants was analyzed by flow cytometry. Monocyte subsets were defined by CD14 and CD16 expression, and the percentage of nonclassical monocytes among total monocytes was quantified. Group differences were assessed using non-parametric statistical tests.
Nonclassical monocytes were significantly reduced in SA, acute COVID-19, and convalescent individuals compared with healthy controls. SA showed a moderate decrease (median=5.5±0.9%). In acute COVID-19, nonclassical monocytes were nearly absent (0.9±0.5%). Convalescents continued to exhibit markedly reduced levels (2.2±1,3%), which did not return to values observed in healthy subjects (15.1±4,5%). SA patients also displayed an increased proportion of intermediate monocytes (8.7% vs. 5.2% in controls).
Nonclassical monocytes constitute a highly sensitive subset reflecting the integrity of resolution-phase innate immunity. The gradient of depletion—from moderate in SA to profound in acute COVID-19—indicates substantial disruption of innate immune homeostasis. Persistently low levels in convalescents suggest incomplete immunological recovery after SARS-CoV-2 infection and support the potential use of nonclassical monocytes as biomarkers of impaired restoration dynamics.

IFN-β and ACKR2 Modulate Tissue Fibrosis and Corresponding Macrophage Subsets in early CCl_4-Induced Liver Injury

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Chronic liver injury drives sustained inflammation and progressive fibrosis, processes shaped by dynamic macrophage remodeling. While macrophage heterogeneity during fibrogenesis has been extensively characterized, the molecular and functional features accompanying fibrosis resolution remain incompletely defined. We examined the impact of IFN-β and/or ACKR2 deficiency on liver fibrosis and the expression of ST2 (IL-33 receptor) and CD107a on hepatic macrophages during recovery from early carbon tetrachloride (CCl_4) induced liver fibrosis and assessed their relationship to inflammatory and fibrogenic signaling. Wild-type, $Ifnb^{-/-}$, $Ackr2^{-/-}$, and $Ackr2^{-/-}Ifnb^{-/-}$ mice were subjected to repeated CCl_4 administration for 2 weeks. Forty-eight hours after the final injection, liver tissue was analyzed by histological staining, multiparameter flow cytometry, quantitative PCR, and ELISA.

Our results showed a progressive tissue fibrosis in $Ackr2^{-/-}$ or $Ifnb^{-/-}$ livers that was exacerbated in the double knockout ones, in comparison to the WT pathology. Resolution was characterized by a progressive shift from $Ly6C^{hi}$ inflammatory macrophages toward $Ly6C^{lo}CX3CR1^{hi}$ phenotypes. ST2 expression was enriched within late-stage $CD11b^{+}F4/80^{hi}$ macrophages, consistent with emergence of a resolution-associated population. CD107a expression was detected within these subsets, suggesting functional remodeling during maturation. Transcriptional analysis revealed modulation of Il1rl1 (ST2), Il33, and fibrosis-associated genes (including Acta2, Col1a1, and Timp1) at the resolution time point, while cytokine profiling demonstrated concurrent inflammatory and counter-regulatory responses. Combined deficiency of IFN-β and ACKR2 altered cytokine output and disrupted ST2 and CD107a expression patterns, indicating coordinated regulation of macrophage phenotype and function. These findings suggest that ST2 and CD107a expression accompany macrophage maturation during fibrosis regression and may reflect functional adaptation influenced by IFN-β and ACKR2-dependent pathways.

Dissecting Platelet-Activating Factor Signalling in Microbial Keratitis Using a Zebrafish Corneal Injury Model

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Microbial keratitis (MK), an infection of the cornea, is a leading cause of blindness worldwide, affecting 1.5–2 million people annually, predominantly working-age adults. Platelet-activating factor (PAF) is a powerful pro-inflammatory mediator but its role in MK remains unclear. Using a zebrafish larval corneal injury model that we have recently developed, we investigated PAF signalling using pharmacological and genetic approaches. Exogenous PAF increased neutrophil and macrophage recruitment, an effect reversed by the PAF receptor antagonist WEB 2086. CRISPR–Cas9 knockdown of the PAF receptor reduced immune cell recruitment and was not restored by exogenous PAF, confirming that the observed effect is mediated through the PAF receptor. To explore endogenous PAF biosynthesis in response to inflammatory stimuli, knockdown of lysophosphatidylcholine acyltransferase (LPCAT) isoforms 1 and 2 was performed. Only LPCAT2 knockdown attenuated immune cell recruitment, supported by dose-dependent inhibition using TSI-01, an LPCAT2 inhibitor. These findings identify PAF as a key mediator of inflammation in MK and highlight it as a potential therapeutic target.

Accumulation of small peritoneal macrophages and dendritic cells during tumor rejection in a concomitant tumor immunity model

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Background: Tumor-associated macrophages may have opposite effects on tumor growth, but their role in the antitumor immunity is not fully understood. In the DBA/2–SL2 concomitant tumor immunity model, secondary tumor growth is suppressed, indicating an important role for local immune responses. This study examined the contribution of peritoneal macrophages and other immune cell populations to tumor control.
Methods: A DBA/2–SL2 concomitant tumor model was used, combining subcutaneous (SC) and intraperitoneal (IP) tumor challenges. Peritoneal immune cells were analyzed by flow cytometry and macrophage function was examined using adoptive transfer and repeated intraperitoneal stimulation.
Results: Secondary IP SL2 tumor rejection was associated with increased numbers of small peritoneal macrophages (SPM) and dendritic cells (DCs) in the peritoneal cavity, whereas primary IP tumors showed tumor cell proliferation. Tumor growth was associated with a decrease in the proportion of myeloid cells and B lymphocytes. Adoptive transfer of peritoneal macrophages alone did not prevent tumor growth, indicating that macrophages alone are unable to control tumor progression. Repeated IP injections of SL2 cells caused regression of 3 out of 5 SC tumors, suggesting a possible systemic immune antitumor effect.
Conclusion: The rejection of secondary IP SL2 tumors in DBA/2 mice was associated with a distinct peritoneal immune environment characterized by increased levels of SPM and DC. Adoptive transfer showed that macrophages alone could not control tumor growth, but repeated IP tumor challenge increased antitumor immune activity, indicating that effective tumor control requires cooperation between different immune cells.

DAY 2 (Tuesday, May 12, 2026)

Session 2
Innate Immunity in Autoimmunity

SBL Mini-symposium 1
Neutrophils in Innate Immunity

IRA Mini-symposium
Therapeutic Targets in Acute and Chronic Inflammatory Memory

Session 3
Innate Immunity and metabolism

Plenary session 3
Innate immune development

Flash talks

Type 1 IFN autoantibodies in autoimmunity

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Autoimmune regulator (Aire) controls the thymic expression of type 1 interferons (T1 IFNs). Patients with Aire mutations often develop neutralizing autoantibodies to interferon-alpha (IFNα), a finding also seen in Aire-deficient rats. In this model, T1 IFN signaling declines early, before autoantibody formation. Once anti-IFNα autoantibodies appear, interferon-stimulated genes are broadly reduced. These autoantibodies are also linked to lower tissue inflammation, suggesting a protective role. Overall, Aire regulation of thymic T1 IFNs is tied to the development of anti-IFNα autoantibodies and reduced autoimmune disease.

From defense to dysregulation: The pleiotropic role of neutrophils in autoimmunity

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Neutrophils, traditionally viewed as short-lived first responders in innate immunity, play a far more complex and pleiotropic role in autoimmunity. While their primary function is host defense through phagocytosis, degranulation, and the release of reactive oxygen species, emerging evidence shows that dysregulated neutrophil activity can contribute to the initiation and progression of autoimmune diseases.
A key mechanism of neutrophils activity involves the formation of extracellular traps (NETs), which can expose intracellular antigens to the adaptive immune system, potentially breaking self-tolerance and promoting autoantibody production. Neutrophils also interact with other immune cells, such as dendritic cells and lymphocytes, shaping adaptive immune responses and amplifying autoimmune pathways.
In autoimmune diseases, neutrophils exhibit altered phenotypes, including increased longevity, enhanced activation, and abnormal signaling. These changes contribute to chronic inflammation and organ damage. Furthermore, subsets of neutrophils with immunosuppressive or proinflammatory properties highlight their functional diversity.
Taking into consideration that neutrophils represent a critical link between innate and adaptive immunity, understanding their dual role - from protective defenders to drivers of dysregulation - offers new insights into autoimmune pathogenesis and may guide the development of novel targeted therapeutic strategies.

Spatially-resolved functional maps of synovial macrophages and structural cells in remission and arthritis

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Rheumatoid arthritis (RA) is a global health burden, characterised by non-resolving chronic or flaring inflammation. Current therapies can reduce inflammation, but fail to establish drug-free remission. Thus, patients stay on lifelong treatment with immunosuppressive and anti-inflammatory drugs. Single-cell omics studies suggest that tissue-resident pro-resolving macrophages drive synovial tissue homeostasis and remission maintenance in RA. However, the functions of distinct macrophage subsets, their synovial tissue niches, and their interactions with structural cells remain unresolved.

We built a multi-omics synovial tissue resource in remission and active RA and characterised the heterogeneity of macrophage and structural cell subtypes across synovial joint niches at single-cell and spatial resolution. We defined macrophage and structural cell phenotypes and functions, localisations to distinct synovial tissue niches, and identified interacting cell partners.

The study enrolled 55 patients with RA or osteoarthritis who donated synovial fluid and synovial tissue, derived from ultrasound-guided synovial biopsy or joint replacement surgery. Synovial fluid and tissue cells were isolated from fresh tissue using our optimised tissue-dissociation protocol, analysed using multi-omics approaches, including CITE-sequencing, single-cell RNA sequencing, and multispectral flow cytometry. To explore cell-cell interactions and tissue architecture, we analysed histology and conducted spatial omics analyses on formalin-fixed, paraffin-embedded (FFPE) synovial tissue biopsies.

We generated a single-cell multi-omics map of the human synovial joint in RA remission, active DMARD-naive, and DMARD-inadequately responding RA. We identified uniquely enriched immune and structural cell subtypes in specific synovial niches across arthritis activity states and inferred their pro-inflammatory or tissue-protective functions and cellular interactions.

Our data uncovered extensive synovial cell complexity, serving as a reference for understanding RA pathobiology. By linking macrophage subsets to tissue-protective functions and tissue niches, we established a strong foundation for drug discovery.

Targeting Extracellular Traps with CIT-013: Linking ET Inhibition, Immune Cell Reprogramming, and Therapeutic Potential in Rheumatoid Arthritis

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Extracellular traps (ETs), formed by neutrophils and other immune cells, are increasingly recognized as central players of chronic inflammation. Local ET accumulation can lead to tissue damage, inflammation and autoimmunity, as is the case in rheumatoid arthritis (RA). This study assesses an anti-citrullinated histone antibody (murine mACHA or human CIT-013) that targets the citrullinated histones H2A and H4 (citH2a/4) component of extracellular traps.

Therapeutic dosing of mACHA to mice with collagen-induced arthritis (CIA) significantly halts joint damage. Furthermore, radiolabeled mACHA shows selective distribution to the inflamed joints of CIA mice proportional to inflammatory burden. In a human model of transient inflammation, challenging healthy volunteers with low-dose lipopolysaccharide, ET formation is rapidly induced. This is significantly inhibited by CIT-013. In RA patients, ET components and citH2a/4 were detected in synovial tissue and serum. During a phase 1 trial, CIT-013 administration to RA patient volunteers associated with clinically meaningful decreases in disease activity and reduction in inflammatory biomarkers.

In mechanistic in vitro studies, a dual mode of action is observed: (1) CIT-013 binds ETs at the terminal stage of trap formation, preventing spreading of ETs, and (2) CIT-013 opsonizes ETs, promoting Fc-dependent clearance by macrophages. This alters the macrophages toward a less inflammatory phenotype.

Collectively, our data establish targeting citH2a/4 within ETs as novel therapeutic approach, with CIT-013 suppressing inflammation and tissue damage. Ongoing phase 2a proof-of-concept studies in RA and hidradenitis suppurativa will further define the clinical relevance of ET targeting as a disease-modifying strategy.

Targeting the cytosolic scaffold of PCNA (Proliferating Cell Nuclear Antigen) in neutrophils to promote the resolution of inflammation

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Neutrophils, also known as polymorphonuclear leukocytes (PMNs), are key effectors of innate immunity. Their lifespan is tightly controlled by a constitutive apoptosis program regulated by dynamic changes in gene expression that balance pro- and antiapoptotic factors. This process is of crucial importance for the resolution of inflammation. Importantly, cytokines and growth factors such as CXCL8 and granulocyte colony-stimulating factor (G-CSF) can delay apoptosis, thereby preserving neutrophil viability during migration and at sites of infection, ensuring optimal antimicrobial activity and efficient pathogen clearance.
Proliferating cell nuclear antigen (PCNA), originally characterized as a nuclear factor involved in DNA replication and repair, has been identified as a key regulator of neutrophil survival by our group (1). During late stages of neutrophil differentiation, PCNA accumulates in the cytosol of mature cells and acts as an antiapoptotic scaffold by binding procaspase-9 and procaspase-3, preventing their activation and thereby prolonging cell lifespan. During apoptosis, PCNA is degraded, allowing caspase activation to proceed. Its stabilization contributes to the prosurvival effects of G-CSF. Structurally, PCNA is a homotrimeric protein which functions as a versatile molecular scaffold. It interacts with a wide range of binding partners, including procaspases and components of the NADPH oxidase complex such as p47phox (2), thereby influencing both apoptosis and antimicrobial functions.
Using proteomic and biochemical approaches, we have identified PCNA as a regulator of glycolysis in neutrophils in response to G-CSF (3). PCNA upregulation is associated with reduced interaction with key glycolytic enzymes. This dissociation leads to increased glycolytic flux and lactate production, indicating that PCNA functions as a negative regulator of glycolysis under resting conditions. Disruption of PCNA interactions using p21-derived peptides reproduces these effects, supporting a model in which PCNA coordinates metabolic reprogramming and survival pathways in activated neutrophils. These observations further support the concept that metabolism, apoptosis, and lifespan are tightly interconnected in neutrophils.
Increased PCNA expression has been observed in inflammatory and infectious settings as illustrated in our studies in severe COVID-19;

Elevated cytosolic levels of PCNA in neutrophils were correlated with increased reactive oxygen species (ROS) production and neutrophil extracellular trap (NET) formation (4). Indeed, PCNA can be associated with PAD4 and histone-3 which have been implicated in NET formation. Pharmacological inhibition of PCNA using the small molecule T2AA suppresses NADPH oxidase activation. Mechanistically, PCNA interacts with the S100A8/S100A9 (calprotectin) complex, regulating neutrophil activation. Disruption of this interaction reduces ROS production and inflammation. In vivo, PCNA inhibition attenuates lung inflammation and promotes resolution of inflammation.

Overall, PCNA emerges as a multifunctional regulator at the crossroads of neutrophil survival, metabolism, and activation. Its broad interactome and capacity to integrate diverse signaling pathways underscore its central role in immune regulation. However, the mechanisms governing binding specificity remain poorly understood, particularly given the wide range of post-translational modifications that modulate PCNA interactions. New studies aimed at deciphering these regulatory networks will be presented to understand PCNA function and to exploit its potential as a therapeutic target in inflammatory and infectious diseases.

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Innate immune exhaustion and rejuvenation in the treatment of disease

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Emerging studies reveal a novel role of innate immune memory during the pathogenesis and treatment of diverse inflammatory diseases. However, the fundamental principles that underlie the generation of innate immune memory are not well understood, thus hindering the effective development of innate-based therapeutics. We have defined the signal-strength and history dependent memory adaptation of innate immune cells including monocytes and neutrophils in both murine and human systems. Analogous to T cell exhaustion which can be induced by repetitive challenges, we demonstrate that innate monocytes can develop an exhaustion memory following repetitive innate challenges. Innate monocyte exhaustion memory can be stably maintained in vitro, ex vivo and in vivo, with epigenetic rewiring of genome DNA methylation. Key signatures of monocyte exhaustion includes a) reduced differentiation; b) pathogenic inflammation, and c) immune suppression, represented by key cell surface markers of reduced CD163, elevated CD38/PD-L1 and reduced CD86. Mechanistically, we defined that the innate membrane adaptor TRAM is required for the establishment of inflammatory and/or exhausted innate memory leukocytes, and that the deletion of TRAM can effectively reprogram innate immune cells into a novel resolving phenotype. Our pharmacological approach independently reveals that the generation of resolving innate immune cells conducive for the treatment of both acute sepsis as well as chronic inflammatory atherosclerosis. Taken together, our studies reveal novel principles of innate memory dynamics, and therapeutic strategies in rejuvenating innate immunity for the treatment of disease.

Targeting eNAMPT/TLR4 signaling to modulate persistent innate immunity dysregulation in the lung

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Persistent dysregulation of innate immune signaling is a central driver of lung injury, fibrosis, and poor clinical outcomes in acute and chronic pulmonary diseases. Among emerging mediators, extracellular nicotinamide phosphoribosyltransferase (eNAMPT) has been identified as a potent damage-associated molecular pattern (DAMP) that amplifies inflammatory responses through activation of Toll-like receptor 4 (TLR4). Elevated circulating eNAMPT levels are strongly associated with disease severity and mortality in conditions such as acute respiratory distress syndrome (ARDS) and progressive pulmonary fibrosis.

This presentation will highlight recent advances in understanding the mechanistic role of eNAMPT/TLR4 signaling in sustaining maladaptive innate immune activation and in perpetuating immune exhaustion. We will review translational and preclinical studies demonstrating that targeting this pathway attenuates inflammatory injury, mitigates fibrotic remodeling and immune exhaustion.

Therapeutic strategies employing eNAMPT-neutralizing antibodies have shown promise in restoring immune homeostasis and improving outcomes in relevant preclinical models. Modulation of eNAMPT/TLR4 signaling represents a novel and potentially transformative approach to improve patient outcomes in high-morbidity conditions with persistent innate immune dysregulation.

The LXR pathway in immune regulation: novel insights from the LXR-CD38 axis

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The liver X receptors (LXRs) are oxysterol-sensing transcription factors that coordinate metabolic and inflammatory programs across immune cells. Our group identified CD38 as a target gene of the LXR pathway, establishing an axis that links lipid metabolism to immune signaling. CD38 is a particularly versatile mediator: depending on the cellular context, it can promote either inflammatory or anti-inflammatory responses, and its coexpression with other ectoenzymes involved in adenosine synthesis further shapes the extracellular metabolic landscape. In this talk, I will discuss how LXR-dependent regulation of CD38 and associated ectoenzyme pathways influences macrophage function and modulates inflammatory responses, with a particular emphasis on the effects observed in the tumor microenvironment.

Immune stimulation can change the phenotype and function of Hematopoietic Stem Cells

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Hematopoietic Stem Cells (HSCs) are the source of blood and immune cells. In healthy life, HSCs are mostly quiescent in the bone marrow. Following inflammation, HSCs can become activated, presumably accelerating the generation of the necessary blood and immune cells. Surprisingly, however, our knowledge regarding the ability of various pathogens to perturb HSCs is minimal. Moreover, the early- and late-effects of such activation are only beginning to be revealed.

We had first revealed surface markers of immune-activated HSCs. Improved identification of activated HSCs provides molecular insights and a better ability to dynamically track the process. We recently found that HSC activation is much faster and broader than previously thought, evident as early as 2 hours after either LPS or pIpC stimulation in vivo. The response is systemic, highly sensitive, and dose-dependent, even at surprisingly low levels of stimulant. Interestingly, other recent studies challenge the concept of HSCs` contribution to emergency hematopoiesis, raising further questions.

Chronic activation may deplete HSCs` potency and increase the risk of malignancies. We find an extraordinary impact following prolonged bacterial infection in mice, and an even more exciting ability for recovery following pathogen clearance. Single-cell analysis provides novel insights into molecular states of naïve, potent-less, and recovered HSCs.

Changes in hematopoietic concepts are fundamental for the generation of new immune cells in health and disease. Maintaining balanced potency is essential for a longer, healthier life.

Neutrophils and NETs induce a pro-inflammatory and matrix-degrading phenotype in cardiac fibroblasts

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Introduction: Following myocardial infarction (MI), cardiac fibroblasts (CFs) undergo phenotypic transitions essential for tissue repair. While leukocytes orchestrate post-MI healing, excessive neutrophil infiltration and neutrophil extracellular trap (NETs) formation have been associated with adverse cardiac remodeling. In this context, we investigated how neutrophils and NETs modulate CFs activation and the underlying signaling pathways.
Methods: Human and mouse CFs were cultured under 3D conditions. CFs were indirectly co-cultured with inflammatory neutrophils using a Transwell system or exposed to NETs generated from PMA-stimulated neutrophils. After 24 h, inflammatory and remodeling markers were assessed in fibroblasts and conditioned media. Gene and protein expression were analyzed by qPCR, immunofluorescence, and multiplex immunoassays. Functional changes were evaluated using xCELLigence assays and wound healing migration assays.
Results: Indirect co-culture of CFs with inflammatory neutrophils induced a pro-inflammatory and matrix-degrading phenotype to CFs, characterized by increased expression of IL-1β, IL-6, MCP-1, MMP-3, and MMP-9. These effects were associated with activation of p38 MAPK and NF-κB signaling pathways. Moreover, fibroblast proliferation was significantly impaired upon exposure to factors released by neutrophils. Similarly, NET treatment triggered upregulation of inflammatory molecules, and DNase-I pre-treatment of NETs attenuated this response. Functionally, NETs reduced proliferation while enhancing the CFs migratory capacity.
Conclusion: Our results indicate that early in inflammation, factors released by neutrophils and NETs activate cardiac fibroblasts, triggering a pro-inflammatory and anti-proliferative phenotype with enhanced invasion capacity, highlighting their role in driving fibrotic remodeling.
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CD5L Inhibition Drives Metabolic and Immune Reprogramming of Human Macrophages

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Tumor-associated macrophages (TAMs) are the most abundant immune cells within the tumor microenvironment, comprising ~30–50% of infiltrating leukocytes. High TAM infiltration is associated with poor prognosis across multiple cancer types, as these cells can promote immunosuppression and tumor progression. CD5L is a protein expressed in macrophages that drives their immunosuppressive phenotype. To develop a novel immunotherapy targeting TAMs, we generated a monoclonal antibody (RImAb) against CD5L, aiming to block its activity. RImAb reprogrammed immune-suppressive macrophages in vitro in human primary blood -derived macrophages and in vivo, in a mouse model of lung cancer.

To investigate RImAb´s mechanism of action, peripheral blood monocytes from five healthy donors (n= 5) were differentiated with MCSF and IL-10 in the presence of RImAb or isotype control. Transcriptomic changes were assessed by mRNA-sequencing and subsequently validated through functional assays.

Differential expression analysis comparing RImAb vs isotype control revealed modulation of genes involved in lymphocyte function and metabolic pathways, consistent with RImAb reprogramming activity. These findings were functionally validated using a three-dimensional co-culture model comprising THP1-derived macrophages or THP1 macrophages transduced to overexpress CD5L, MRC-5 fibroblasts and liver (Huh7) or lung (H23) cancer cells. Fluorescence microscopy coupled to image analysis, qPCR, and flow cytometry-based viability assays showed phenotypic changes consistent with the transcriptomic signatures. Additionally, T-cell co-culture assays using CFSE-based flow cytometry showed that RImAb treatment restores macrophage immunostimulatory function thereby enhancing T-cell proliferation, indicating reversal of the immunosuppressive phenotype.

Collectively, these results support CD5L blockade as a promising strategy to reprogram TAMs and improve anti-tumor immunity.

Transcriptional control of the macrophage repair program

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Macrophages are key players in the response to tissue damage and are involved in all stages of the repair process. Despite its fundamental importance and its conservation across repair contexts, how the macrophage post-injury response is coordinated at the transcriptional level remains poorly understood. I will present our latest work identifying a transcription factor controlling the macrophage repair program in both skin wound healing and skeletal muscle regeneration.

Flow Cytometric Comparison of Different Human Myeloid Cell Enrichment Strategies

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Peripheral blood mononuclear cells (PBMCs) isolated via Ficoll density gradient centrifugation are widely utilized across immunological research, including cell-based therapeutic applications. PBMCs comprise T and B lymphocytes, NK cells, and monocytes in ratios that vary significantly between donors. The choice of enrichment method for specific cell populations can profoundly influence final yield, purity, and the potential for unintended cellular activation.

As part of a broader myeloid differentiation study, this research evaluates six isolation methods (plastic adherence, cold aggregation, density gradient centrifugation, immunomagnetic positive and negative selection, suspension-based cell clumping, and a total PBMC control) to determine the optimal balance between myeloid enrichment, final cellular yield, and phenotypic integrity as evaluated by flow cytometry.

Our preliminary results suggest that physical selection methods provide cost-efficient and higher yields but at the expense of purity while immunomagnetic selection assays result in higher purity but lower absolute yields and an increased potential for myeloid cell activation.

Association between the IFN-γ/IL-4 ratio in saliva and self-reported health status and factors affecting it in patients with Sjogren's disease

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The aim of the study was to evaluate association between IFN-γ/IL-4 ratio in saliva and the self-reported health status and factors affecting it in patients with Sjogren's disease (SD).
Patients and methods: The study included 41 patients with SD, with a median age of 53 years. IFN-γ and IL-4 concentrations in saliva were determined by ELISA. The information about health was provided by the patients themselves.
Results: 65.9% patients had extraglandular onset. Stress and infection were the most commonly reported triggering factors. Median IFN-γ/IL-4 ratio was significantly higher in patients reporting infection as a triggering factor compared with an unknown cause. Regarding the IFN-γ/IL-4 ratio, significant associations were found with time from symptom onset to confirmed diagnosis, indicating that each additional year was associated with an increase of 10.60 units in the IFN-γ/IL-4 ratio. GERD presence, which was associated with an average increase of 49.73 units in the ratio and sialometry, indicating that each additional 1 mL/5 min increase in salivary flow was associated with an increase of 51.58 units in the IFN-γ/IL-4 ratio. The study revealed that age was positively associated with the ESSPRI score. Age remained an independent predictor of ESSPRI score, indicating that for each additional year of age, the ESSPRI score increased by approximately 0.40 units.
Conclusions:
1. IFN-γ/IL-4 ratio is higher in patients with SD reporting infection as a triggering factor.
2. IFN-γ/IL-4 ratio is associated with time from symptom onset to confirmed diagnosis in SD patients.
3. A higher IFN-γ/IL-4 ratio ratio indicates a risk of having GERD in SD patients.

Characterization and application of monoclonal antibodies to human calnexin

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Calnexin (CNX) is a type I integral endoplasmic reticulum (ER) membrane chaperone involved in glycoprotein folding and quality control. In addition to its established role in protein folding, recent studies suggest that CNX participates in the regulation of ER stress, autophagy, and immune signaling pathways. Changes in CNX expression have been observed in cancer, inflammatory, and neurodegenerative diseases. Therefore, CNX could be a potential biomarker in diseases associated with ER stress and immune dysregulation. Monoclonal antibodies (mAbs) are widely used biotechnological tools for the detection and functional analysis of various proteins. The aim of this study was to characterize mAbs developed against human CNX and to evaluate their specificity, affinity, and suitability for quantitative detection. A set of mAbs was purified and tested for affinity and specificity using ELISA and Western blot. The majority of mAbs demonstrated high affinity for CNX, with no cross-reactivity to the related chaperone calreticulin. Most mAbs recognized native CNX in human cell lysates by Western blot and in fixed cells by immunofluorescence, and several were suitable for immunoprecipitation. Optimal antibody pairs were identified, test conditions were optimized for the development of a sandwich ELISA for CNX quantification. The developed assay enables quantitative detection of CNX in cell lysates and provides a tool for further studies of the role of CNX in ER stress and disease pathogenesis.

Plasma concentrations of short-chain fatty acids (SCFAs) in women with endometriosis

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Endometriosis is a significant gynecological disease characterized by the presence of endometrial cells at ectopic sites outside the uterine cavity. Its clinical manifestations include intermenstrual bleeding, heavy menstrual bleeding, severe pelvic pain, infertility, and other related symptoms. SCFAs are metabolites of gut bacteria that reach organs via the bloodstream, and playing a major role in the interaction between the microbiota and human health; altered microbiota and SCFA may reflect various pathological conditions.

The study included plasma samples from 80 patients with endometriosis with different stages of diseases according to the ASRM classification (I, II, III and IV) and 30 control women. The study was approved by the Institutional Bioethics Committee (approval no. KB-18/2025). Plasma samples were processed for SCFAs extraction, derivatized, and analyzed by high-performance liquid chromatography (HPLC) using a C18 column with UV detection at 400 nm and statistical analyses were conducted with GraphPad Prism 10. Analysis revealed significantly higher plasma concentrations of acetic, propionic, and butyric acids (nmol SCFA compound/µL plasma) in patients with endometriosis compared to the control group. Acetic acid showed the most pronounced increase in the early stages of the disease (I and II; $p<0.0001$), suggesting its potential utility in early diagnostics. Propionic acid reached its highest concentrations in the most advanced stage (IV; $p=0.001$), which may correlate with increased inflammation. Butyric acid displayed a consistent upward trend with disease progression, reaching peak values in stages III and IV ($p=0.010$) for the combined group).

Elevated systemic SCFA levels in women with endometriosis may indicate intestinal barrier dysfunction and altered microbial metabolism, while also reflecting chronic inflammation, as SCFAs actively modulate immune system function and can both influence and be influenced by inflammatory processes.

Development and validation of sensitive immunoassays to quantify human serum C-reactive protein

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C-reactive protein (CRP) is an acute-phase protein, an inflammatory marker mainly synthesized in the liver. CRP levels in the blood reflect acute or chronic inflammation. Elevated CRP levels are commonly associated with chronic inflammatory diseases. However, sensitive CRP quantification is associated with a significant indication in cardiovascular disease (CVD) risk management. Serum CRP below 2 mg/mL shows no CVD risk, whereas 2-3 mg/L indicates moderate risk and above 3 mg/L indicates a high future risk of CVDs. However, current detection methods are not sufficiently sensitive. Hence, we have optimized a sensitive radioimmunoassay (RIA) and a direct ELISA for CRP. First, a highly sensitive RIA assay (0-200 ng/mL) is developed, which can measure the CRP level in human serum as low as 3 ng/mL. For competitive RIA, CRP was radiolabelled using the chloramine-T method, and the separation phase was based on magnetizable cellulose particles. The optimized assay included two pipetting steps and an incubation time of 1 h at room temperature. In another direct ELISA, the anti-CRP antibody was conjugated with HRP using the sodium periodate oxidation method. The optimized assay protocol is as follows: Add 25 µL standards (0 to 100 ng/mL) to each well and incubate for 2 hrs at RT, followed by plate wash. The blocking was carried out for 30 minutes, followed by washing. Then, add 100 µL of anti-CRP-pAb-HRP to each well and incubate for 1 hr. Afterwards, 50 µL TMB was added to the wells, 0.2M H_2SO_4 was added to stop the reaction, and the absorbance was measured at 450 nm to plot the standard graph. Optimized assays were analyzed and validated for assay sensitivity, precision, dilution linearity, recovery, and cross-reactivity. The assay covers the clinical range of human CRP analysis for clinical tests.

Clinical case of Nijmegen breakage syndrome: diagnostic challenges in early infancy

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive primary immunodeficiency caused by defects in DNA repair mechanisms. It is characterized by microcephaly, growth retardation, immune dysregulation, and increased susceptibility to infections and malignancies. Inflammatory complications in NBS include chronic, non-infectious conditions like granulomatous inflammation, polyarthritis and skin manifestations. Managing inflammatory conditions in NBS is challenging due to a high predisposition of NBS patients to malignancies and increased sensitivity to radiation and chemotherapy agents. Early diagnosis of NBS is essential but may be challenging due to clinical heterogeneity, especially in early infancy.
Case presentation: A female infant, born from the second pregnancy, presented with severe neonatal complications, including respiratory distress, bacterial sepsis, disseminated intravascular coagulation, and prolonged mechanical ventilation. Routine vaccinations were postponed due to her clinical condition. At the age of 5 months, microcephaly (head circumference 38 cm) and a positive family history of microcephaly prompted further evaluation for a genetic immunodeficiency. Despite a phenotype highly suggestive of NBS, targeted genetic testing did not confirm the diagnosis. The patient remains under close multidisciplinary follow-up by a pediatrician and immunologist. Live attenuated vaccines have been withheld due to the potential risk.
Conclusions: This case emphasizes the importance of thorough immunological evaluation and multidisciplinary management in infants presenting with microcephaly and severe infections. In regions with a relatively higher prevalence of NBS, particularly in Eastern Europe and Ukraine, increased clinical awareness is essential. According to available data, up to 84 cases have been reported in Ukraine, highlighting the regional significance of this condition. Importantly, clinical suspicion should guide management decisions even in the absence of molecular confirmation. Early recognition enables individualized vaccination strategies, careful monitoring for complications, and timely referral to specialized care. Further research is needed to improve diagnostic approaches and understanding of NBS pathogenesis.

Investigation of Potential Biomarkers Predicting Acute or Chronic Progression of Inflammatory Diseases

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Background: Kidney dysfunction frequently accompanies chronic inflammatory, metabolic, oncological, and infectious diseases and contributes to immune dysregulation. The onset of renal impairment significantly complicates the course of nearly any disease and worsens clinical outcomes; therefore, early assessment of kidney function is critically important in clinical practice. This study investigates the potential of L-arginase (L-arg) and cholinesterases (ChE) as rapid, functional biomarkers of renal dysfunction severity using a novel electrochemical approach.
Methods: We developed two types of amperometric biosensors based on choline oxidase and urease detection platforms, designed to measure ChE and L-arg activity in serum. Mild and advanced nephropathy were induced in rat models using folic acid and doxorubicin. The biosensor performance was validated against standard biochemical markers of nephropathy.
Results: The results demonstrate the successful development and application of amperometric choline and urea biosensors for the determination of ChE and L-arg activity in rat serum samples.
Conclusion: ChE activity showed a strong correlation with the severity of nephropathy, increasing as the disease progressed. ChE activities were closely associated with established nephropathy severity markers, including serum creatinine and urea concentrations. Meanwhile, L-arg activity measurements show promising potential as an additional functional biomarker in rat serum samples.

DAY 3 (Wednesday, May 13, 2026)

Plenary Session 4
Innate Immunity in Precision Medicine

Session 4
Innate Immunity in the Clinic

Session 5
The Future of Innate Immunity (short talks)

Joint iiSIAR-EU-RESOLVE presentation

Innate Immune Checkpoints Revisited: Alternative Splicing, Novel Targets and the Glycan Dimension

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Immune checkpoint regulation extends beyond the classical receptor–ligand interactions that form the basis of current cancer immunotherapies. Increasing evidence indicates that immune inhibition is strongly influenced by ligand context, receptor isoform usage, and post-translational modifications. In this presentation, we summarize recent work highlighting how these layers shape immune checkpoint function and therapeutic response. Firstly, membrane-associated PCNA is identified as an inhibitory ligand for the innate NKp44 (NCR2) receptor, and antibody-mediated blockade of this interaction increases NK-cell effector functions in vitro and in vivo. I will present alternative splicing of NKp44 and other natural cytotoxicity receptors as a source of inhibitory innate checkpoint receptor isoforms with context-dependent expression. Then, the advantages of anti-ligand immune checkpoints will be discussed with emphasis of a novel antibody to a primary innate checkpoint ligand: HLAE complexed with a subset of tumor-associated peptides. Antibody recognition of HLA-E presenting tumor-associated peptides selectively disrupts inhibitory NKG2A signaling while preserving NKG2C-mediated activation, enhancing NK-cell responses against multiple myeloma cells. Finally, the role of the glycan dimension in checkpoint biology will be discussed. N-glycosylation of PD-L1 significantly affects its interaction with PD-1 and the efficacy of PD-1/PD-L1–directed antibodies. Specific glycosylation sites alter antibody blocking efficiency, the generation of soluble and extracellular vesicle–associated PD-L1, and downstream T-cell activation and cytotoxicity. These findings indicate that PD-L1 glycosylation status, in addition to expression level, contributes to immune suppression and variability in clinical responses.
Together, these studies illustrate how splice variants, ligand specificity, and glycosylation contribute to immune checkpoint regulation and inform more precise immunotherapeutic approaches.

Innate immunity and local cytokine network implicate male reproductive failure

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Although it has been well known that long-Covid 19 syndrome operates through enhanced systemic cytokine secretion deteriorating both male and female reproductive functions it is relatively low information available concerning local cytokine network within reproductive tract. Over 300 hundreds participants subdivided into five infertile groups (idiopathic infertility, professional drivers, males with past cryptorchidism, varicocoele, bacterial infections) versus healthy controls were studied in seminal plasma samples by Luminex for over 16 cytokine/growth factors, semen classical features (sperm number, motility, viability, morphology), oxidative stress parameters (total antioxidant capacity – TAC, SOD, CAT and MDA (malonylodialdehyde) levels as well as antisperm antibodies presence by common detection tests (SpermMar And flow cytometry). Although in respect to cytokine levels there were generally lower levels in seminal plasma than in systemic inflammatory circulation – interesting pattern has been detected in some of infertility entities. Compiled heat maps of correlations including all studied parameters revealed the following: in idiopathivally infertile group CATalase occurred to be most prominent factor entering all the possible correlations (including semen parameters, cytokines, other red-ox fetaures), in a group with bacterial infections correlations with TAC and sperm morphology have been dominant, similarly to a group with cryptorchidism (correlations of sperm morphology and TAC with addition of CAtalse). Groups of professional drivers and males with varicocoele readily displayed overall correlation pattern when concerning the number of leukocytes in semen versus other parameters studied. When analyzing correlations among cyokines there were revealed negative correlations in a group with idiopathic infertility, between IL-18 and TRAIL, MIF and IL-13, and MIF and IL-4. In a group with cryptorchidism between MIF and IL-13, while in groups of professional drivers as wells as males with varicoclele between TGF beta and IL-4. There was not detected significant antisperm antibody levels in any of the groups. This preliminary set of observations indicates the potential of cytokine analysis (innate immunity activation) for delineating particular infertile entity which may be used both for diagnosis as for biologically-sensitive treatment.
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Macrophages: Sentinels, Guardians, Soldiers, and Sabotoeurs of the Immune System

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Macrophages, the principal phagocytes of innate immunity, exhibit remarkable functional plasticity and play central roles in immune homeostasis, host defense, and disease pathogenesis. Depending on environmental cues and the nature of stimulation, they may exert protective or detrimental effects in infections, cancer, and chronic inflammation.
This lucture will provide a comparative overview of key macrophage phenotypes and activation states, including resident (sentinel) vs . inflammatory macrophages (soldiers); classically activated M1 macrophages (soldiers) vs . alternatively activated M2 macrophages (guardians); and primed vs . trained macrophages ("super soldiers"). Special attention will be given to biofilm-associated macrophages (BAMs) and tumor-associated macrophages (TAMs) - two macrophages subtypes that may act as "saboteurs:. These populations illustrate how tissue context and microenvironmental signals can redirect macrophage functions towards either hyperinflammation and tissue damage or immune suppression and tumor progression.
Finally, experimental data will be presented demonstrating how beta-glucan-induced macrophage training enhances antimicrobial functions, reduces the development of bacterial biofilm infections, and contributes to the concept of herd trained immunity. These findings highlight the therapeutic potential of targeting macrophage plasticity to modulate immune responses in infectious and neoplastic diseases.

Dysregulated Immunometabolism in SLE Neutrophils is Reversible with Anifrolumab Therapy

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Neutrophil dysregulation is a key feature of systemic lupus erythematosus (SLE). Low-density neutrophils (LDNs) are elevated, pro-inflammatory, and linked to vascular damage. While their phenotype is described, their immunometabolism is not. As metabolism dictates cellular function, we characterized the immunometabolism of SLE neutrophils and the impact of targeted therapy.

Peripheral blood was collected from SLE patients and healthy controls (HC). LDNs and normal-density neutrophils (NDNs) were isolated via magnetic selection and density centrifugation. Metabolism was assessed using the Seahorse Mitostress and Glycolytic Rate assays. In a proof-of-concept intervention, two patients with severe, refractory cutaneous SLE were treated with monthly anifrolumab (300 mg IV). Blood was drawn pre- and post-treatment for metabolic analysis alongside clinical assessment (CLASI).

At baseline, SLE LDNs exhibited a hypermetabolic, glycolytic, and metabolically flexible state. Inhibiting mitochondrial ATP synthase increased glycolytic (HK2, PKM2) and proinflammatory (IL1B, IL6) gene expression. Following anifrolumab, a profound metabolic reprogramming occurred. LDNs and NDNs showed markedly reduced oxidative phosphorylation (decreased basal and maximal OCR, ATP production). The hyperglycolytic phenotype normalized, shifting cells toward a quiescent metabolic state. This metabolic silencing coincided with dramatic clinical improvement, with CLASI activity scores decreasing from 43 to 8 and 47 to 12.

This work provides the first evidence that SLE LDNs are metabolically dysregulated and that this pathogenic state is reversible. Anifrolumab therapy induces a metabolic recalibration of neutrophil subsets, functionally silencing them. These findings identify the normalization of neutrophil immunometabolism as a novel downstream cellular mechanism underlying successful therapeutic intervention in SLE.

Neutrophils and monocytes research parameters as initial evaluation of innate immune response in common variable immunodeficiency (CVID) and hypogammaglobulinemia

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Neutrophils and monocytes are effector cells of innate immunity, responsible for early responses and regulation of inflammation. In patients with primary immunodeficiencies, functional and structural alterations of innate immune cells may remain underrecognized.
To evaluate neutrophil-related parameters and selected monocyte parameters in patients with common variable immunodeficiency (CVID) and hypoammalobulinemia the context of innate immune dysregulation.
The study included CVID patients (n=22), patients with hypogammaglobulinemia (n=13) and healthy controls (n=28). Complete blood counts and neutrophil research parameters were assessed using a Sysmex hematology analyzer: granularity intensity (NEUT-GI), neutrophil reactivity intensity (NEUT-RI), neutrophil size (NE-FSC) and neutrophil/monocyte data relating to granularity, activity and volume (NE-WX/MO-WX, NE-WY/MO-WY, NE-WZ/MO-WZ, MO-X, MO-Y, MO-Z).
Absolute neutrophil counts did not differ significantly between the study groups; however, significant differences were observed in neutrophil-related parameters. Both CVID and hypogammaglobulinemia patients exhibited significantly reduced NE-FSC values compared with healthy controls ($p<0.001$). In contrast, NE-WZ values were significantly increased in both patient groups ($p<0.001$). Monocyte analysis revealed differences limited to the overall patient cohort, including reduced monocyte counts and a significant alteration in the monocyte complexity parameter MO-Z ($p<0.05$).
Extended hematology analyzer parameters revealed early innate immune abnormalities not detectable through standard blood counts, including altered neutrophil size, increased activation and structural variability. Although CVID and hypogammaglobulinemia are classified as adaptive immune disorders, impairment of humoral immunity may promote persistent activation and remodeling of innate immune cells. These findings highlight the value of incorporating advanced neutrophil and monocyte parameters into the diagnostic evaluation of patients with antibody deficiencies.

Toll-like receptor 9 activation contributes to Alzheimer`s disease pathology

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Alzheimer`s disease (AD) is marked by amyloid-β (Aβ) plaques, tau hyperphosphorylation, neurodegeneration, and cognitive decline. Toll-like receptors (TLRs), major initiators of immune activation, are potentially decisive in modulating AD pathology, yet their exact roles are unknown. TLR9 is a lysosomal receptor which recognizes microbial and host-derived double-stranded DNA (dsDNA) to induce cellular immunity. TLR9 is interesting in AD, phospholipase D3 and progranulin whose genes are linked to predisposition of AD are controlling TLR9 activation in immune cells. To address the role of TLR9 in AD, we generate a mouse model of AD lacking TLR9. We found that extranuclear dsDNA accumulates in mice and human AD brains, activating TLR9 and driving microglial dysfunction. TLR9 deficiency in mice reduced Aβ plaque burden, cognitive deficits, and neuronal loss. TLR9-deficient microglia maintained homeostasis, showed anti-inflammatory M2a reprogramming, and improved lysosomal integrity, enhancing Aβ clearance. Sustained TLR9 activation disrupted lysosomal function, while inhibition restored lysosomal fitness in human microglia. Our findings highlight TLR9 critical role in modulating microglial activation and lysosomal function, suggesting that targeting TLR9 signaling and lysosomal function could be a promising strategy to mitigate AD pathology.

Reframing Neurodegeneration: Protein Aggregation as a Maladaptive Antimicrobial Response

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Neurodegenerative diseases such as Alzheimer's and Parkinson's share hallmark protein pathologies, such as amyloid plaques and tau tangles, whose origins remain unclear. Emerging evidence suggests that microorganisms may act as triggers that seed and propagate these protein misfolding events. To visualize microbial involvement directly, we applied decrowding expansion pathology (dExPath), an advanced form of expansion microscopy that enables super-resolution imaging (~40 nm) and exposure of previously inaccessible epitopes. Using dExPath, we detected pathogen-derived proteins colocalizing with disease-associated aggregates in human brain tissue from multiple neurodegenerative conditions. Furthermore, infection of human brain organoids and rodent neuronal cultures with candidate pathogens recapitulated these pathologies, indicating that microbial exposure is sufficient to induce neurodegenerative signatures. These findings suggest that (1) neuronal infection can initiate or accelerate hallmark protein pathologies, (2) these aggregates may have antimicrobial and neuroprotective functions, and (3) neurodegeneration may represent a maladaptive immune-like response of neurons to infection. Our ongoing work seeks to define the molecular mechanisms underlying this neuron-protective process, reframing neurodegenerative diseases as infectious and adaptive rather than purely degenerative disorders.

CRISPR-Cas9 screening in human iPSC-derived microglia identifies novel drivers of microglial health and CNS disease susceptibility

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Background: Microglia are essential for brain homeostasis and disease. Despite advances in defining microglial identity, the regulators of their maintenance, resilience, and survival across homeostatic and disease states remain incompletely characterized.
Methods: Human iPSCs were differentiated into iPSC-derived microglia (iMicroglia) through doxycycline-inducible expression of macrophage transcription factors (PU.1, MAFB, CEBPα, CEBPβ, IRF5, IRF8) with IL-34, TGF-β1, GM-CSF, and M-CSF. Microglial identity and Cas9 expression/activity were assessed by microscopy and spectral flow cytometry. To identify regulators of differentiation and survival, a pooled lentiviral CRISPR-Cas9 knockout screen (n = 3) was performed using a custom library of 3,402 experimental and control gRNAs selected through unbiased analysis of human tissue macrophage scRNA-seq atlases. Genes were knocked out in iPSCs before differentiation. gRNA abundances were compared between iMicroglia and iPSCs to identify enriched or depleted gRNAs. Top hits were analyzed using published microglial and brain omics datasets.
Results: Laminin 511–differentiated iMicroglia exhibited ramified morphology, robust IBA1 expression, high MERTK, and moderate CD14. Cas9 activity was confirmed by effective B2M knockdown. Screening identified known (CSF1R) and novel (GPX4, ZEB2) regulators. Top hits included genes associated with oxidative stress (SOD2), ferroptosis (GPX4, PEBP1), cytoskeletal organization and brain structural traits (DAAM1), brain injury (GPX4), and rare neurological disorders (ZEB2). Validation is ongoing.
Conclusions: Oxidative stress, ferroptosis, and cytoskeletal remodeling are key pathways in microglial differentiation and health, informing therapeutic target discovery for CNS disorders involving microglial dysfunction.

Targeted Delivery of Specialized Pro-Resolving Mediators Reduces Atherosclerosis and Induces Durable Innate Immune Reprogramming

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Atherosclerosis (AS) is a chronic inflammatory disease characterized by defective resolution pathways within the vascular wall. Specialized pro-resolving mediators (SPMs) actively modulate inflammation, but their therapeutic use is limited by instability and poor plaque targeting. We evaluated the therapeutic efficacy of SPM-loaded lipid nanocarriers dual-targeted to VCAM-1 and collagen IV (T-LN/SPMs) in ApoE-deficient mice with established lesions (12 weeks on a high-fat diet) over 4 weeks of treatment with free SPMs, non-targeted LN/SPMs, or targeted T-LN/SPMs. Biodistribution, flow cytometry of circulating and bone marrow immune cells ($CD11b^+$, $Ly6G^+$, $Ly6C^+$), plasma cytokines, and histological plaque assessment (Masson's trichrome) were performed. Bone marrow-derived macrophages (BMDMs) were isolated from treated mice and differentiated ex vivo without further nanocarrier exposure; reparative marker CD206 and inflammation-associated proteins (cytokines, TGF-β1) were evaluated. T-LN/SPMs preferentially accumulated in the aorta, reduced circulating neutrophils, and promoted an anti-inflammatory monocyte phenotype, resulting in decreased lipid deposition and plaque burden. Notably, BMDMs from T-LN/SPMs–treated mice retained an enhanced reparative phenotype ex vivo, with increased CD206 and IL-10 expression and a decreased pro-inflammatory cytokine profile, despite the absence of continued treatment. These findings indicate that in vivo administration of targeted SPM nanocarriers not only reduces atherosclerosis but also induces durable innate immune reprogramming consistent with a pro-resolving trained phenotype, highlighting their potential as a therapeutic strategy in cardiovascular disease.
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Impaired resolution of LPS-induced endothelial dysfunction: role of GPR18 receptor and systemic inflammatory response

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Sepsis remains a major cause of mortality, particularly in elderly patients, and is strongly associated with persistent endothelial dysfunction. However, mechanisms governing the resolution phase of inflammation-induced vascular dysfunction are still insufficiently understood.
The aim of this study was to investigate the role of the GPR18 receptor in the development and resolution of endothelial dysfunction in a murine model of endotoxemia.
Experiments were conducted in young C57BL/6 mice and GPR18 knockout (GPR18-KO) mice after lipopolysaccharide (LPS, 3 mg/kg) administration. Endothelial function was assessed using MRI-based measurements of vasomotor responses to acetylcholine. Additionally, systemic inflammatory and biochemical parameters, including serum amyloid A (SAA), hematological profiles, and nitrate levels, were evaluated at multiple time points (baseline, 12, 24, 48, and 72 hours post-LPS) in both WT and GPR18-KO mice.
Young WT mice exhibited transient endothelial dysfunction with recovery observed within 24 hours. In GPR18-KO mice, endothelial recovery was delayed, up to 48 hours after LPS injection. Preliminary analyses of systemic inflammatory markers indicate dynamic, time-dependent changes following LPS administration, with differences observed between WT and GPR18-KO mice. These findings suggest that GPR18 signaling may modulate not only vascular function but also the systemic inflammatory response during endotoxemia.
In conclusion, our results support a critical role of the GPR18 receptor in the resolution of endothelial dysfunction and suggest that its impairment leads to delayed recovery of vascular function and may contribute to prolonged inflammatory responses.
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Neutrophil extracellular traps promote a pro-resolving macrophage phenotype characterized by inflammasome suppression and enhanced phagocytosis

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Background: Neutrophil extracellular traps (NETs) contribute to antimicrobial defence but are increasingly recognized as mediators of sterile inflammation and tissue injury. Beyond their role against pathogens, NETs have been shown to modulate the function of vascular cells, promoting endothelial dysfunction, thrombogenicity, cytokine release, and extracellular matrix remodelling. Despite these well-established effects on vascular and stromal compartments, the impact of NETs on macrophage functional reprogramming remains incompletely understood.
Methods: Human THP-derived macrophages were exposed to NETs, and the impact of NETs on macrophage transcriptomic signature was evaluated by RNAseq. Gene and protein expression of inflammasome-related mediators and resolution-associated markers were assessed by qPCR and Western blot. Secreted IL-1β, MMP-1, MerTK, and osteopontin levels were measured by ELISA, and phagocytic capacity was quantified using an assay kit.
Results: The transcriptomic and validation results demonstrated that NET stimulation induces a pro-resolving macrophage phenotype characterized by reduced expression of NLRP3, IL-1β, and IL-18, indicating suppression of inflammasome signaling, and by decreased soluble osteopontin levels, a marker associated with inflammatory macrophages. Moreover, NET-exposed macrophages exhibit increased expression of the efferocytic receptors CX3CR1 and MerTK, as well as the PPARγ transcription factor, resulting in enhanced phagocytic capacity. Nevertheless, MMP-1 expression was reduced by NETs, suggesting that NETs mediated controlled extracellular matrix remodeling.
Conclusions: Our findings demonstrate that NETs induce macrophage phenotypic reprogramming characterized by enhanced phagocytic capacity and suppression of inflammasome signalling, accompanied by reduced osteopontin and MMP-1. This integrated response suggests attenuation of inflammatory and pro-fibrotic macrophage programs, supporting the concept of a resolution phenotype.

Can computational flow cytometry reveal specific immune resolution signatures during the resolution of intestinal inflammation?

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The resolution of inflammation is coordinated and regulated by certain mediators. Pro-resolution involves distinct immune pathways that conventional flow cytometry analyses may not fully capture. A self-resolving dextran sulphate sodium (DSS)-induced colitis mouse model was used to assess if computational cytometry analysis can identify overlooked immune resolution signatures that cannot be detected by conventional gating. Mice were treated with either the natural extract Nigella Sativa fixed oil (NS), probiotic kefir, or the specialised pro-resolving factor Maresin-2 (MaR2). OMIQ software was used to analyze the composition of colonic lamina propria leukocytes. First, conventional flow cytometry gating was performed to identify known immune populations. These results were then compared with a non-supervised analysis using computational approaches, including UMAP and FlowSOM. Using manually defined gates, both NS and MaR2 showed a significant up-regulation of M1 pro-inflammatory macrophages, while other subpopulations, such as M0 newly arrived and M2 anti-inflammatory macrophages, did not show significant changes compared to DSS-untreated mice. In contrast, kefir did not significantly affect macrophage populations. Computational analysis, however, revealed distinct treatment-specific phenotypic landscapes and immune resolution signatures not identified by conventional gating. Notably, for each treatment, there was a differential expansion of specific subpopulations, that were previously undetected. NS modulated macrophage subclusters affecting multiple phenotypic states across M0, M1, and M2 compartments. Conversely, MaR2 and kefir treatments induced changes in a more restricted but distinct subset of macrophage clusters. Computational approach provided unique immune subsets specific to each pro-resolving factor during resolution of intestinal inflammation that were not identified by conventional gating, indicating the heterogeneity and agent dependency of this process. This highlights the utility of high-dimensional computational analysis in identifying novel therapeutic strategies for colitis.

Tumor Antigen Presentation Defects: Impact on Immunotherapy Response and Strategies for Pharmacologic Modulation in Preclinical Models

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The limited clinical response rates to cancer immunotherapy underscore the need to elucidate tumor-intrinsic resistance mechanisms, develop rational strategies to overcome them, and identify predictive biomarkers to guide patient stratification. We investigated how the functionality of the tumor antigen processing and presentation machinery (APM) shapes responses to dendritic cell vaccination (DCV) and anti–PD-1 therapy, and whether cyclophosphamide (CY) can pharmacologically modulate tumor APM to improve chemoimmunotherapy efficacy in two syngeneic C57BL/6 mouse tumor models: Lewis lung carcinoma (LLC1) and glioma (GL261). LLC1 cells displayed an APM-impaired phenotype, characterized by reduced expression of APM-related genes and low surface MHC-I, which was associated with poor intratumoral CD8+ T-cell infiltration and resistance to DCV and anti–PD-1. In contrast, APM-functional GL261 tumors elicited cytotoxic immune responses and were sensitive to both DCV and anti–PD-1. CY increased type I interferon signaling and APM-related gene expression in both cell lines and elevated surface MHC-I levels in LLC1 cells in vitro . However, in vivo , CY-driven APM activation and enhanced CD8+ T-cell responses were observed only in GL261 tumors, whereas APM restoration in LLC1 remained limited, suggesting additional microenvironmental and/or epigenetic constraints. Combining CY with DCV and/or anti–PD-1 improved efficacy in both models, resulting in complete regression and durable immune memory in GL261, while in LLC1 the main benefit was improved tumor control and enhanced antitumor T-cell properties. Overall, APM dysfunction emerges as a driver of immunotherapy resistance, shapes CY-driven immunomodulation, and may serve as a predictive biomarker of response to CY-based chemoimmunotherapy. Future studies will define the mechanisms limiting APM restoration in LLC1 and develop rational combination strategies to overcome these barriers.

Fibronectin-targeted nanoparticles mediate accumulation of HSP90 inhibitors in the fibrotic heart with possible neutrophil contribution

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Cardiac fibrosis is characterized by excessive extracellular matrix (ECM) deposition and inflammatory cell recruitment, leading to myocardial dysfunction. HSP90 supports pro-fibrotic signaling via the TGF-β/SMAD3 pathway, making it a therapeutic target, while circulating immune cells infiltrate injured myocardium and may influence nanoparticle biodistribution. We aimed to develop fibronectin-targeted lipid nanoparticles for the selective delivery of HSP90 inhibitors to the fibrotic myocardium and investigate their interactions with circulating immune cells. Nanoparticles (~200 nm; ζ-potential −40 mV) encapsulating HSP90 inhibitors were prepared via oil-water emulsification and sonication, and fibronectin-binding peptides were conjugated using maleimide-thiol chemistry. Biodistribution and immune cell uptake were assessed in an Ang II-induced cardiac fibrosis model using near-infrared imaging and flow cytometry, while TGF-β1-activated cardiac fibroblasts were treated with free or nanoparticle-encapsulated inhibitors to evaluate anti-fibrotic responses by qRT-PCR. Ang II treatment elevated circulating $CD11b^+$ myeloid cells and neutrophils, indicating systemic immune activation, and caused capillary obstruction, tissue damage, and deposition of neutrophil elastase (NE) in the heart. Fibronectin-targeted nanoparticles preferentially accumulated in the heart compared with non-targeted nanoparticles, demonstrating high cardiac specificity. Flow cytometry revealed enhanced uptake by circulating immune cells, predominantly neutrophils. Functionally, fibronectin-targeted nanoparticles delivering HSP90 inhibitors attenuated cardiac hypertrophy and suppressed periostin, fibronectin, and collagen III expression in activated fibroblasts. These findings suggest that fibronectin-targeted nanoparticles enhance cardiac specificity and anti-fibrotic effects, with circulating neutrophils potentially facilitating their delivery.
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An Image is Worth a 1000 Words: Machine Learning-Assisted Quantification of Lung Fibrosis

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Background: Idiopathic Pulmonary Fibrosis (IPF) is characterized by excessive collagen deposition and changes in lung tissue architecture. Conventional histological scoring is subjective and does not include important parameters, such as alveolar gas exchange potential. Quantifying features like gas exchange potential or collagen coverage manually are extremely time-consuming and impractical, highlighting the need for an objective, high yield approach.
Objective: To quantitatively assess fibrotic changes and tissue remodeling in bleomycin-induced lung fibrosis across experimental groups and models, using a single, standardized analysis pipeline to ensure consistent measurements across all samples.
Methods: Lung fibrosis was induced by subcutaneous injections of 50 ug bleomycin (3 injections per week for 4 weeks). Then, lungs were harvested, fixed, paraffin-embedded and sectioned for histological analysis. Sections were stained with Picrosirius Red for collagen visualization or Hematoxylin and Eosin for general morphology, and scanned as whole-slide images. Machine learning (ML) classifiers in QuPath were trained to segment collagen, tissue, and alveolar spaces. Quantitative features extracted included collagen coverage area, compressed tissue fraction, and potential gas exchange space. Metrics were calculated across multiple images per subject and compared between experimental groups.
Results: ML classifiers identified collagen and tissue regions, quantifying differences in collagen deposition, tissue compression, and gas exchange potential across test groups' lungs. ML-assisted analysis allowed analysis of large datasets that would be impractical to perform manually, and reduced observer bias.
Conclusion: ML-assisted pathologic analysis provides a uniform framework for detailed, reproducible quantification of lung fibrosis beyond conventional pathological scoring. This approach quantifies both collagen deposition and tissue architectural changes relevant to tissue function, enabling comprehensive assessment of fibrotic remodeling in preclinical studies.

Bioethical aspects of the development process of innate immunity

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The development and functioning of innate immunity raise several important bioethical considerations, particularly as scientific advances increasingly allow researchers to manipulate immune responses. In conditions when innate immunity is the body's first line of defense against pathogens, it is involving physical barriers, immune cells, and molecular mechanisms that respond rapidly to infection. While studying and modifying these processes can lead to major medical breakthroughs, it also introduces ethical questions related to safety, fairness.

One of the most stringent bioethical aspect concerns experimentation and research ethics. Ethical guidelines in preclinical research require that animal trials follow such principles as reduction, refinement, and replacement (3R) to minimize suffering and demonstrate respect. Similarly, human research must respect informed consent, protect participant welfare, and ensure that risks are justified by potential benefits. These principles are especially relevant when testing new therapies that stimulate or suppress innate immune responses.

Another issue involves emerging technologies that modify immune function, including gene editing and immunomodulatory drugs. Researchers must carefully balance innovation with caution.

Equity and access also represent a major bioethical challenge. Therapies that enhance or regulate innate immunity are often expensive and may not be equally available worldwide, for all social categories. This creates disparities between high-income and low-income populations, raising questions about global justice and the fair distribution of medical benefits.

In conclusion, bioethical oversight, regulatory frameworks, and open dialogue with society help ensure that advances in innate immunity research contribute responsibly to human health.

PROS1-TAM signalling in microglia regulate microglia development, homeostasis and sterile inflammation

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Introduction & Objectives: The TAM (Tyro3, Axl, Mertk) receptors constitute a central anti-inflammatory axis active regulating inflammation through efferocytosis and gene regulation. Although GAS6 and Protein S (PROS1) were identified as TAM ligands, the role of PROS1 in resolution biology in general, and particularly in the nervous system is incompletely understood. Here we explore the roles of *Pros1* in microglia - the brain immune cells in development, homeostasis and inflammatory-related conditions.
Methods: Pros1 was either conditionally deleted at early microglial developmental stages (*Pros1*-cKO) or in adulthood using a Cre-ER system following tamoxifen induction. Microglial development and function was assessed by immunohistochemistry, gene expression and histology. The role of PROS1 in microglial response to inflamed diseased states was also assessed.
Results: *Pros1*-cKO mice had fewer microglia throughout the brain at 2 months of age due to abnormal microglia development. Additionally, key microglia functions such as phagocytosis and regulation of inflammation are impaired at steady state. Remarkably, loss of PROS1 within microglia alone resulted in inflamed brain tissue at steady state. Moreover, we find that PROS1 is key to efficient resolution of inflammation following systemic challenge. Finally, inflammation and microglia-derived PROS1 play a role in regulating neurogenesis in adult mice, impacting normal brain function.
Conclusions: Through studying the PROS1-TAM axis in the CNS we reveal the ongoing need for active anti-inflammatory pathways at steady state. Using *Pros1*-cKO mouse models, we identify microglia-derived PROS1 is necessary for microglia development and for microglial resolution-related immune functions both at steady state and following systemic inflammation. This study reveals the role of the PROS1-TAM axis in maintaining a healthy, functional brain during development, and in resolving inflammation at steady state and following a systemic challenge.
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