14th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE (Tatra 2026)

June 13 – 17, 2026
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Program and Abstracts

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Individual consultations
On **15 June 2026**, **Dr. Kašpárková** will be available for **one-on-one sessions** with junior participants to further discuss workshop topics and address questions beyond the scope of the main lecture.

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06:00 - 11:00 **Departure**

Fueling Minds in Nature: Walking, Talking and Thinking with the Professors and Peers

(Compiled by Jan Dobeš)

Organized Conference Excursions

We will organize two optional special bus trips during the conference:

1. The first is a short fifteen-minute shuttle ride to the nearby village of **Pavlov**, which caters beautifully to both outdoor and history lovers. Hikers can choose from several trails of various lengths that wind through the unique natural landscapes of the **Pálava Hills** and up to historic castle ruins. If you prefer a more relaxed pace, Pavlov is a famous hub for local wine production, where local winemakers open their cellars for tastings. The village is also home to **Archeopark Pavlov**, a highly modern, interactive underground museum built directly on an ancient Paleolithic site where mammoth hunters once lived.
2. Our second excursion is a twenty-minute drive to the **UNESCO-listed Lednice complex**. This trip offers the chance to take a guided tour of the stunning, fairy-tale-like **Lednice Chateau**. Or you can stroll through the expansive English gardens, an experience that can be beautifully paired with a scenic boat ride along the estate's historic canals.

Individual Activities

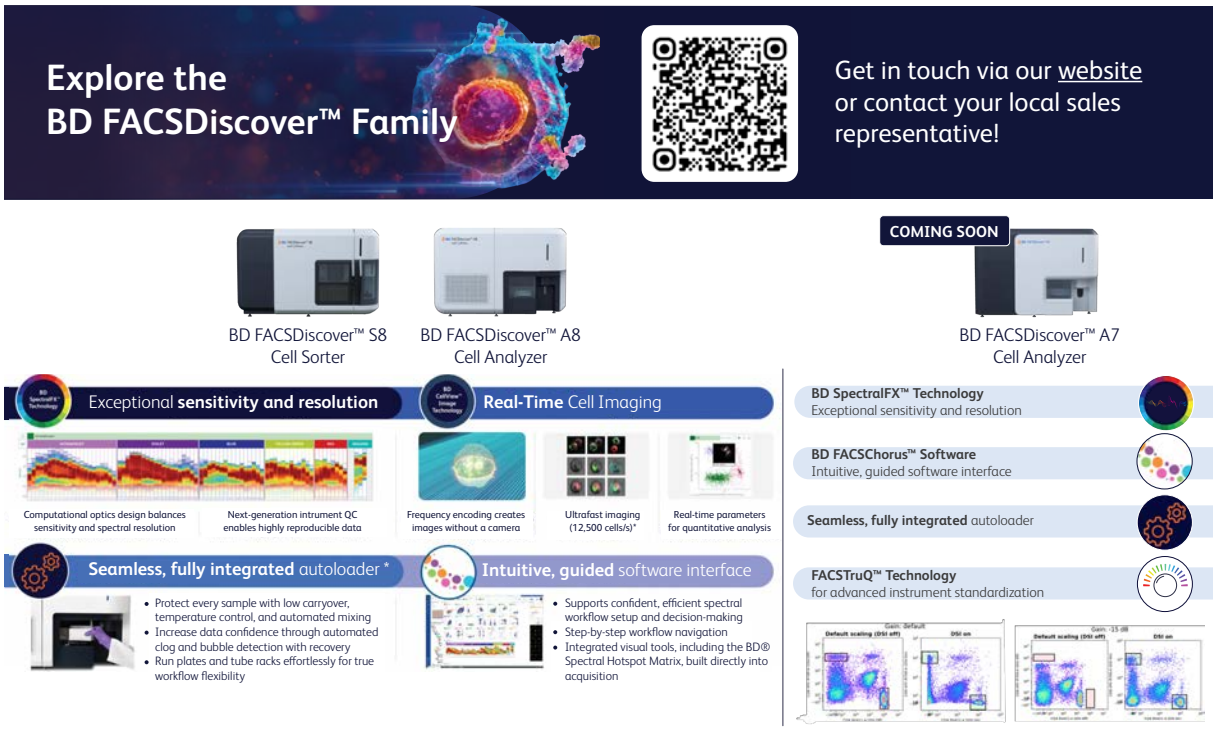
We have three tips for you:

1. **Welcome to Mikulov and Its Surroundings**
   Nestled in the sunny hills of South Moravia right by the Austrian border, Mikulov is a town where history and nature seamlessly blend. The entire skyline is dominated by the Mikulov Castle, which sits on a rocky cliff overlooking cobblestone streets. Surrounded by endless vineyards and situated right at the edge of the beautiful Pálava Hills, the town offers a wonderfully laid-back vibe. Whether you spend your free time exploring the historic Jewish quarter, hiking up *Svatý kopeček* for panoramic views, or relaxing in a local wine cellar, you will find plenty to enjoy between conference sessions.

2. **Exploring the Town Center**
   If you choose to stay directly in Mikulov, several remarkable sights are just a short walk away. The prominent **Mikulov Castle** houses a museum with exhibitions dedicated to regional art, ancient local history, and the area's deep-rooted wine production. Right on the main square, you will find the **Dietrichstein Tomb**, the historic funeral chapel of the prominent local family. Mikulov also boasts a rich Jewish heritage, and you can easily spend an afternoon wandering through the historic **Jewish quarter**, visiting the local Synagogue, or exploring the old Jewish cemetery, which is one of the largest in the region. For those looking to stretch their legs, a charming walking path leads up **Svatý kopeček**, offering the absolute best panoramic views of the town and its countryside. Alternatively, if you prefer to explore underground, **the Na Turoldu Caves** on the edge of town offer a unique look at local geological formations and subterranean domes.

3. **Sports and Relaxation**
   For those who want to stay very active, the region is famous for its exceptional cycling trails. You can **rent a bicycle** right across the street from the conference hotel to explore the vineyards at your own pace. If you prefer **swimming**, hotel itself features an excellent wellness center and pool. However, if you are looking for a unique outdoor swimming experience, you can take a dip in **Lom Janičův vrch**, a beautifully flooded limestone quarry.

Advertisement Waters Biosciences (Formerly BD Biosciences)



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ABSTRACTS SPEAKERS

S.01

From Promiscuous Gene Expression to Thymic Mimetic Cells

Jakub Abramson[1]

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The thymus is essential for the establishment of central immune tolerance, yet the cellular diversity and regulatory mechanisms that underlie this process remain incompletely understood. Our research program aims to uncover the principles governing thymic function and their broader implications for immune homeostasis.

A central focus of our work has been the role of Aire^{+} medullary thymic epithelial cells (mTECs), which promote tolerance through the ectopic expression of hundreds of tissue-restricted antigens. More recent findings indicate that these cells possess not only unprecedented transcriptional plasticity, but also a remarkable degree of developmental flexibility. mTECs can give rise to a wide spectrum of highly specialized cell types, collectively termed “thymic mimetic cells,” including populations resembling skin, endocrine, and muscle lineages, as well as diverse epithelial subtypes characteristic of mucosal tissues, such as tuft cells, microfold (M) cells, ionocytes, and ciliated cells.

This combination of extensive transcriptional promiscuity and the capacity to adopt highly divergent cell identities positions mTECs as a unique model of epithelial plasticity. However, the molecular mechanisms that enable such extraordinary flexibility remain poorly understood. In this presentation, I will discuss recent insights into the regulation of mTEC transcriptional and developmental plasticity, alongside emerging evidence defining the physiological roles of thymic mimetic cells in shaping central tolerance.

S.02

Systemic and Mucosal Immunity: Separate Roles and Integration Strategies

Rita Carsetti[1]

1. Bambino Gesù Children Hospital IRCCS, Rome, Italy

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Systemic vaccination induces serum antibodies and circulating memory B cells (MBCs) but provides limited protection in the upper respiratory tract (URT), where many respiratory pathogens initiate infection. How systemic MBCs contribute to mucosal immunity remains unclear. Using multiparametric flow cytometry, single-cell RNA and V(D)J sequencing, and functional analyses of paired blood and nasal/oropharyngeal samples, we characterized human B cells across systemic and mucosal compartments. Swab-derived B cells transcriptionally overlap with circulating activated MBCs while exhibiting distinct features of activation, tissue retention, and spontaneous IgA/IgG secretion. Approximately 6% of mucosal B-cell clones were shared with blood, indicating systemic–mucosal connectivity. Both infection and vaccination expanded two circulating antigen-specific activated MBC subsets, whereas antigen-specific B cells accumulated in the URT only following local inflammation. The finding that B-cell recruitment is reactive rather than preemptive may explain the limited efficacy of parenteral vaccines and provides a rationale for developing integrated systemic–mucosal vaccination strategies.

S.03

Cytoplasmic NAD/H Synthesis via NRK1 Regulates Inflammatory Capacity and Promotes Survival of $CD4^+$ T Cells

Sarah Dimeloe[1]

1. University of Birmingham, Birmingham, United Kingdom

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T cell metabolism increases upon activation, underpinning immune effector functions. Nicotinamide adenine dinucleotide (NAD/H) is an essential redox cofactor for glycolysis and mitochondrial substrate oxidation. Its phosphorylation to NADP/H regulates reactive oxygen species (ROS) abundance. NAD/H levels increase upon T cell activation, but synthesis pathways and implications are not fully characterised. In this talk, I will discuss the role of the NAD/H-synthesis enzyme nicotinamide riboside kinase 1 (NRK1), the expression of which increases upon stimulation of both human and murine $CD4^+$ T cells. Functionally, NRK1 activity restrains activation and cytokine production of $CD4^+$ T cells while promoting survival. These activities are linked to increased NRK1 expression in the cytoplasm, where it locally raises NAD/H levels. This supports glycolysis, but more profoundly impacts cytoplasmic NADP/H generation, thereby controlling ROS abundance and nuclear NFAT translocation. During fungal and viral infection, T-cell-intrinsic NRK1 maintains effector $CD4^+$ T cell abundance within affected tissues and draining lymph nodes, supporting infection control. Taken together, these data confirm that subcellular regulation of immune cell metabolism determines immune responses at the level of whole organism.

S.04

Regulation of Adaptive Immune Responses by Non-Classical Antigen Presenting Cells

Jan Dobeš[1,2]

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2. Cell Biology Group, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

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Adaptive immune responses rely on the vast diversity, precise activation, and context-specific polarization of helper T cells. This functional heterogeneity is driven by instructive signals from a diverse array of antigen-presenting cells. While conventional dendritic cells have long been considered the primary messengers between innate and adaptive immunity, recent evidence highlights the critical role of specialized (and often overlooked) antigen-presenting cell types that exert a fundamental impact on T cell fates.

In this presentation, I will explore the influence of non-conventional antigen-presenting cells on mucosal immunity. Specifically, I will discuss how populations such as extrathymic Aire-expressing cells and intestinal epithelial cells function beyond classical paradigms to shape adaptive responses. By examining these cells in the context of host-pathogen interactions, I will describe how their unique niche-specific signals orchestrate immune defense, thereby redefining our understanding of the mucosal immune landscape.

S.05

Regulation of TNF-Induced Cell Death

Peter Draber[1]

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TNF is a potent proinflammatory cytokine that can induce cell death by activating the kinase RIPK1. The adaptor proteins TANK and AZI2 protect against cell death by recruiting TBK1 to the TNF receptor signaling complex, thereby inhibiting RIPK1. While deficiency of either adaptor alone is well tolerated, combined loss of TANK and AZI2 results in partial embryonic lethality and severe TNF- and RIPK1-driven autoinflammation. Here, we show that TANK/AZI2-deficient mice exhibit a striking expansion of regulatory T cells (Tregs), most of which display an effector phenotype with high expression of immunosuppressive genes. Although thymic Treg generation is modestly increased, Tregs arise predominantly in the periphery through a largely cell-intrinsic mechanism. The marked accumulation of effector Tregs suggests that the T-cell compartment may limit TNF-driven pathology in this model. Supporting this, T cell ablation in TANK/AZI2-deficient mice markedly exacerbates disease progression and enhances TNF-driven, RIPK1-mediated inflammation. Similarly, T cells protect against acute TNF-induced systemic inflammatory response syndrome by limiting RIPK1-mediated cell death. Together, our findings identify TANK and AZI2 as negative regulators of Treg formation, demonstrate that T cells restrain TNF-driven inflammation by limiting RIPK1-dependent cell death, and suggest that this protective effect is mediated primarily by Tregs.

S.06

Spatial Immune Niches as Determinants of Anti-Tumor Immunity in Ovarian Cancer

Jitka Fucikova[1,2]

1. Department of Immunology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic
2. Sotio Biotech, Prague, Czech Republic

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Introduction: High-grade serous ovarian carcinoma (HGSOC) remains poorly responsive to immune checkpoint blockade (CPI) despite the presence of tumor-infiltrating immune cells. This suggests that the spatial organization and functional coordination of immune populations within the tumor microenvironment (TME) constrain effective antitumor immunity.

Methods: Spatial transcriptomics and multiplex immunofluorescence characterized the immune architecture of HGSOC and mapped spatial interactions among TLS, $CD8^+$ T cells, and NK cells. Functional studies evaluated NK–T cell crosstalk and effects of NKG2A blockade in preclinical models.

Results: Spatial analyses revealed that antitumor immunity in HGSOC is organized within discrete immune niches and aggregates with varying degrees of maturation. Specifically, mature TLS as forming only in 16% of HGSOC. Limited TLS maturation was associated with a $CD8^+$ T-cell compartment dominated by CPI-resistant $PD\text{-}1^+TIM3^+$ effector cells, whereas CPI-responsive $TCF1^+PD\text{-}1^+$ progenitor $CD8^+$ T cells were rarely observed. A similar pattern of functional restraint characterized the NK cell compartment. HGSOC tumors were enriched in CD56bright NK cells expressing inhibitory receptors NKG2A and TIM3. Spatial mapping showed NK cells localize within tumor cores and TLS-associated niches, where they frequently interact with $CD8^+$ T cells. Tumor cells displayed HLA-E expression, the canonical ligand for NKG2A, limiting NK cell–mediated cytotoxicity. Functional studies showed NK cells and $CD8^+$ T cells engage in reciprocal crosstalk essential for antitumor immunity. Disruption of this interaction contributed to immune dysfunction in ovarian cancer, whereas therapeutic NKG2A blockade restored NK cell effector function, enhances $CD8^+$ T-cell responses, and improves PD-1 blockade efficacy in preclinical models.

Conclusions: Spatial immune niches defined by limited TLS maturation and inhibitory T and NK-cell states shape immune dysfunction in ovarian cancer. Targeting the NKG2A–HLA-E axis may restore coordinated NK–T cell immunity and enhance responses to CPIs in HGSOC.

S.07

From an Idea to a Tradition: The Story of the TATRA Conference

Juraj Ivanyi[1], **Georg Wick**[2]

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2. Innsbruck University, Innsbruck, Austria

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While attending the Congress of the Czechoslovak Immunology Society in 1991 in a mountainous region of Slovakia, my (J.I.) nostalgia for the region, combined with experience of attending in 1964 one of the American 'Gordon Conferences' in a nature setting, set my mind to imitate, with an Immunology conference in the Tatra mountains. After Juraj Svac from Banska Bystrica, Slovakia, agreed to act as local organiser, I invited Georg Wick from Innsbruck and Jaroslav Sterzl from Prague to involve the Austrian and Czechoslovak Immunology Societies. Importantly, Georg Wick suggested to run the conferences in continuity, i.e. bi-annually. After obtaining key funding from EFIS (promoted by Klaus Eichmann), the first Tatra Immunology conference took place in 1994. The 4-day scientific programmes of all 13 past conferences with 25-30 speakers and about 100 participants had sessions ranging from fundamental to clinical immunology. Poster presenting participants were led to engage in temperamental discussions by Adrian Hayday from London. Reports on selected themes have been published in different journals. The break since 2020 was caused by the Covid pandemic.

The Tatra mountains offered mid-day hiking and visiting rustical villages or castle ruins. At night, participants enjoyed the 'Shepard's Hut' bar and Slovakian folk music at final dinner. The 1st Conference entitled Molecular determinants of T cell Immunity opened by Avrion Mitchison from London produced some truly exciting moments. The main organizer for the 2nd conference (1996) was Georg Wick. From the 3rd Conference in 1998, Vaclav Hořejši from Prague achieved upgrading to 'Regular' Conferences under the auspices of EFIS and the European Journal of Immunology (Tatra-EFIS-EJI) and organized the scientific profile of the all subsequent 11 conferences, until 2018. Local organizing was managed mostly by Stanislava Blažičková: starting in the Low Tatras, moving to 2 different venues in the High Tatra mountains enabled water rafting along the Slovakian-Polish border.

S.08

Probing the Monocyte-to-Macrophage Axis in Health and Disease

Steffen Jung[1], **Sebastien Trzebanski**[1], **Jung-Seok Kim**[1]

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Monocytes are short-lived myeloid immune cells that arise from adult hematopoiesis and circulate for a short time in the blood. Monocytes comprise two main subsets, in mice defined as Ly6C^{high} and Ly6C^{low} cells. Classical Ly6C^{high} monocytes (CM) are critical players in inflammation and can serve as precursors of tissue-resident macrophages (TRM). Non-classical Ly6C^{low} monocytes (NCM) derive from CM and can be considered vasculature-resident macrophages. Recent studies revealed that CM themselves are heterogeneous, including cells with neutrophil- and dendritic cell (DC)-like transcriptomic signatures, that arise from distinct myeloid lineages. Using a fate characterization of these GMP- and MDP-derived CM involving adoptive cell transfers and mixed BM chimeras revealed that these cells, surprisingly, differentially seed selected tissues, including the lung and the dura mater during homeostasis and following challenge. Collectively, these data establish a novel link between CM dichotomy to tissue macrophage heterogeneity.

Aside from their role in inflammation, monocytes replenish selected peripheral macrophage compartments, replacing the original YS-derived ‘embryonic’ TRM (eTRM). Whether eTRM and monocyte-derived TRM (moTRM) display distinct functions remains largely to be defined. Focusing on the brain parenchyma, we recently reported that monocyte-derived microglia (MoMg) that accumulate with age are on transcriptomic level identical to the original microglia. However, as opposed to the latter, MoMg can be afflicted by somatic mutations associated with clonal hematopoiesis. Indeed, we establish in a respective mouse model that mutant Momg can cause brain pathology.

S.09

Role of B Cells in Central T Cell Tolerance

Ludger Klein[1], **Joao Freitas**[1], **Dario Zebcevic**[1], **Nadia Khouja**[1], **Iliana Siamishi**[1]

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Tolerance to self-antigens is a fundamental property of the immune system. During central tolerance induction in the thymus, autoreactive T cells are either eliminated or diverted into the regulatory T cell lineage. Both processes are dependent on the presentation of self-antigens by thymic antigen presenting cells (APCs). Commonly, dendritic cells and medullary thymic epithelial cells are believed to represent the key APCs for central T cell tolerance. The thymus also harbours a distinct population of B cells, raising the possibility that thymic B cells serve a non-redundant APC function in central T cell tolerance induction. I will discuss our ongoing work to test this hypothesis through large scale comparisons of the TCR composition of 'normally' selected T cell repertoires versus repertoires selected in the absence of B cells, Dendritic cells, or both.

S.10

Contribution of Non-Hematopoietic Cells to Tissue Immune Responses during Infection

Mustapha Jaiteh[1,2], Rob ter Horst[1,2], Henrique Colaço[1,3], Stephan Reichl[1,2], Jake Burton[1,2], Philipp Muench[1], Anne-Christine Orts[1], Francesco Piras[1,2], Adele Nicolas[1], Wentao Li[1], Anna Hofmann[3], Aubrey Burrett[3], Maria Guarini[1], Andrea Pääro[1], Andrzej Gruza[4], Marion Bokor[4], Caroline Lassnig[4], Birgit Strobl[4], Thomas Decker[5], Mathias Müller[4], Andreas Bergthaler[1,3], Thomas Krausgruber[1,2,6], Christoph Bock[1,2,6]

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6. Shared senior authors

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Most mammalian cells can respond to infection-linked signals via the interferon pathway, but the regulatory processes have been studied mainly in hematopoietic immune cells. Here we present a systematic dissection of the epigenetic and transcriptional programs of interferon signaling in structural cells (endothelial and epithelial cells, fibroblasts) across five organs (colon, kidney, liver, lung, spleen). We observed broad regulatory changes upon abrogated interferon signaling in knockout mice for key mediators of interferon signaling (IFNGR1, STAT1, TYK2) already under homeostatic conditions. Moreover, mice with STAT1 knockout in non-hematopoietic cells surprisingly died when challenged with systemic viral infection using the lymphocytic choriomeningitis virus (LCMV) model, while mice with STAT1 knockout in hematopoietic immune cells survive despite poor viral clearance. We found that STAT1 knockout led to downregulation of antiviral immune response genes in structural cells resulting in and an overshooting T cell response with vascular leakage and lethal immune pathology. In summary, our results highlight the importance and complexity of interferon signaling in structural cells and uncover an essential role of immune regulation in non-hematopoietic cells to protect against a lethal response to viral infection.

S.11

A Granzyme K-Positive T Cell Program Links Peripheral EBV Priming to CNS Autoimmunity

Kilian Schober[1]

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Epstein-Barr virus (EBV) infection is necessary but not sufficient for multiple sclerosis (MS). T cells are central to EBV control, yet how peripherally primed EBV-specific T cells are connected to central nervous system (CNS) autoimmunity remains unresolved. We here analyzed paired blood (PB) and cerebrospinal fluid (CSF) from MS patients at diagnosis and (non-)inflammatory controls. We identified a $GZMK^+CCR7^+GPR183^+$ effector-memory program in PB that predicted clonal expansion in the CSF, which was more pronounced in MS. This CSF-biased signature was enriched for EBV specificity and conserved across CD4 and CD8 T cells. In the CSF, $GZMK^+$ T cells preferentially interacted with B cells and plasmablasts, enhancing their machinery for antigen presentation and metabolic reprograming. Overall, our findings suggest that $GZMK^+$ EBV-specific T cells adopt a CSF-biased phenotypic program in the periphery and contribute to CNS autoimmunity through non-cytotoxic interactions with B cells.

S.12

Functional Diversity of T Cells

Eva Salyova[1], **Ondrej Stepanek**[1]

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Memory T cells provide protection against reinfections of the same or related pathogens. Accordingly, there is an urgent need to optimize vaccines to be potent memory T cells inducers. A key advantage of memory T cells is that they substantially increase the number of pathogen-specific T cells in the body. However, little is known about the differences between naive and memory T cells on a per cell basis. Our research revealed that memory T cells have an early bias towards the formation of effector T cells, which persists in the memory phase and leads to the formation of numerous effector memory T-cell progenies. In contrast, antigen-specific T cells retain stemness properties and are biased towards the formation of central memory T cells.

S.13

Thymflammation: A Novel Concept of Central Immune Tolerance

Matouš Vobořil[1]

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Central immune tolerance relies on the thymus to eliminate self-reactive T cells and generate regulatory T cells (Tregs), preventing autoimmunity. While this process is well described for tissue-restricted self-antigens presented by medullary thymic epithelial cells, how the immune system establishes tolerance to antigens expressed only during inflammation, such as interferon-stimulated genes (ISGs) or cytokine-regulated proteins, has remained unclear. Here I present the concept of "thymflammation": the thymus maintains a state of sterile inflammation to enable T cell selection against inflammation-associated self-antigens.

We show that medullary thymic epithelial cells produce type I and III interferons (IFNs) in a sterile, Aire-dependent manner, driving ISG expression in thymic antigen-presenting cells. Loss of IFN signaling impairs Treg generation, reduces TCR repertoire diversity, and compromises tolerance to ISG-derived antigens. We further identify a CD301b$^+$ dendritic cell population maintained by steady-state IL-4/IL-13 that mediates clonal deletion, establishing tolerance to type-2-inflammation-associated antigens. Finally, we describe a novel CX3CR1$^+$ transitional DC subset that continuously immigrates into the thymus from the blood, positioned near thymic microvessels, and is uniquely capable of acquiring and presenting peripheral antigens.

Together, these findings support a model in which the thymus mirrors peripheral inflammatory environments to tolerize T cells against context-dependent self-antigens, with broad implications for autoimmunity.

S.14

Early-Life Origin B Cells in the Adult Immune System

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The adult immune system is composed of cells with diverse lifespans that arise at distinct stages of ontogeny. For the past decade, work in our laboratory has centered on understanding how developmental origin shapes the composition of the adult B cell pool. Using multiple lineage tracing approaches, we have stratified murine adult B cells by the timing of their output, finding that a substantial proportion is seeded during a restricted neonatal window. Within this early-life compartment, we find not only the innate-like B-1 cells but also IgA plasma cells that are sustained by B cell memory within gut chronic germinal centers. Remarkably, this layer of neonatally derived B cells contributes to the majority of IL-10 potent regulatory B cells in the adult immune system that protects from inflammatory insults later in life.

A defining feature of these early-life B cells is expression of the fetal-specific post-transcriptional regulator Lin28B. We show that Lin28B enhances positive selection while restraining receptor editing during B cell maturation, effectively licensing the outcome of self-reactive $CD5^+$ B cells early in life. Paradoxically, it is precisely these self-reactive $CD5^+$ B-1 cells that are the most potent producers of IL-10 later in life. We propose that the transient expression of Lin28b defines a narrow but consequential window in which B cells with regulatory potential are incorporated into the long-lived B cell pool. More broadly, these findings contribute to a growing view that the neonatal period is a formative window for immune calibration. Regulatory T cells have long been appreciated as early architects of tolerance, but our data suggest that B cells established during this same period are equally important in setting the threshold for immune responsiveness later in life.

S.15

TGF-β Restricts GSDME-Mediated IL-1α Release to License Th17 Cells for Enhanced Pathogenicity

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Transforming growth factor-β (TGF-β) has potent immunosuppressive functions but is also involved in the generation of inflammatory Th17 cells with roles in autoimmunity and clearance of fungal infections. Here, we identify a previously unrecognized function of TGF-β in restraining T cell-mediated inflammation through intracellular sequestration of the unconventional danger signal IL-1α, limiting its extracellular inflammatory bioactivity. This IL-1a entrapment promotes an inflammatory preparedness in Th17 cells, enabling enhanced secretion of stockpiled IL-1a upon secondary TCR stimulation. Mechanistically, we show that TGF-β suppresses cleavage of the membrane pore-forming molecule gasdermin E (GSDME) in T cells via the c-Flip-Casp8-Casp3 axis, thereby blocking the exit route for IL-1α but not for other Th17 cell-associated effector cytokines. Innate IL-1b, but not other cytokines, could abrogate the TGF-β-mediated intracellular sequestration of IL-1a, leading to its continuous bioavailability. Our findings reveal a novel facet of TGF-β signaling, in which its anti-inflammatory effects extend to the fundamental mechanism of unconventional cytokine release, with far-reaching implications for Th17 cell-mediated immunopathology.

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Dive deep into spatial biology – MACSima and UltraMicroscope Platforms

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Spatial biology is an emerging field that studies the spatial organization of cells and molecules within tissue to better understand complex biological processes. Currently available methods may only provide information from thin tissue sections, limiting analysis to rather roughly selected areas. Our workflow combines 3D imaging with multiplex spatial analysis to get an overview of complex large samples, identify target structures within them, and to further analyze a carefully selected region in depth with hundreds of markers – all on the same valuable specimen. In this study, we present 3D-immunofluorescence (3D-IF) staining and tissue clearing to prepare mouse brain hemispheres for 3D light sheet imaging with the **UltraMicroscope Blaze™**. With the full specimen's 3D view, we were able to identify target regions in order to prepare tissue sections that precisely cover these specific parts of the specimen, a process termed light sheet guided histology. The tissue sections were further analyzed with **MACSima™ Imaging Cyclic Staining (MICS)**, providing expression levels of up to hundreds of protein markers from individual cells on a single sample.

ABSTRACTS PARTICIPANTS

P.01

Targeting the Catalytic Activity of HDAC1 in T-Cells Protects Mice against Experimental Autoimmune Encephalomyelitis

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Histone deacetylases (HDACs) are key epigenetic regulators controlling T cell mediated immunity. T cell specific deletion of Hdac1 ($HDAC1^{cKO}$) protects mice against experimental autoimmune encephalomyelitis (EAE), a preclinical disease model resembling human multiple sclerosis (MS). Some HDACs have non-enzymatic functions in addition to their catalytic activity, thus knockout of HDAC protein expression does not mimic HDAC isoform selective pharmacological inhibitor treatment. Therefore, whether inhibiting HDAC1 enzymatic activity and thus mimicking HDAC1 inhibitor treatment is sufficient to block EAE induction remains unclear. We generated a novel mouse strain expressing catalytically inactive HDAC1 ($HDAC1^{Off}$) in $HDAC1^{cKO}$ CD4 T cells to mimic selective inhibition of HDAC1 enzymatic activity *in vivo*. Control mice expressed wildtype HDAC1 in $HDAC1^{cKO}$ CD4 T cells ($HDAC1^{On}$).

$HDAC1^{Off}$ mice exhibited normal T cell homeostasis. $HDAC1^{Off}$ and $HDAC1^{On}$ forms were comparably expressed in CD4 T cells, equally-well incorporated into co-repressor complexes, with similar interactomes. As expected, deacetylase activity was markedly reduced in $HDAC1^{Off}$ CD4 T cells. IL-17A expression was reduced in *in vitro* differentiated $HDAC1^{Off}$ TH17 compared to $HDAC1^{On}$, and in contrast to normal IL-17A levels in $HDAC1^{cKO}$ TH17. $HDAC1^{Off}$ mice were fully protected from EAE, exhibiting a significant reduction in leukocyte infiltration into the CNS, and a profound decrease in pathogenic CD4 T cells. $HDAC1^{Off}$ CD4 T cells expressed substantially lower levels of proinflammatory cytokines, indicating impaired T cell effector function. Further transcriptomic analysis uncovered a broad downregulation of gene pathways governing T cell activation, inflammatory response, and T cell migration, supporting functional impairment of $HDAC1^{Off}$ T cells driving autoimmune inflammation. Our study demonstrates that inhibiting the catalytic activity of HDAC1 in T cells is sufficient to achieve a clinical benefit in EAE development. This raises the exciting translational perspective that targeting HDAC1 enzymatic activity might represent a promising therapeutic strategy in treating human T cell mediated autoimmune diseases.

P.02

IFNG-Producing Self-Reactive CD4⁺ T Cells Drive Autoimmune Adrenalitis in a Mouse Model of Addison's Disease

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Autoimmune Addison's disease (AD) is a rare, life-threatening endocrine disorder caused by immune-mediated destruction of the adrenal cortex and frequently occurs in patients with Autoimmune polyglandular syndrome type 1 due to AIRE deficiency. The pathogenesis of AD remains poorly understood, largely because of the limited availability of mechanistically tractable animal models.

We established a mouse model of Experimental Autoimmune Adrenalitis (EAA) by targeting the adrenal self-antigen CYP11A1. Immunization with CYP11A1-derived peptides induced expansion of antigen-responsive CD4⁺ T cells and promoted adrenal infiltration by CD4⁺ and CD8⁺ T cells together with myeloid cells. Inflammation progressed to a granulomatous lesion and culminated in adrenal insufficiency, characterized by impaired adrenal corticosterone production, particularly in AIRE-deficient mice with defective central tolerance.

To directly assess the pathogenic capacity of autoreactive T cells, we performed an adoptive transfer of polyclonal CYP11A1-restimulated CD4⁺ T cells prior to EAA induction. Transfer into T cell-deficient recipients accelerated adrenal inflammation and dysfunction, demonstrating that activated CD4⁺ T cells are sufficient to drive disease. In contrast, IFNG-deficient CD4⁺ T cells induced only mild granulomatous inflammation and failed to cause overt adrenal insufficiency, identifying CD4⁺ T cell-derived IFNG as a critical effector mechanism in adrenal autoimmunity.
Ongoing analysis of expanded T-cell clones suggests heterogeneous T-cell receptor (TCR) specificities toward CYP11A1-derived epitopes. These studies aim to further define the clonal architecture and antigen specificity of pathogenic CD4⁺ T cells.

Together, these findings establish a tractable model of autoimmune adrenalitis and highlight IFNG-producing CD4⁺ T cells as central mediators of adrenal pathology.

P.03

Integrative Spatial and Immune Profiling Identifies Predictors of Response to Neoadjuvant anti-PD-1 Therapy in NSCLC

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Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality. While neoadjuvant anti-PD-1 immune checkpoint blockade shows promising results in resectable NSCLC, patient responses remain heterogeneous. Emerging evidence suggests tertiary lymphoid structures (TLS) as critical mediators of anti-tumor immunity, improving outcomes in various cancers. We propose that defining the composition of TLS, and the transcriptomic and phenotypic states of intratumoral immune cells, may determine precise predictive biomarkers of response, which could enable better stratification of patients likely to benefit from neoadjuvant immunotherapy. Here, we performed multiplex immunofluorescence (mIF), RNA sequencing and spatial transcriptomics on a cohort of patients with resectable NSCLC. At baseline, RNA sequencing of baseline samples showed enrichment of genes linked to antigen presentation and T-cell immunity in tumors of patients who later achieved pathological complete response (pCR), while digital pathology analysis revealed a higher density of cytotoxic $CD8^+$ T cells and an increased number of $PD\text{-}1^+$ $CD8^+$ T cells in responders, suggesting a pre-existing immune-active microenvironment associated with treatment sensitivity. Spatial transcriptomic analysis on TLS-rich areas of samples after therapy further showed distinct immune programs between pCR and non-pCR patients. Tumors of pCR patients showed enrichment of immune activation and effector function pathways, while non-responders displayed increase in immunosuppressive cell populations, including regulatory T cells and $SPP1^+$ macrophages, which were also detected in eTLS and mTLS areas. Combining mIF with spatial transcriptomics enables detailed characterization of cellular composition and antitumor functions of the lung immune microenvironment. Understanding the differences in pCR and non-pCR patients could facilitate a better stratification of patients who will benefit from neoadjuvant anti-PD-1 immunotherapy.

P.04

Continuous LCK Signaling Maintains Mature Regulatory T Cell Function and Reveals a Homeostatic Compensation Mechanism

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The SRC-family kinase LCK is a proximal gatekeeper of T cell receptor (TCR) signaling and is indispensable for thymic T cell development. While its developmental role is well established, whether mature peripheral regulatory T cells (Tregs) require continuous LCK-dependent signaling for their maintenance and function remains unclear. Because Tregs constantly encounter self-antigen in the periphery, defining how TCR–LCK signaling sustains their homeostasis is essential for understanding immune tolerance.

To address this, we generated inducible, lineage-restricted LCK deletion models that ablate LCK in mature peripheral T cells without affecting thymic development. In Foxp3-CreERT2 $Lck^{flox/flox}$ mice, tamoxifen induces efficient, time-dependent loss of intracellular LCK in $FOXP3^{+}$ Tregs. In parallel, CD4-CreERT2 $Lck^{flox/flox}$ mice allow deletion across the CD4 lineage.

Despite efficient deletion, overall Treg frequencies remain stable. Instead, LCK ablation in Tregs results in two coexisting populations: LCK-deficient Tregs and LCK-sufficient “escapee” Tregs. LCK-deficient Tregs display impaired proximal signaling and reduced responsiveness to cognate antigen *in vivo*. Conversely, escapee Tregs exhibit a hyperactivated $CD44^{hi}$ $Ki\text{-}67^{+}$ phenotype, consistent with proliferative expansion. These findings reveal a compensatory set-point mechanism in which LCK-sufficient Tregs expand to maintain the functional Treg pool.

Comparative analysis shows that this compensatory response is stronger in Tregs than in conventional CD4 T cells, indicating that Tregs are particularly sensitive to LCK loss and more dependent on continuous TCR signaling for homeostasis. Functional assays further demonstrate that LCK ablation alters Treg suppressive capacity and metabolic fitness.

Together, our data establish that mature Tregs require sustained LCK-mediated signaling and uncover a dynamic compensation mechanism that stabilizes immune tolerance when proximal TCR signaling is disrupted.

P.05

Interplay of Cytoskeleton and Membrane Morphology During Formation of Immunology Synapse

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T-cells are key players in adaptive immunity; however, it is not fully understood how membrane architecture and the distribution of critical molecules influence their response to antigens. We propose that changes in membrane morphology at the T-cell–APC interface can control early signaling events. Our main goal is to determine how membrane dynamics and the underlying cytoskeleton drive these processes.

Using advanced microscopy, we visualized the formation of the immunological synapse (IS) and observed robust accumulation of the plasma membrane at the contact site between T-cells and APCs. Utilizing high-pressure freezing with TEM or FIB-SEM microscopy further revealed previously uncharacterized ultrastructural details of an early IS, which is formed by a surprisingly complex meshwork of intertwined membrane protrusions of interacting cell conjugates. This observation supports the notion that increased membrane surface area within the forming IS (including signalling proteins) appropriates microvilli and cytoskeleton geometry and provides the optimal microenvironment for the initial events that lead to the recognition of cognate antigen during T-cell:APC interaction.

Since we previously documented the critical role of spatio-temporal organization and dynamics of Lck and other signalling molecules in proximal TCR signalling, we have now focused on the identification of molecules which link these signalling modules to the cytoskeletal network. We discovered α-actinin-1 and 4 as cytoskeletal components of Lck-RACK1 complexes, which are transiently formed during the initiation of T-cell activation in a Lck kinase activity-dependent fashion. Deletion of α-actinin-1 and 4 resulted in altered responsiveness of T-cells upon activation indicating that α-actinins are essential regulators of T-cell signalling.

Taken together, our data adds new pieces to the puzzle showing the importance and complexity of the interplay of the cytoskeleton, membrane morphology, and signalling molecules during IS formation.

P.06

Mapping Yolk Sac Hematopoiesis Reveals a Common Hemato-Endothelial Progenitor

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During mouse embryogenesis, blood cell production is sustained by three independent hematopoietic waves. The first two originate in the yolk sac (YS), while the third arises intra-embryonically and is driven by hematopoietic stem cells (HSCs), which later establish lifelong hematopoiesis. However, HSCs are dispensable prenatally, highlighting the importance of YS-derived progenitors for early development and potential prenatal therapies.

Our study aims to map YS-derived hematopoietic lineages, investigate their origins, and assess their contribution to myeloid cell production. Using single-cell RNA sequencing (scRNA-Seq), we identified a common hemato-endothelial ancestor for YS-derived waves. We show that the first wave generates cells with megakaryocyte-erythrocyte (MkE) potential, while the second wave, driven by erythro-myeloid progenitors (EMPs), gives rise to both MkE progenitors and the first myeloid cells. To further characterize EMP-derived blood production, we identified differentially expressed genes and searched for potential surface marker candidates. Notably, we found complement receptor CD88 to be a marker of the early EMP subset, which could enhance flow cytometry-based identification. Additionally, we are developing a novel lineage-tracing model to distinguish between hematopoietic waves across different niches.

Our findings suggest that YS-derived waves share a hemato-endothelial ancestor and that EMPs are responsible for the first myeloid cells in embryogenesis. Ongoing *in vivo* validation of this model will provide deeper insights into early hematopoietic development and its clinical implications.

P.07

Same Tau, Different Matrix: Glial and Vascular Remodelling under Pharmacological and Diet-Induced Extracellular Matrix Modulation

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Extracellular matrix (ECM) composition is a key regulator of innate immune tone and cerebrovascular function, yet how discrete ECM perturbations gate glial–vascular responses in the tau-affected brain remains unclear. We used three complementary *in vivo* models to test how pharmacological and diet-driven ECM remodeling reprograms microglial and astrocytic responses, astrocyte–vascular coupling, and neurovascular structure in the context of tau pathology. In WKY rats, inhibition of hyaluronan synthesis with 4-methylumbelliferone (4MU) reduced brain hyaluronan, a backbone component of ECM, and promoted a predominantly homeostatic, surveillant microglial state. This was accompanied by astrocytic shrinkage but preserved astrocytic endfoot coverage of cerebral vessels, and coincided with an expanded vascular network and reduced perfusion, consistent with increased vessel number and angiogenesis-like remodeling. In tau-transgenic WKY72 rats, the same 4MU intervention largely maintained homeostatic-like microglia and astrocytes, albeit less pronounced than in wild-type WKY, indicating that tau partially constrains but does not abolish the pro-homeostatic impact of ECM modulation on innate immune and vascular tone. In contrast, Western diet–fed R3m4 tau-transgenic mice displayed a maladaptive innate immune profile with exhausted, lipid-laden microglia, reduced vascular density, increased hyaluronic acid deposition, and loss of neurons and parvalbumin-positive interneurons. Rather than increasing total tau burden, Western diet shifted tau toward more mature, aggregation-prone pathology, consistent with impaired clearance in an ECM-altered, metabolically stressed milieu. Together, these data show that “the same tau” can drive distinct microglial, astrocytic, and vascular programs depending on how the brain’s matrix is reshaped, highlighting ECM composition as a tractable node to re-tune innate immune programs and neurovascular responses in tauopathies

P.08

Fever Range Hyperthermia Induces Cell Cycle Progression in Calcium-Macrophages

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Introduction: Increased extra-cellular concentration of calcium and phosphate leads to formation of calciprotein particles (CPPs). The uptake of CPPs via macropinocytosis in absence of growth factors lead to differentiation of monocytes into calcium-macrophages. Calcium-macrophages are characterised by their distinctive needle-like shape and resemble macrophages with an osteopontin signature in rheumatoid arthritis (RA) synovium where concentration of calcium ions is high.

Objective: In this study, we observed the differentiation of calcium-macrophages in fever range hyperthermia (39°C) since RA can be associated with local febrile range temperatures.

Results: Calcium-macrophages show increased macropinocytosis and increased cell count over the course of differentiation in febrile temperature. We also observed significantly higher cell cycle activity in calcium-macrophages at 39°C through proliferation assays like ki-67 and EdU. Proteomic analysis of calcium-macrophages differentiated at 37°C and at 39°C also confirmed the increase in cell cycle reactomes in the latter. In normal temperature, mTORC1 is inactivated in calcium-macrophages and the glucose levels remain unchanged over the course of differentiation unlike control macrophages like M-CSF and GM-CSF macrophages. However, at 39°C, we observed an increase in mTORC1 activity and also glucose dependency during differentiation. When mTORC1 was pharmacologically activated, we observed a further increase in cell cycle markers and vice versa. When glucose was removed from the media, the cell cycle was abrogated in both normal and febrile temperature. While differentiation in normal temperature was not affected, poor differentiation of calcium-macrophages at 39°C suggested that glucose dependency play a role in the increased cell cycle activity.

Conclusion: Calcium-macrophages undergo an increase in cell cycle activity in febrile temperatures through an increased mTORC1 activity. Calcium-macrophages also switch to utilising glucose for differentiation and maintenance at 39°C unlike 37°C which aids cell cycle progression.

P.09

Defining the Role of AXL in Treating Intermediate-Stage HCC Patients

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Background: Hepatocellular carcinoma (HCC) is a global health burden due to high incidence and mortality rate. HCC is predominantly diagnosed at intermediate and advanced stages, which are treated with local transarterial chemoembolization (TACE), using the chemotherapeutics Doxorubicin (DOX) or Epirubicin (EPI). As the majority of patients show a poor response to TACE, we aim to better understand the molecular mechanisms behind drug effects in HCC. In this study, we particularly focus on the receptor tyrosine kinase AXL, which associates with drug resistance and activation of anti-inflammatory pathways. We hypothesize that AXL facilitates the escape from drug-induced cytotoxicity in HCC by promoting anti-inflammatory and thus tumor-evading mechanisms.

Methods: Publicly available RNA sequencing data of human HCC samples before/after TACE using Epirubicin were analyzed. AXL-expressing HCC cells treated with either DOX or EPI were characterized *in vitro*. DOX/EPI-driven pathways were examined by Western blotting, ELISA, and flow cytometry. AXL activation was pharmacologically or genetically inhibited using Bemcentinib or CRISPR/Cas9 editing, respectively.

Results: Analysis of RNA-seq datasets of EPI-treated versus untreated TACE patients revealed a significant activation of inflammation in the presence of AXL expression, including interferon production and DC differentiation. *In vitro,* DOX/EPI-treated AXL-positive HCC cells exhibited activation of AXL and STAT1/STAT3. However, activation and signaling of AXL were independent of STAT1/STAT3. Furthermore, DOX/EPI treatment stimulated S6 kinase, which was strongly reduced by Bemcentinib, suggesting AXL-dependent activation of the mTOR-induced translational machinery.

Conclusions: In contrast to the working hypothesis, these findings indicate a DOX/EPI-driven AXL activation correlating with the upregulation of inflammatory processes. The data suggest that HCC patients expressing AXL provide a better treatment response to TACE.

P.10

Mesenchymal Stem Cells Regulate Bone Marrow Plasma Cell Survival through Soluble Factors and Contact-Dependent Mechanisms

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Long-term serological immunity relies on bone marrow–resident plasma cells (PCs), which reside in specialized niches that support their survival and function. Although these cells were previously thought to decline with age, recent studies indicate that PC numbers may in fact increase over time. This observation raises an important question: how does the aging bone marrow microenvironment adapt to sustain long-lived PCs, and which factors contribute to this support? To address this, we used cryo-sectioning, immunofluorescence imaging and adoptive transfer assays in young and aged mice to characterize PC niches in long bones. Imaging analyses consistently showed co-localization of PCs with mesenchymal stem cells (MSCs), particularly within lower marrow regions. To investigate this interaction further, we developed an *in vitro* co-culture system using MSCs and PCs under defined conditions. Compared to media-only controls, co-culture with MSCs exhibited significantly enhanced PC survival as well as higher antibody secretion levels. Transwell experiments and exposure to MSC-conditioned media demonstrated that soluble factors contribute to PC survival; however, maximal functional support required direct cell–cell contact. Overall, these findings identify MSCs as key regulators of plasma cell survival within the bone marrow microenvironment.

P.11

Cellular Model Utilizing Primary Human Fibroblasts, Bronchoalveolar Lavage Fluid, and a Defined Fibrotic Cytokine Cocktail to Study Idiopathic Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease driven by the sustained activation of lung fibroblasts and excessive extracellular matrix deposition. Increasing evidence suggests that fibroblasts are not merely passive responders to lung injury but represent a key regulator of disease progression, hence a feasible therapeutic target. However, the development of effective antifibrotic strategies is limited by the lack of relevant and reproducible *in vitro* models. Here, we present a cellular model of IPF based on primary human lung fibroblasts that integrates either the IPF patients' bronchoalveolar lavage fluids (BALF) or a defined fibrotic cytokine cocktail to induce the fibroblast activation. The activated fibroblasts are cultured to generate the fibrosis-associated secretome and analyzed subsequently by mass spectrometry for potential biomarkers. In parallel, the model is employed to assess the effects of molecular modulators on the fibroblast activation. Not only this dual platform enables the mechanistic study of fibroblast-driven fibrotic responses but also the compound screening to identify biomarkers and therapeutics. Our model provides a versatile experimental framework bridging bench and bedside in IPF.

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P.12

The Protective Effect of Intranasally Administered Extracellular Vesicles Derived from the Probiotic *Escherichia coli* O83:K24:H31 in Murine Allergic Airway Inflammation

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Early colonization with microbiota is crucial in the development of mucosal tolerance, a central mechanism of allergy prevention. This effect can be supported through application of appropriately chosen probiotic bacteria, such as the probiotic strain of *Escherichia coli* O83:K24:H31 (EcO83) which is known to prevent allergy after early postnatal oral administration. To achieve a stronger effect in the context of respiratory allergy, intranasal administration represents a more effective route while maintaining the good safety profile of EcO83. Nevertheless, introduction of viable microorganisms intranasally is a potential risk due to the anatomical and physiological proximity of the brain. A promising, fully safe alternative are bacterial extracellular vesicles (bEVs) derived from probiotic bacteria.

Bacterial EVs are ~100 nm large membrane vesicles which can contain all elements of the parent bacterium while being completely non-infectious. They contain components of the bacterial membrane and cell wall, cytosolic elements including bacterial nucleic acids and other immunoactive MAMPs such as LPS. Numerous studies have shown that probiotic bEVs can retain the probiotic effects of the parent microorganisms. Various approaches of bEV modifications are being developed to make the effect more specific, stronger or safer. Thus, bEVs can serve as an intrinsically bioactive delivery agent of various cargo (including potential allergens) to elicit highly effective immune modulation such as tolerance induction.

EcO83-derived bEVs have been able to decrease the severity of allergic airway inflammation in a murine model of ovalbumin-induced allergic airway inflammation and exerted complex immunomodulatory effects in the lung tissue, including IL-10 induction as well as TLR4-dependent activation of macrophages, with the effect being comparable to EcO83 itself. Therefore, EcO83 bEVs serve as a plausible, safer alternative to live EcO83 in the context of respiratory mucosa.

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P.13

Immunomodulatory Effects of Encapsulated Probiotics on Adaptive Cellular Response in Rainbow Trout Infected with *Aeromonas salmonicida*

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The goal of this study was to investigate the effect of a newly developed form of fish feed containing an encapsulated autochthonous probiotic strain on the adaptive cellular immune response of fish infected with *Aeromonas salmonicida*. The probiotic strain, *Lactiplantibacillus plantarum* R2 Biocenol™ CCM 8674, was isolated from the gut of healthy rainbow trouts.

Fish with an average weight 77,74 ± 0.38 g were divided into two groups. Both of them were infected with one of the most siginficant pathogen in aquaculture – *A. salmonicida*. In the probiotic group, probiotic feed was continuously administered. The fish were supplemented with probiotics throughout the experiment (11 weeks total). In the control group, no treatment was used, and the fish were fed a commercial food. An infection was introduced in the 7th week through an immersion bath (7.33×105/ml). Samples were collected from various organs—specifically, the head kidney, spleen, skin, and gills—during weeks 8 and 10. The study focused on examining the gene expression levels of CD4 and CD8 molecules.

In the probiotic-supplemented group, secondary immune organs, including the spleen, gills, and skin, showed a significant up-regulation in surface glycoprotein levels on T-helper lymphocytes, particularly during the second sampling. In contrast, the head kidney, which functions as the primary immune organ in fish, demonstrated this increase during the first sampling. For the marker of T-cytotoxic lymphocytes, there was a significant increase in CD8 mRNA levels exclusively in the spleen and head kidney in both samplings.

These results therefore point to a possible immunomodulatory effect of the immune response after pathogen attack and to the mitigation of the overall course of infection. Notably, no excessive immunostimulation was observed during continuous application, suggesting that probiotic feed can be used in aquaculture during risk periods for disease prevention and periods of increased stress.

This study was supported by the Slovak Research and Development Agency under contract no. APVV-19-0234 and APVV-24-0348.

P.14

Primed for Residency: A Novel Systemic Memory CD8⁺ T Cell Subset Emerges Post-Respiratory Infection

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Immunological memory is a hallmark of adaptive immunity. Upon antigen recognition, CD8⁺ T cells differentiate into short-lived effector cells and long-lived memory subsets. Although memory heterogeneity is well established, the mechanisms guiding the formation of distinct memory populations remain unclear. We investigated whether pathogen type and route of infection influence the differentiation and functional properties of memory CD8⁺ T cell subsets.

We used transgenic OT-I mice expressing a TCR specific for ovalbumin (OVA) and infected them with OVA-expressing pathogens: *Listeria monocytogenes* (Lm-OVA) as a bacterial model, *Lymphocytic choriomeningitis virus* (LCMV-OVA), and Influenza A (PR8-OVA) as systemic and respiratory viral model. Effector OT-I cells were monitored in peripheral blood by flow cytometry. At memory time points (>30 days p.i.), spleen and lung populations were analyzed using single-cell RNA sequencing and flow cytometry. Endogenous CD8⁺ T cell responses were assessed to confirm physiological relevance. Functional properties were examined through adoptive transfer of memory subsets into naïve hosts followed by infection, and subsequent analysis of trafficking and tissue localization.

Distinct infection contexts preferentially generated specific memory phenotypes: bacterial infection induced central memory T cells (Tcm; CD62L⁺CCR7⁺), systemic viral infection favored effector memory cells (Tem; CX3CR1⁺KLRG1⁺), and respiratory viral infection promoted lung tissue-resident memory cells (Trm; CD69⁺CXCR6⁺). Importantly, respiratory infection also gave rise to a previously undescribed splenic memory subset (TmX) characterized by IFITM, LGALS3, and ITGA1 expression. This population was validated by flow cytometry and partially induced by respiratory LCMV infection. Although TmX cells expanded less robustly than Tcm upon recall, they efficiently trafficked to peripheral tissues and acquired Trm-associated markers.

Pathogen type and infection route critically shape memory CD8⁺ T cell formation. The identification of TmX subset reveals systemic protection upon re-infection. These results underscore the importance of considering the route of antigen exposure in vaccine design, as it may shape the quality and composition of immunological memory.

P.15

Reversible Extracellular Matrix Remodeling by Hyaluronan Depletion Reshapes Microglial and Vascular Organization

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Hyaluronan (HA), a major glycosaminoglycan of the brain extracellular matrix (ECM), is a key structural and signaling component that regulates extracellular diffusion, synaptic stability, and glial organization, yet its role in modulating microglial phenotype and glial–vascular interactions in the healthy adult brain remains poorly understood. To dissect HA-dependent regulatory mechanisms, we pharmacologically inhibited HA synthesis using dietary administration of 4-methylumbelliferone (4-MU) in adult rats. HA depletion induced pronounced but reversible ECM remodeling, including reduced perineuronal net integrity and matrix compaction. These structural changes were accompanied by significant alterations in microglial morphology, characterized by increased branching, larger cell area, and greater structural complexity, consistent with a shift toward a surveillant, homeostatic phenotype. Microglial density was unchanged, consistent with phenotypic modulation rather than cellular expansion. Despite astrocytic shrinkage, astrocyte endfoot polarity and vascular coverage were preserved, suggesting that HA depletion modifies ECM–glial signaling without disrupting blood–brain barrier integrity. At the vascular level, HA inhibition increased capillary network complexity while reducing cerebral perfusion, consistent with ECM-dependent structural remodeling. Importantly, all changes were fully reversible after treatment washout. These findings identify HA as an important regulator of extracellular matrix–mediated control of microglial phenotype and vascular organization in the adult brain, with controlled modulation of HA synthesis inducing reversible changes that reveal a previously underappreciated capacity for ECM-driven plasticity.

P.16

Development of an Ex-Vivo Human Whole Blood Assay for Profiling T Cell–Dependent Inflammatory Responses

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In contrast to single-cell or isolated lymphocyte assays, whole blood assays preserve a physiologically relevant environment that incorporates multicellular crosstalk, paracrine signalling, and plasma-mediated regulatory mechanisms, all of which critically influence inflammatory responses.

In this study, we aimed to optimise an ex vivo human whole blood assay using stimuli relevant to T cell–driven inflammatory diseases. Whole blood from healthy donors was stimulated with titrated concentrations of two reagents: a soluble anti-CD3/CD28 antibody (ImmunoCult™), to mimic T cell receptor (TCR) activation, and a TCR–MHC cross-linking reagent (CytoStim™), to emulate antigen-presenting cell–mediated T cell activation. Cytokine production was quantified using multiple platforms (HTRF™, MSD, ELISA, and LegendPlex™) at 4-, 24-, and 48-hour timepoints to assess response kinetics and assay performance.

The TCR–MHC cross-linker consistently elicited stronger and more reproducible cytokine responses compared to anti-CD3/CD28 stimulation, demonstrating clearer dose dependency and reduced donor variability. IL-8 was robustly detected using HTRF, while IFN-γ was reliably quantified by ELISA. Treatment with cyclosporin A resulted in a dose-dependent inhibition of both IFN-γ and IL-8 production, confirming the assay's sensitivity to immunomodulatory intervention. Optimal assay conditions were defined as 24-hour stimulation with 2.5 µL of the TCR–MHC cross-linker, providing a robust signal, strong reproducibility, and an appropriate assay window.

Overall, this platform establishes a physiologically relevant and scalable framework for evaluating T cell–dependent inflammatory responses, with defined optimal conditions and validated readouts suitable for drug discovery and the profiling of immunomodulatory compounds.

P.17

A Graph-Based Multi-Omics Platform for Causal Hypothesis Generation in Tumor Immunobiology

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Advanced cancer research generates vast amounts of multi-omic data, yet integrating and interpreting these diverse datasets remains a major challenge. In this project we have developed a causal analysis platform, DAIMOND, designed to integrate different data types into interconnected biological knowledge map, enabling the generation of testable hypotheses about cancer mechanisms and therapy. Here, we apply DAIMOND to study tumor hypoxia—a hallmark of solid tumors that shapes the tumor microenvironment and drives immunosuppression. By leveraging DAIMOND's integrative capabilities, we aim to elucidate hypoxia-mediated molecular networks and identify novel therapeutic strategies to overcome immunosuppression.

DAIMOND integrates genomic, transcriptomic, proteomic, pathway, disease, and experimental data into a common data model stored in the Neo4j graph database. In this model, all data are connected according to their known or predicted biological relationships forming the knowledge base. The knowledge base was constructed using KNIME workflows and data from GEO, Reactome, UniProt, NCBI, HumanNet, KEGG Disease, and in-house microarray datasets. GEO datasets were specifically selected for hypoxia- and cancer-related studies of patient tumor and adjacent normal tissues. Our microarray datasets contain all genes differentially expressed upon CA9 silencing (siCA9) in fibrosarcoma tumor cells, obtained by Clariom analysis. A key component of our workflow is AI-assisted literature curation, which includes PubMed searching and comparing hypotheses generated in DAIMOND with existing scientific literature.

The generated hypothesis suggests a convergent route where IFNα signaling connects to NK activation through CA9 silencing and downregulation of HYOU1 in cancer cells. CA9-silencing changes cytokine/chemokine secretion profile of cancer cells modulating antitumor immune responses. Loss of HYOU1 enhances IFNα signaling, which upregulates NK activating receptors and increases NK cell cytotoxicity that is typically suppressed in the hypoxic tumor microenvironment. The second causal reading suggests that hypoxia/ER-stress chaperone HYOU1 influences NKG2D abundance or signaling competence, which then affects NK cytotoxicity. Directionality is inferred from database edges and should be experimentally verified in the relevant cellular context.

This approach reduces the complexity of multi-omic cancer datasets while preserving causal and biological context. The platform aims to support the discovery of hypoxia-related mechanisms, candidate biomarkers, and potential therapeutic targets, contributing to more precise diagnostics, treatment design, and drug discovery in oncology.

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P.18

Dysregulated Sympathetic Nervous System in Systemic Lupus Erythematosus Promotes the Autoimmune Phenotype of B cells

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Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune disease, highly prevalent in females of reproductive age. Despite major improvements in survival rates due to better treatment options, there is still unmet need for more efficacious treatments to reduce disease activity, limit dependence on high-dose steroids, and improve quality of life. SLE development is not only due to genetic and intrinsic factors, but it relies also on environmental triggers. Specifically, stressful events have been linked to disease onset and flares, introducing stress-management and sympathetic nervous system (SNS) manipulation as potential therapeutic strategies. Nevertheless, the lack of knowledge of neuroimmune pathogenetic mechanisms and their role in SLE impedes clinical translation. The aim of our study is to delineate SNS activity, focusing on its key neurotransmitter norepinephrine (NE), during SLE development and to decode its effect on B cells, the major SLE pathogenic immune cell subset. Transcriptomic analysis revealed enrichment of SNS/NE-related pathways in whole blood of SLE patients compared to healthy individuals, as well as in the kidneys of pre-diseased (3-months-old) compared to diseased (6-months-old) lupus-prone NZBW/F1 mice. Accordingly, NE levels were higher in the kidneys of NZBW/F1 compared to control mice. Importantly, they declined as disease progresses, showing a 1.4-fold decrease in kidneys and 2.5-fold decrease in the spleen comparing diseased to pre-diseased mice. Exploring NE signaling pathway, several immune cells from NZBW/F1 mice presented elevated adrenergic receptor expression compared to control mice. Moreover, pre-diseased NZBW/F1 splenic and renal B cells, macrophages, neutrophils and Ly6Chigh monocytes displayed significantly higher expression of a1 and b2 receptors compared to diseased mice. Focusing on B cells, NE promoted their activation and differentiation to antibody-secreting cells. Thus, for the first time, we correlated altered NE levels – immune cell interaction with SLE disease stages and showed that NE may facilitate the switching to the autoimmune phenotype of B cells. We propose that sympathetic hyperactivity at early disease stages may introduce NE as an early mediator of immune dysregulation and SLE development with therapeutic potential.

P.19

Tick-Borne Encephalitis Virus Infection is Associated with Autophagy-Related Structures in Human Keratinocytes

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Tick-borne encephalitis virus (TBEV), a neurotropic arbovirus of the *Flaviviridae* family, represents an important cause of viral encephalitis, a potentially fatal neuroinfection prevalent in Europe and Asia. During natural infection via tick bite, the virus first encounters skin-resident cells, including keratinocytes, which represent an early barrier to infection. Autophagy is an evolutionarily conserved cellular process involved in the cellular homeostasis and antiviral responses and has been shown to be modulated by several flaviviruses. However, its role during TBEV infection in human keratinocytes remains poorly understood. Based on our results from transmission electron microscopy, when we observed the presence of autophagy-related structures in infected cells, we focused on examination of autophagy-related genes. HaCaT cell line of human keratinocytes was infected with TBEV (strain Hypr). The expression of selected autophagy-related genes was subsequently analysed by real-time PCR at different time points post infection. Cell proliferation and viability were monitored using real-time cell analysis (RTCA), and ultrastructural changes were examined by transmission electron microscopy. Although transmission electron microscopy demonstrated an increased presence of phagophores and autophagosome-like structures in TBEV-infected cells, analysed autophagy-related genes showed expression levels comparable to non-infected controls. ATG5, ATG7, ATG13 and SQSTM1 did not display consistent transcriptional changes and a light transient increase in ATG12 expression was observed. Real-time cell analysis revealed no significant differences in proliferation or viability between infected and non-infected keratinocytes or between infections performed at MOI 1 and MOI 5. These findings indicate that TBEV infection in keratinocytes is associated with ultrastructural changes in the autophagic pathway without major transcriptional changes in core autophagy-related genes or detectable effects on cell viability. This suggests that TBEV may modulate autophagy primarily at the post-transcriptional level during early virus–host interactions in skin cells and raises the possibility that autophagic membranes could contribute to the cellular environment supporting viral replication.

P.20

Essential Role of the SLC15A4-TASL Complex in Systemic Lupus Erythematosus

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Endolysosomal Toll-like receptors 7, 8 and 9 (TLR7/9) detect nucleic acids and their degradation products leading to the activation of NF-κB, MAPK and IRF signalling and the induction of protective antimicrobial responses. Conversely, aberrant activation of these receptors contributes to several autoimmune diseases, including systemic lupus erythematosus (SLE). The SLC15A4-TASL complex mediates IRF5 activation downstream of TLR7/9, and genetic ablation of *Tasl* and its paralogue *Tasl2* (*Tasl*-DKO double knockout) impairs IRF5-dependent cytokine production and antiviral responses *in vivo*, while preventing disease manifestations in a chemically-induced SLE model.

Here we assessed the relevance of this signalling axis in three distinct and complementary genetic SLE models, reflecting different disease aetiologies. In the *Fas*-lpr model, *Tasl*-KO largely prevented and *Tasl*-DKO fully normalized autoimmune manifestations, including splenomegaly, autoantibody production and immunocomplex deposition in kidney glomeruli. Furthermore, we show that *Slc15a4*- and *Tasl*-deficient mice are fully protected in the Tlr7-kika model, in which a knock-in gain-of-function mutation in *Tlr7* identified in SLE patient drives spontaneous systemic autoimmunity. Notably, SLC15A4 and TASL deficiency blunted the generation of pathogenic age-associated B (ABC) cells in both autoimmune models as well as in aged animals. Lastly, through *Tasl*-DKO splenocyte transfer in lymphopenic *Rag1*-deficient DNASE1L3 knockout mice, we demonstrate that the critical pathogenic function of the SLC15A4-TASL pathway extends to this DNA-driven, TLR9- and TLR7-dependent model, strongly supporting a B-cell intrinsic role mediating disease development.

Collectively, these data demonstrate the central role of the SLC15A4-TASL complex in SLE, supporting its potential as druggable target for this and related diseases.

P.21

Multi-Level Orchestration of γδ T Cell Development and Central Nervous System Inflammation by Retinoic Acid

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The vitamin A metabolite retinoic acid (RA) is critical for the maturation and function of the immune system, however, our knowledge regarding its role in gamma delta (γδ) T cells is limited. By specifically inactivating the RA receptor alpha (RARα) in type 3 lymphocytes and using a combination of fetal thymic organ cultures and single-cell RNA-sequencing we found that RA strengthened TCR signaling to limit the embryonic development of interleukin(IL)-17-producing γδ T cells (γδT17), while at the same time it promoted their survival after birth. In adult mice, RA imposed a multi-level transcriptional program allowing γδT17 cells to become activated, survive and sense interferon. Consequently, cells with inactive RARα responded poorly to immunization and produced low level of cytokines leading to near disease resistance in the experimental autoimmune encephalomyelitis model. Finally, RA was required for optimal expression of the α4β1 integrin and homing to the brain. Collectively, these studies suggest that by acting at various stages during the lifetime of γδ T cells, RA supports their normal development, their survival and their ability to respond to inflammatory stimuli.

P.22

Disruption of the Antibody Repertoire after CD19 CAR T-Cell Therapy in Patients with Rheumatic Autoimmune Diseases

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Background and Objectives: Rheumatic autoimmune diseases cause chronic, destructive inflammation. Conventional treatments often require lifelong use and carry significant side effects, and many patients either do not respond or experience relapse. CD19 chimeric antigen receptor (CAR)-T cell therapy has emerged as a transformative approach for B cell-driven diseases, achieving superior B cell depletion and potentially leading to sustained, drug-free clinical remission. We used phage immunoprecipitation sequencing (PhIP-Seq) to characterise the long-term effects of this novel therapy on the antibody repertoire in patients.

Methods: We screened serum samples from 33 patients with various rheumatic autoimmune diseases (including rheumatoid arthritis and systemic lupus erythematosus) from *Charité – University Hospital Berlin* and the *University Hospital Erlangen* using PhIP-Seq. For this purpose, we employed a library of 357,000 antigens, including self-antigens, microbiota, and pathogens. We quantified the stability of the antibody repertoire over time and analysed changes in its composition by comparing serum samples taken before and after CAR T-cell therapy.

Results: Our analysis shows that CD19 CAR T-cell therapy rapidly disrupts antibody repertoire stability: Patients experience a shift equivalent to a decade of natural change in healthy adults that persists for up to 12 months. Furthermore, decreased antibody responses against self-antigens and microbiota were observed, while responses against viral pathogens increased after therapy.

Conclusion and Outlook: These findings align with the current understanding that CAR T-cell therapy induces an "immunological reset" in patients with rheumatic autoimmune diseases. The observed disruption to the repertoire stability suggests a transition from a disease-associated antibody state, characterised by high levels of antibodies against self-antigens and microbiota, to a newly established repertoire. Future studies utilising larger cohorts and specialised PhIP-Seq libraries will further validate these trends and explore their clinical implications.

P.23

Itaconate as a Novel Immunometabolic Modulator of Testicular Inflammation in an Ex Vivo Fragment Model

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Infertility is a growing global health problem, affecting up to 15% of couples worldwide. Testicular inflammation is a major contributor to male infertility, yet current treatments remain symptomatic and often fail to address the cause of inflammation-driven testicular damage. This gap highlights an urgent need for novel, mechanism-based therapeutic strategies targeting local immune and metabolic pathways in the testes.

We established a novel *ex vivo* testicular fragment model from mouse and human tissue that preserves tissue architecture, key somatic cell populations and robust inflammatory and metabolic responses. This system provides a powerful and physiologically relevant platform to dissect testicular inflammation and to test immunometabolic interventions.

Using this model, we uncovered a protective role of the immunometabolite itaconate in the testes. While itaconate is classically associated with macrophage-driven anti-inflammatory responses, its function in the testes has not yet been explored. We demonstrate, for the first time, that the itaconate pathway is active not only in testicular macrophages but also in Sertoli cells. Modulation of this pathway with itaconate, its derivative and pharmacological inhibitors during LPS-induced inflammation revealed that itaconate limits inflammation and helps preserve metabolic activity in testicular fragments.

Our data identify the testis as a new site of itaconate action and highlight both itaconate signalling and our *ex vivo* model as promising tools for developing strategies to alleviate testicular inflammation and protect male fertility.

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P.24

Assessment of *P. falciparum* SLTRiP Immunogenicity and Identification of Protective and Immunodominant T-Cell Epitopes

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Malaria continues to be one of the top causes of morbidities worldwide due to the emergence of drug-resistant *Plasmodium* strains and the lack of a highly efficacious vaccine. Vaccination with irradiated sporozoites confers complete sterile protection against malaria. However, their large-scale deployment is logistically impractical, shifting the focus towards the development of subunit vaccines. In our study, we have evaluated *P. falciparum* Sporozoite and Liver Stage Tryptophan-Rich Protein (SLTRiP), a novel antigen expressed during the pre-erythrocytic stage, as a potential vaccine candidate. In a murine model, immunization with the SLTRiP protein resulted in a reduction in liver-stage parasite burden and a delayed onset of parasitaemia upon challenge, thereby providing strong protective efficacy. It contains PEXEL motifs that enable this protein to be exported into host hepatocytes. *Pf*-SLTRiP was expressed in *E. coli* BL21 RIL cells and purified via sequential affinity and ion-exchange chromatography. A transgenic *P. berghei* parasite expressing the *P. falciparum* gene was generated via the double homologous recombination method and was used as a rodent malaria challenge model in this study. Upon performing endpoint titre ELISA on sera, *Pf*-SLTRiP immunized mice showed very high antibody titres. Intracellular staining (ICS) showed a statistically significant increase in antigen-specific $CD4^+IFN\text{-}\gamma^+$, $CD4^+TNF\text{-}\alpha^+$, $CD8^+IFN\text{-}\gamma^+$, and $CD8^+TNF\text{-}\alpha^+$ T cells in immunized mice compared to non-immunized mice. Additionally, the long-term T-cell effector and central memory responses generated by *Pf*-SLTRiP immunization were also studied. Furthermore, the protein was divided into sub fragments based on T-cell epitopes, which were individually expressed, purified, and used for immunization, thereby identifying the most protective and immunodominant regions of the protein. Thus, SLTRiP demonstrates significant potential as a vaccine candidate and can be strategically integrated with other stage-specific malaria antigens to enhance immunogenicity and expand its protective efficacy. This approach could result in a dual-antigen formulation that aligns with current multi-stage malaria vaccine strategies.

P.25

Rethinking the Inflammaging Concept: The IL-10-Resistin Paradox and the Unique “Immunobiographies” of Men and Women

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Inflammaging is a systemic, sterile, low-grade chronic inflammation that characterizes the aging process and is often viewed as a unidirectional decline. However, we see a more detailed and complicated picture with “immunobiographies”: an individual's immune status represents the lifelong, cumulative result of adaptations to antigenic and inflammatory challenges. This study investigated these changes within a healthy Georgian cohort (n=150, ages 18-90), uncovering important sex related differences in immune-metabolic aging. In women, the inflammatory background is tightly regulated by the adipokine axis. Specifically, the pro-inflammatory adipokine resistin positively correlates with IL-18, IL-6, and TNFR. Female leptin levels show a dualistic pattern: they are positively correlated with IL-6 while maintaining a significant inverse relationship with IL-9 ($p=0.001$). In contrast, the male "immunobiography" is defined by a distinct metabolic-immune profile. In men, ghrelin levels correlate specifically with TNFR ($p=0.008$), and both adiponectin and ghrelin maintain an inverse relationship with body weight; this correlation is absent in the female cohort. Perhaps most interesting is the IL-10-Resistin Paradox identified in the elderly. Resistin levels rise alongside pro-inflammatory markers, but there is a simultaneous rise in the anti-inflammatory IL-10. This suggests that successful aging depends not only on reducing inflammation but also on maintaining a balanced, well-regulated immune state. After the age of 60, the association between resistin and systemic markers (IL-18, IL-6, TNFR) significantly intensifies ($r=0.512$-0.566, $p<0.0001$). These data highlight the need for a new perspective: we must standardize immune-metabolic profiles by both gender and age to accurately identify biomarkers and optimize individualized therapeutic interventions.

P.26

BHLHE40/DEC1 Programs Epidermal Langerhans Cell Identity

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Epidermal Langerhans cells (LCs) are long-lived, self-renewing dendritic cells that maintain immune tolerance and respond to environmental signals. They form dense networks in stratified epithelia and migrate to draining lymph nodes, yet the transcriptional mechanisms governing their identity remain unclear. Here, we identify the transcription factor BHLHE40 (DEC1) as a key positive regulator of epithelial LC differentiation. Transcriptomic profiling showed rapid induction of BHLHE40 downstream of TGF-β1–TGFBR1/ALK5 signaling in monocyte-committed precursors derived from human hematopoietic progenitors. Lentiviral perturbation demonstrated that BHLHE40 promotes LC differentiation, whereas its loss skews cells toward a monocyte fate. BHLHE40 enhanced transcriptional programs linked to epithelial adaptation and immunoregulation, reflected by altered activation states and epithelial/mesenchymal features. In human skin, BHLHE40 is expressed in steady-state LCs but reduced in psoriatic, monocyte-derived LCs, consistent with IL-4–mediated repression during moDC differentiation. Supporting a conserved role, Bhlhe40-deficient mice showed disruption of the epidermal LC network and reduced LC accumulation in skin-draining lymph nodes. Together, these findings establish BHLHE40 as a conserved regulator connecting constitutive canonical TGF-β1 signaling to epithelial LC homeostasis and implicate its dysregulation in inflammatory skin disease.

P.27

Deciphering Human Epitope-Specific T Cell Responses in Tonsils

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T cells recognize antigens via peptide–HLA (pHLA) complexes presented to their T cell receptors (TCRs). Although epitope-specific $CD8^+$ T cell responses have been extensively studied, current knowledge is largely derived from peripheral blood, which contains <2% of the total T cell pool and thus represents only a limited view of human immunity. In contrast, secondary lymphoid organs, such as tonsils, are central sites of T cell priming and differentiation but remain poorly defined with respect to repertoire composition and epitope specificity.

We hypothesize that T cell responses differ substantially between blood and secondary lymphoid tissues in terms of targeted epitopes, phenotypic states, and TCR repertoires. To address this, we performed single-cell RNA sequencing (scRNA-seq) of $CD8^+$ T cells from paired blood and tonsil samples of three HLA-A*01 positive donors with idiopathic tonsillitis. Epitope specificity against 16 common HLA-A*01-restricted pathogen-derived epitopes was assessed using DNA-barcoded pHLA dextramers, together with phenotypic profiling and TCR repertoire analysis.

To define spatial organization, we are currently conducting spatial transcriptomics combined with TCR sequencing on tonsil sections to map epitope-defined clones within their tissue context. In parallel, we implemented a high-throughput TCR deorphanization platform (ENTER: epitope-specific T cell recognition through engineered lentiviral particles), which has been described recently by multiple groups. This system employs engineered lentiviruses which have a mutation in their envelope protein and are displaying defined pHLA complexes. Viral entry occurs exclusively upon cognate TCR engagement, enabling simultaneous identification of TCR sequences and their specific epitopes by scRNA/TCR-seq of infected cells.

We are currently screening TCRs against a library of >2000 ENTER-encoded epitopes. This approach will substantially expand the number of functionally annotated TCRs and enables a comprehensive comparison of epitope specificity, phenotype, clonality, and spatial distribution of $CD8^+$ T cell responses in blood and tonsils. These insights will have direct implications for the development of new vaccines and immunotherapies.

P.28

Application of the CRISPR/Cas9 Method in the Preparation of MPO-Deficient Hematopoietic Progenitor Cells

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Myeloperoxidase (MPO)-ANCA glomerulonephritis is a rare autoimmune disease characterized by a loss of immune tolerance, leading to the production of anti-neutrophil cytoplasmic antibodies (ANCA) targeting the neutrophil-derived protein MPO. Affected individuals experience severe inflammation of small blood vessels, resulting in impaired kidney function that can progress to kidney failure. Current treatment typically involves a combination of glucocorticoids with other immunosuppressive agents (e.g., methotrexate, rituximab, or cyclophosphamide). However, these conventional therapies are broadly immunosuppressive and often associated with significant toxicity and adverse effects.

The primary task of our study was to prepare MPO knock-outs using the CRISPR/Cas9 method. The 32D cell line was initially utilized to optimize electroporation parameters for the most efficient transfection. Following the isolation and sorting of transfected clones, sequencing revealed one successful 32D MPO KO, which was subsequently verified at the protein level using Western blot and MPO activity assays.

After the successful preparation of MPO KO on 32D cells, the approach was translated to lineage-negative (Lin-) cells isolated from mouse bone marrow. Following further optimization, MPO-deficient Lin- cells were successfully generated with a transfection efficiency above 60% and validated at the DNA level.

The poster will also include data from a successful experimental mouse transplantation using the CD45.1/CD45.2 congenic system, done in cooperation with the group of Prof. Adrian Schreiber at Charité Berlin.

These results show preliminary data for a novel potential therapy for MPO-ANCA glomerulonephritis, using the CRISPR/Cas9 method to create MPO-deficient neutrophils in affected individuals with the aim of reducing the pathological immune response.

P.29

A20: A Checkpoint Regulator in KRAS-Driven Lung Adenocarcinoma

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Background: Lung cancer is the leading cause of cancer-related deaths and despite the success of immune checkpoint inhibitors, resistance remains a major challenge, highlighting the need for improved immunotherapies. A20, an anti-inflammatory protein, has emerged as a potential modulator of the tumor microenvironment. This study investigates how partial systemic downregulation of A20 influences immune responses in KRAS-driven lung cancer.

Methods: We used a KRAS-driven lung adenocarcinoma mouse model with an A20-heterozygous background, combined with adoptive transfer of antigen-specific T cells and *ex vivo* characterization of T cells in response to A20 downregulation.

Results: Our study shows that systemic A20 reduction in A20-heterozygous mice promotes an anti-tumorigenic microenvironment, marked by increased infiltration of $CD4^+$ and $CD8^+$ T cells and dendritic cells in lung tumors. *Ex vivo*, A20 downregulation in $CD8^+$ T cells enhanced their proliferation and cytotoxic activity. Adoptive transfer of antigen-specific A20-heterozygous $CD8^+$ T cells led to greater tumor control in $Rag2^{-/-}$ mice. Likewise, A20-heterozygous recipients showed improved tumor reduction after receiving wild-type $CD8^+$ T cells compared to wild-type hosts. Accordingly, our data suggests that A20 knockdown is both boosting cytotoxic activity and facilitating infiltration of these cytotoxic T cells into the tumor microenvironment.

Conclusions: These findings highlight the translational potential of A20 as a promising therapeutic target to optimize cellular and immunotherapies. Targeted modulation of A20 in immune cells could significantly strengthen anti-tumor immunity. Furthermore, combining A20 knockdown with immune checkpoint inhibitors may offer a synergistic strategy to boost treatment efficacy. This work lays the foundation for further exploration of A20-targeting strategies in cancer therapy.

P.30

Synthetic Polymer-Based iBodies Targeting the CD73 Metabolic Checkpoint for Cancer Immunotherapy

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Extracellular adenosine in the tumor microenvironment (TME) acts as a potent metabolic checkpoint, suppressing T-cell infiltration and effector function. CD73 (ecto-5′-nucleotidase), the rate-limiting enzyme in this pathway, is therefore an attractive target of immunotherapy. Although monoclonal antibodies (mAbs) against CD73 are in development, they face limitations in production costs, immunogenicity, and tissue penetration. We address these challenges by developing iBodies—synthetic polymer antibody mimetics based on N-(2-hydroxypropyl)methacrylamide (HPMA) scaffold that enables controlled, multivalent presentation of high-affinity ligands.

We first performed a comprehensive biochemical characterization of human and mouse CD73 to map kinetics and substrate specificity. We then adapted the DIANA (DNA-linked Inhibitor Antibody Assay) for high-throughput screening of chemical libraries and performed structure–activity relationship (SAR) optimization to nominate lead inhibitors. Lead compounds were conjugated to the HPMA backbone to generate CD73-targeted iBodies, which were evaluated in enzymatic assays, cellular binding studies, and *in vitro* T-cell activation models.

The resulting iBodies potently and selectively inhibited both human and mouse CD73 and robustly neutralized membrane-bound CD73 across various cancer cell lines. Functionally, CD73-targeted iBodies effectively abrogated immunosuppressive purinergic signalling, significantly enhancing T-cell proliferation and cytokine production compared with the corresponding small-molecule inhibitors. Together, these findings establish a biochemically optimized iBody strategy to modulate the metabolic landscape of the TME, providing a preclinical foundation for next-generation immunotherapy CD73-directed immunotherapy.

P.31

Lactoferrin-Mediated Modulation of Serine Protease in an *In Vitro* Model of Idiopathic Pulmonary Fibrosis

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Serine proteases play a central role in many physiological processes; however, when dysregulated, they contribute to various pathologies. For example, proteolytic degradation of extracellular matrix proteins and activation of cytokines by serine proteases are critically implicated in idiopathic pulmonary fibrosis (IPF). IPF is a progressive and ultimately fatal interstitial lung disease driven by aberrant fibroblast activation and pathological extracellular matrix deposition. Previously, we identified a natural inhibitor of serine proteases in milk glycoprotein lactoferrin. Here, we investigate the effects of lactoferrin-based interventions on fibroblast activation, with a focus on potential proteolysis-related mechanisms, by means of an *in vitro* IPF cellular model developed by us. In parallel, we attempt to elucidate the structural determinants of the underlying interaction between lactoferrin and the serine protease plasminogen. Our results indicate that lactoferrin modulates selected fibrotic markers under profibrotic conditions, supporting a protective role of lactoferrin in IPF.

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P.32

The Role of AIRE in Female Fertility

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The Autoimmune regulator (AIRE) is expressed in thymic epithelium where it acts as an essential factor for the establishment of central tolerance. Patients with mutations in the AIRE gene suffer from Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, i.e. multiorgan autoimmunity) syndrome, which is often manifested, among other symptoms, by infertility. Up to 70% of women with APECED develop primary ovarian insufficiency (POI) which leads to premature follicular exhaustion; a phenotype which has also been reproduced in Aire knockout mice. It is believed that this phenotype is a consequence of the breach of central tolerance caused by the malfunction of thymic AIRE.
To elucidate the cellular effectors involved in immune-mediated mechanisms, we characterized the composition and frequency of various lymphoid and myeloid cells in the ovaries of wild-type and Aire–/– mice aged 5 to 28 weeks. Compared to wild-type controls, Aire–/– ovaries exhibited increased numbers and frequencies of activated $CD4^+$ and $CD8^+$ T cells, supporting the role of autoimmunity in the development of POI. Notably, up to 10% of activated $CD4^+$ and 4% of activated $CD8^+$ T cells have MHC class II molecules on their surface in Aire–/– mice. Advanced data analysis further revealed a subset of B cells with high MHCII expression present in Aire–/– ovaries but absent in wild-type mice. These findings suggest that MHCII expression on several hematopoietic cell subsets could be a critical factor in AIRE-mediated POI pathogenesis.
Additionally, in 12-week-old Aire–/– mice, we observed that approximately 20% of ovarian non-hematopoietic cells were highly positive for surface MHCII, in contrast to only 1–2% in wild-type ovaries. These cells also very likely contribute to antigen presentation and, consequently, to the development of ovarian autoimmunity. To better understand their role in POI, we currently work on detailed transcriptomic and functional characterization of all ovarian hematopoietic and non-hematopoietic $MHCII^+$ cell subsets. Together with previous findings, our data highlights the complex etiology of POI and underscore the importance of further investigation into the role of AIRE in female reproductive health.

P.33

Cellular Vaccines in Preclinical Models: Immune Modulation from Cancer to Endometriosis

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Background and Objective: Cellular vaccines are promising innovative approaches for treatment of immune-dependent pathologies. Numerous preclinical and clinical studies have proved their potential utility in cancer therapy. The antigens on vaccine cells' surface can alert and activate the immune system, but in some cases an additional trigger is needed to boost an adequate immune response. Various interleukins can act as such adjuvant factors. Here, we summarise the effect of genetically modified, cellular vaccines in preclinical cancer models and translate this approach to endometriosis.
Methods: A genetically modified cellular vaccine was developed. Numerous *in vitro* methods, including RNA-sequencing, and a rat endometriosis model were employed to study its therapeutic efficacy. To investigate potential mechanisms of action, we assessed by FACS multiple aspects of the immune response in vaccinated animals.
Results: Cellular vaccines can serve as effective treatment methods not only in cancer but also in other immune-dependent pathologies, like e.g. endometriosis. They work in a context-dependent manner, modulating the immune system response by affecting the infiltration of immune cells (Tregs, NKs, DCs) and/or stimulation of cytokine secretion. Here we show the efficiency of engineered cellular vaccine in endometriosis treatment. We find that interleukin-specific modification of the vaccine affects cells' transcriptomes and increases its immunologic potential.
Conclusion: Cellular vaccines represent promising therapeutic strategies for cancer and endometriosis, with the ability to modulate the immune system at multiple levels. However, despite insights gained from preclinical studies, their precise mechanisms of action remain to be fully elucidated.

P.34

Synthetic Lipid Raft-Induced Plasma Membrane Phase Separation Drives T Cell Activation

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Lipid rafts are heterogeneous, dynamic, and compartmentalized membrane domains that influence protein sorting and trafficking. In T cells, lipid rafts coalesce at the immunological synapse, forming a signaling platform. T cell receptors (TCRs) and other signaling molecules are recruited into lipid rafts, while the phosphatase CD45 is excluded during early T cell activation. Cholesterol is a key component of lipid rafts, and modulation of cholesterol homeostasis profoundly influences T cell function. However, evaluating how the spatial arrangement of cholesterol affects lipid raft formation and function in live cell membranes remains challenging due to technical limitations. Here, we design a DNA origami-based cholesterol nanopatch (CNP) that organizes multiple cholesterol molecules in a programmable manner to mimic lipid rafts. Upon binding to the outer leaflet of the cell membrane, CNPs spontaneously form large, stable clusters. These cholesterol-rich synthetic rafts colocalize with the raft-associated protein flotillin-1 (Flot1) and stabilize its dynamics on the T cell membrane. CNP-induced rafts also drive the spatial segregation of CD45 while recruiting TCR, Lck and LAT, leading to ZAP-70 phosphorylation and CD69 expression. These results demonstrate that localized cholesterol enrichment drives the formation of large, polarized lipid rafts that promote T cell activation independently of antigen stimulation. Moreover, tunable control over cholesterol number, spacing, and pattern on DNA origami enables systematic investigation of lipid raft formation and its functional consequences. This platform provides a powerful tool for studying lipid raft biology across diverse cell types.

P.35

Decoding Microglia–T Cell Communication in Hypoxic Neuroinflammation

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Microglia are myeloid-derived innate immune cells residing in the central nervous system (CNS) and play a central role in regulating inflammatory responses within the neural environment. Hypoxia modulates microglial activation through HIF-dependent signaling pathways, leading to complex changes in morphology, functional phenotype, and inflammatory output. This study aimed to investigate whether chemically induced hypoxia modulates the immunological crosstalk between human microglia and T cells. Accordingly, we evaluated changes in microglial costimulatory molecule expressions (CD80, CD86, CD40), T-cell activation markers (CD25, CD69, CD40L), and the inflammatory cytokine milieu, with a particular focus on IL-10 and IL-6. Jurkat T cells were co-cultured with $CoCl_2$-treated HMC3 human microglial cells to mimic hypoxic conditions and to assess microglia–T cell interactions. In parallel, Jurkat T cells were stimulated with PMA/ionomycin to induce activation. Surface expressions of costimulatory and activation markers were analyzed by flow cytometry, while cytokine levels were measured by ELISA. Comparative analyses under normoxic and hypoxic conditions were performed to determine how hypoxia influences microglia–T cell signaling. CoCl2 treatment increased HIF-1α levels, confirming the successful induction of a hypoxia-like state in HMC3 cells. PMA/ionomycin stimulation also successfully activated Jurkat T cells, as demonstrated by the increased expression of CD69 and CD25. In the microglia–T cell co-culture system, the expression of activation-associated surface markers (CD80, CD86, CD40 in microglia; CD40L, CD69, CD25 in Jurkat T cells) was altered by hypoxic conditions. Although IL-10 was not detected, likely due to a cellular artifact, the reduction in IL-6 suggests a shift toward a less pro-inflammatory state. These findings provide new insights into how hypoxia- driven microglial signaling reshapes microglia-T cell communication.

P.36

Reciprocal NOD2/CARD9 Regulation in EoL-1 Cells and Human Eosinophils in Crohn's Disease Following Viral TLR7/8 Stimulation

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Background: Crohn's disease (CD) is a chronic immune-mediated inflammatory bowel disease influenced by genetic susceptibility and dysregulated innate immunity. Genome-wide association studies have identified NOD2 and CARD9 as major CD risk genes. In the context of viral exposure, these molecules participate in TLR7/8-associated innate immune signaling. Although eosinophils are classically linked to antiparasitic immunity, emerging evidence suggests a potential role in antiviral responses. However, the regulation of the NOD2/CARD9 axis in eosinophils under viral stimulation remains unclear.

Objective: To investigate the reciprocal regulation of NOD2 and CARD9 in eosinophil-like EoL-1 cells and primary human eosinophils following TLR7/8-mediated stimulation.

Methods: NOD2 or CARD9 were silenced in EoL-1 cells using siRNA, followed by stimulation with the TLR7/8 agonists R848 and ssRNA40. Gene expression was analyzed by qRT-PCR and protein levels by semi-quantitative immunocytochemistry. In parallel, peripheral blood eosinophils from CD patients and healthy controls were stimulated ex vivo for 24 hours, and NOD2 and CARD9 mRNA levels were assessed. Viral responses were further evaluated by measuring Killer cell Immunoglobulin-like Receptors (KIRs) on primary eosinophils using flow cytometry.

Results: In EoL-1 cells, viral ligand stimulation did not significantly alter basal NOD2 or CARD9 mRNA levels after knockdown; however, reciprocal regulatory patterns were observed following single-gene silencing. In primary eosinophils, unstimulated CD samples exhibited higher NOD2 expression than healthy controls. TLR7/8 stimulation significantly upregulated NOD2 and CARD9 compared to non-stimulated cells. Notably, NOD2—but not CARD9—expression was significantly higher in CD patients than in controls after stimulation. Among KIR receptors, activating KIR2DS4 and KIR2DS3 were regulated upon stimulation in healthy controls, whereas CD patients showed no regulation of KIR2DS4. No changes were observed in inhibitory KIR receptors in either group.

Conclusion: These findings support a role for eosinophils in TLR7/8-associated innate immune signaling and suggest differential regulation of the NOD2/CARD9 axis in CD, potentially contributing to altered antiviral responses.

P.37

Exploring the Role of RAGE in the Diagnostics of Fibrotic Processes

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Diffuse parenchymal lung diseases (DPLD) are characterized as a heterogeneous group comprising more than 200 diseases, with inflammatory and/or fibrotic processes causing parenchymal scarring and a suffocating cough.

Our study included 262 patients who came from National Institute of Tuberculosis, Lung Diseases and Thoracic Surgery, Vyšné Hágy, and Department of Pneumology and Phthisiology, Faculty of Medicine, Comenius University and University Hospital, Bratislava. Patients were divided into two groups according to the phenotype: DPLD accompanied by fibrosis and inflammatory DPLD. The first group included patients with idiopathic pulmonary fibrosis (IPF; n=57), hypersensitivity pneumonitis (HP; n=21), sarcoidosis – stage IV (s (IV); n=6) and a group of less common fibrotic DPLD (DPLDf; n=10), a total of 94 patients. The second group of inflammatory DPLD (total 174 patients) included patients with hypersensitivity pneumonitis (HP; n=39), sarcoidosis stage I-III (s (I-III); n=91) and a group of patients with less common inflammatory DPLD (DPLDi; n=14) classified as inflammatory phenotype. The control group (ctrl; n=24) consisted of individuals who underwent bronchoalveolar lavage (BAL) for diagnosis and in whom the disease was not proven. The results of our study point to an important role of the soluble molecule sRAGE in distinguishing between fibrotic and inflammatory phenotypes. We also found that the concentration of sRAGE in BAL fluid increased with advanced stage of sarcoidosis. Furthermore, *in vitro* experiments showed that lung fibroblasts produced sRAGE to a greater extent than lung epithelial cells.

A positive correlation was demonstrated between sRAGE and HRCT images of ground-glass opacities (GGO) in fibrotic phenotypes, and a significantly negative correlation of sRAGE in patients with inflammatory phenotypes.

We can therefore conclude that the association of sRAGE with DPLD phenotypes, cellular correlates, HRCT features, and functional parameters has contributed to a better understanding of the role of RAGE in the context of DPLD, but further systematic studies are needed to understand the RAGE/sRAGE axis.

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Regulatory T Cell Signatures Predict Response to Immune Checkpoint Inhibitors and Uncover Metabolic Programs Shaping Treg Function

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Immune checkpoint inhibitors (ICI) have shifted the paradigm of cancer treatment and revolutionized cancer immunotherapy. Nevertheless, more than half of the patients do not respond, while treatment is frequently accompanied by severe autoimmune toxicities. Therefore, new therapeutic strategies to harness anti-tumor immunity and predictive biomarkers of clinical responses are urgently needed. Regulatory T cells (Treg) are ideal candidates for addressing this dual challenge, as they represent a key mechanistic hub of ICI therapy; while important for maintaining peripheral homeostasis, within tumors they serve a major immunosuppressive role, dampening anti-tumor responses and impeding immunotherapy success.

Accordingly, we conducted a comprehensive single-cell multi-omic interrogation of over 150,000 Treg from peripheral blood mononuclear cells, as well as tumor specimens of melanoma patients before ICI therapy. Single-cell transcriptomic analysis identified a Treg signature predictive of response to ICI therapy, marked by downregulation of activation- and function-associated genes, termed “RegSign”, in Treg from responders. Of note, PD-1-expressing peripheral Treg with tissue-homing features exhibited the greatest differences in the “RegSign” signature between responders and non-responders, yielding superior predictive accuracy across cancer types. High-dimensional phenotypic analysis (CyTOF) and functional profiling of circulating Treg validated responders’ Treg dysfunction. Metabolomic analysis and single-cell metabolic profiling uncovered increased reliance on fatty acid oxidation and impaired triacylglycerol (TG) breakdown in the responder’s Treg. Importantly, disruption of TG breakdown phenocopied responders’ Treg features, including diminished “RegSign” signature expression and reduced suppressive function.

Collectively, we identify “RegSign” as a readily accessible, clinically implementable predictive biomarker capturing a conserved state of Treg dysfunction in responders across diverse cancer types. Mechanistically, responders’ Tregs are defined by distinct metabolic states marked by altered lipid metabolism, and perturbation of these pathways compromises Treg function while recapitulating the features of responders’ Treg.

P.39

Modulation of Selected Immune Markers after Application of Humic Substances to *Apis mellifera*

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Unlike vertebrates, honey bees possess only an innate immune response. Antimicrobial peptides (AMPs) and their related genes are integral components of the defense mechanisms of this response. Relish is a transcription factor in honey bees that regulates the expression of antimicrobial peptides genes, namely abaecin and hymenoptaecin. As honey bees lack adaptive immunity, they have evolved an effective innate defense mechanism against pathogens. In general, AMPs exhibit a broad spectrum of antimicrobial or antifungal activities. Humic substances (HS) are products of natural origin and are widely used today in veterinary medicine and agriculture. As there is still insufficient information about the effect of HS on the honey bees immune, the aim of our study was primarily to test the effect of humic substance on selected immune markers. We focused mainly on evaluating changes in the relative gene expression of selected genes (*abaecin, apidaecin, defensin 1* and *relish*), as well as determining the relative abundance of abaecin, defensin and hymenoptaecin using the ELISA method. HS significantly reduced the relative gene expression of abaecin. However, no significant changes were observed in relative abundance of all tested AMPs.

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P.40

Transitional Dendritic Cells as New Players in T Cell Tolerance

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Myeloid cells, including dendritic cells and macrophages, are essential for establishing central tolerance in the thymus by promoting T cell clonal deletion and regulatory T cell generation. Previous studies suggest that the thymic dendritic cell (DC) pool comprises plasmacytoid DC (pDC), $XCR1^+$ DC1 and $SIRP\alpha^+$ DC2. Yet the precise origin, development, and homeostasis, particularly of DC2, remain unresolved.

Our preliminary data indicate that the thymus harbors a novel thymic population of transitional DC (tDC) amongst DC2 that shares a developmental origin with pDC and possess thymus-immigrating capacity. This project aims to elucidate the origin and characteristics of tDC in the thymus and uncover their specific role in the mechanism of T cell tolerance in steady-state and during inflammation.

Specifically, the project employes intravenous labeling of cells and *in vitro* co-culture systems to determine tDC capacity to acquire and process blood-borne antigens for presentation. Using photoconvertible transgenic mice and adoptive transfer experiments, the migratory potential of tDC will be characterized under steady-state and during peripheral inflammation. To assess the importance of tDC in T cells selection, the project will utilize tDC-depleted mouse models and test the impact of tDC deficiency on T cell clonal deletion, regulatory T cells generation and subsequent development of autoimmunity under both steady-state conditions and during tissue-specific inflammation. For that, RNA sequencing of T cell receptor (TCR) repertoire, TCR cloning and the generation of T cell hybridomas will be performed.

P.41

Synthetic Antibody Mimetics as a Potential Therapeutic Tool for Targeting Acute Myeloid Leukemia Cells

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Acute myeloid leukemia (AML), the most common acute leukemia in adults, is characterized by clonal expansion of immature myeloid blasts in the bone marrow and peripheral blood, leading to bone marrow failure and ineffective hematopoiesis. The disease remains difficult to cure due to relapse driven by molecular heterogeneity and therapy-resistant clones. Current targeted strategies are limited by the lack of AML-specific antigens; however, CD64, a high-affinity IgG receptor (FcγRI), may represent a promising target. CD64 is a lineage-restricted antigen expressed exclusively on cells of the myeloid lineage, with minimal to no expression on hematopoietic stem cells. It is highly expressed in a subset of AML cells—typically those with monocytic differentiation—and is used for diagnostics and immunophenotyping, further supporting its therapeutic potential.

We described the development of CD64-targeted synthetic polymer antibody mimetics (iBodies) and cytotoxic polymer-drug conjugates (PDCs) based on an N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer scaffold. We engineered anti-CD64 iBodies that bind to CD64-positive cells, including human monocytes and AML cells. To enable targeted elimination, we conjugated a cytotoxic payload to the HPMA backbone via a cathepsin-cleavable linker, generating anti-CD64 PDCs. Upon binding, PDCs are internalized and induce apoptosis, as evidenced by caspase-3/7 activation. Our *in vitro* data suggest that CD64-positive AML cell lines are susceptible to these PDCs. Importantly, *in vivo* studies show anti-leukemic activity in cell line-derived xenografts (CDX) established in NSG-SGM3 mice. Together, CD64-targeted HPMA iBodies and PDCs provide a modular platform with promise for targeted AML therapy.

P.42

Neuroinvasive Bacteria Induce Altered Immune Response Profiles in Human Astrocytes

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Astrocytes, predominant glial cells in the brain, are a key regulators of central nervous system homeostasis and play a critical role in immune response during infection. The aim of this study was to evaluate astrocytic responses to neuroinvasive pathogens *in vitro* using RNA sequencing transcriptomic analysis. Primary human astrocytes were incubated separately with 6 neuroinvasive strains of bacteria (*Borrelia garinii, Escherichia coli K1, Klebsiella pneumoniae, Listeria monocytogenes, Neisseria meningitidis,* and *Streptococcus pneumoniae*) for 6 hours at 37°C and 5% CO2. Libraries of isolated RNA for sequencing were prepared (QuantSeq 3′ mRNA-Seq) and sequenced on Illumina NextSeq, single-end 75 bp, to a minimal depth 13 million reads per sample. RNA-seq results were validated with qRT-PCR, followed by pathway enrichment analysis using Reactome server of EMBL. The analysis focused on immune response revealed significant overexpression of pattern recognition receptor genes, such as *TLR2*, *TLR3*, and *NOD2*. In contrast, *TLR4* and *TLR5*, which are classically involved in the recognition of bacterial lipopolysaccharide and flagellin, respectively, were not induced. Activation of pattern recognition receptors triggers intracellular signaling cascades involving signaling molecules (MAP kinases, NF-κB, RIPK2) leading to the induction of cytokine expression. Our data showed significant changes in the expression of *IL1B*, *IL6*, *IL7*, *IL11*, *IL15*, *IL23A*, *IL26*, and *IL32*. The main pro-inflammatory molecule, *IL6*, remained unchanged in astrocytes infected with *K. pneumoniae*, *N. meningitidis*, or *S. pneumoniae*, but was increased by other infections. Most chemokines, including *CXCL1*, *CXCL2*, *CXCL3*, *CXCL8*, *CXCL10*, *CCL2*, *CCL4*, and *CCL20*, were upregulated. Conversely, the presence of *S. pneumoniae* did downregulated *CXCL3* and *CXCL20*. Despite the fact that astrocytes produce complement regulatory factors and receptors, only minor alterations in the gene expression were detected. These results could contribute to understanding the immune-related signaling events occurring in astrocytes in response to different neuroinvasive bacteria.
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P.43

Solutions for Simultaneous Single Cell Transcriptome Analysis and Immune Receptor Profiling

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The adaptive immune system is composed of a diverse array of antigen binding T-cells and B-cells. The diversity in T-cell and B-cell receptor sequences are governed by the recombination and somatic hypermutation in the V (variable), D (diversity) and J (joining) gene segments. Advancements in multi-omics approaches at a single cell resolution has led to adapting the cutting-edge GEXSCOPE® microwell-based technology, to provide gene expression data combined with the T-cell or B-cell receptor sequences, including the sequence information on the complementarity determining region 3 (CDR3). The improved performance of our GEXSCOPE® Single Cell V(D)J Kit enables high pairing rate of immunoreceptors. For full length immunoreceptor single cell sequencing, Singleron offers the sCircle® Single Cell Full Length TCR or BCR Sequencing Library Kit, which enables full length V(D)J region sequencing at a single cell level. Characterization of therapeutical antibodies or T cell therapies at a single cell level can now be conveniently performed on both, human and mouse immunoreceptors. Singleron's immune profiling kits, offer unique high-throughput single cell solutions for clonal analysis, antibody discovery, or developing CAR-T therapies, which can be used without the necessity of specialized equipment.

P.44

A Novel Immunoregulatory Axis: N-Terminal Ectodomain Interaction between PD-1 and E-Cadherin Drives T Cell Inhibition

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Rationale: E-cadherin, a member of the cadherin superfamily, is widely recognized for its role in mediating cell-cell adhesion. Beyond its structural function, emerging evidence highlights its immunomodulatory role, particularly through its interaction with the inhibitory receptor KLRG1 expressed on NK cells and T cells. Using biophysical and *in vitro* cell-based studies, we have identified a novel interaction between E-cadherin and PD-1, an inhibitory receptor predominantly expressed on T cells.

Methods: The N-terminal ectodomains of E-cadherin and PD-1 were expressed and purified followed by interaction analysis using Surface Plasmon Resonance. Fluorophore-conjugated E-cadherin tetramers were used to check for interaction with PD-1 expressed on T cells via flow cytometry. The effect of E-cadherin on T cell activation and differentiation was investigated to understand the functional role of the interaction. The available structures of PD-1 and E-cadherin were analyzed followed by molecular docking and mutagenesis studies to map their binding interface.

Results: Surface Plasmon Resonance analysis using purified N terminal ectodomains of E-cadherin and PD-1 revealed a direct and specific interaction between the two proteins. A high avidity E-cadherin probe further validated the interaction on PD-1 expressing murine T cells. Furthermore, we have demonstrated the effect of this interaction on T cell activation, differentiation, and effector functions through cytokine profiling and cell functionality studies. SPR-based comparative binding analyses showed that the residues within the canonical homodimeric interface of E-cadherin are involved in heterotypic binding to PD-1. Also, the mutants disrupting this interface abrogated PD-1 binding and reversed the inhibitory effect of the interaction on T cells, further corroborating the biophysical studies.

Conclusion: Our findings uncover a previously unrecognized immunoregulatory axis wherein E-cadherin directly binds PD-1 via their N terminal ectodomains to suppress T cell function. Given the widespread expression of E cadherin in the peripheral tissue as well as in certain tumors, the novel PD-1: E-cadherin interaction may have important implications in immune tolerance and tumor immune evasion.

P.45

Defining the Macrophage and Dendritic Cell Potential of Classical Monocytes in the Mouse

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Pioneering work of the Goodridge lab showed that classical monocytes can have discrete developmental origins, and we recently extended this finding to establish that this heterogeneity can impact the phenotypes and function of the tissue-resident macrophages (TRM) the monocytes give rise to. GMP-derived monocytes (GMP-Mo) are enriched for transcripts associated with neutrophils, whereas MDP-derived MDP-Mo display a transcriptomic signature akin to dendritic cells (DC). Adoptive transfer and fate-mapping studies have shown that GMP-Mo and MDP-Mo display distinct homing and/or differentiation potential, a finding recently corroborated by others. However, directly linking progenitor origin to monocyte progeny, including TRM but also monocyte-derived DC (MoDC) remains challenging. Here we will report our efforts to use Cre-recombinase-based genetic heritable barcoding, such as the LoxCode approach, to track the fate of GMP- and MDP-derived individual monocyte clones over time. This should help resolve lineage relationships and map the GMP/MDP axis at a higher resolution both in steady state and following challenge, as well as robust translation to human settings.

P.46

Safeguarding Early-Life Microbiota: The Role of Stem-Like Sca-1$^+$ RORγt$^+$ Tregs

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A balanced immune system relies on regulatory T cells (Tregs), an antigen-specific tolerogenic component of the adaptive immune system that evolved to prevent excessive immune responses. While thymus-derived Tregs generated during negative selection of self-reactive T cells play a role in self-tolerance maintaining, however a subset of microbiota-specific, peripherally induced RORγt$^+$ Tregs has emerged as a critical regulator of intestinal immunity and host–microbiota crosstalk. RORγt$^+$ Tregs begin to arise during microbiota colonization at weaning and, according to the prevailing paradigm, these weaning-derived RORγt$^+$ Tregs are considered to be a long-lived population that imprints robust oral tolerance that persist into adulthood. In contrast, RORγt$^+$ Tregs generated outside the weaning period are thought to be transient and insufficient to sustain long-term oral tolerance.

Using inducible Foxp3 lineage tracing, bone marrow chimeras, and gnotobiotic mouse models, we have identified RORγt$^+$ regulatory T cells (Tregs) as being the most clonally stable Treg population in mesenteric lymph nodes, a site where microbiota-reactive T cells are deleted or diverted into the Treg lineage in a process analogous to thymic negative selection. We show that RORγt$^+$ Tregs form a stem- and memory-like compartment characterized by high expression of Ly6A (Sca-1), which is consistent with their long-term persistence even under conditions of limited antigenic stimulation. Using S1P receptor inhibition, we provide evidence that mucosa-infiltrating RORγt$^+$ Tregs are derived from this Sca-1$^+$ pool. Once established, this compartment limits the induction of newly arising RORγt$^+$ Tregs, resulting in a clonally rigid regulatory niche. Notably, formation of a stable and suppressively competent Sca-1$^+$ RORγt$^+$ Treg compartment occurs upon first microbiota exposure, independent of developmental timing, and is followed by long-term stabilization of the RORγt$^+$ Treg pool.

Our findings suggest that RORγt$^+$ Tregs represent a previously unrecognized form of tolerogenic “memory” that safeguards beneficial primordial microbiota, supports microbial colonization resistance as a passive defense against pathogen establishment, that defines a new paradigm of gut immune homeostasis.

P.47

Breastfeeding-Derived Maternal Microchimerism: Characterization of Maternal Immune Cells in Offspring Tissues

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Maternal microchimerism (MMc) is a widely accepted physiological phenomenon in which maternal cells migrate into and persist within the tissues of the offspring. This transfer occurs during two key developmental periods–pregnancy and lactation. Although MMc has long been recognized, the presence of maternal cells in breast milk and their capacity to migrate into offspring tissues has only recently come into focus. The functional consequences of MMc remain a matter of ongoing debate. Maternal cells may beneficially affect the offspring in enhancing protection against infections, supporting immune system maturation or compensating for immunodeficiencies. Conversely, MMc has been implicated in harmful outcomes, including potential contributions to the pathogenesis of autoimmune and inflammatory diseases in susceptible individuals. This project aims to comprehensively characterize maternal-newborn microchimerism established during breastfeeding, with a particular focus on immune cell populations across various organs. Using methodologies like flow cytometry and confocal microscopy, we have identified maternal cells transferred during breastfeeding in various lymphoid and non-lymphoid organs of the offspring, with the highest frequencies detected in Peyer's patches and the highest numbers detected in the spleen and thymus. The frequency of these cells varies not only between organs but also between individuals, mouse strains, and developmental stages. Special emphasis is placed on the phenotypic characterization of maternal cells present in the thymus and spleen of 12-day-old mouse pups using single-cell RNA sequencing. The predominant maternal cell phenotype was that of T cells, but maternal NKT cells, B cells, plasma cells, and cells of the myeloid lineage were also detected. In addition, immune cells present in murine milk were analyzed to further define the cellular populations capable of migrating to the offspring and contributing to the establishment of MMc.

P.48

Prediction of CD4⁺ vs. CD8⁺ T cells from Murine Single Cell TCR Sequence Data using Machine Learning Approaches

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The surge in available single-cell data, coupled with recent advances in machine learning, has paved the way for studies that were previously difficult to conduct, including those involving T cell receptor (TCR) sequences. However, despite recent advances the significant breakthrough in predicting the specificity of a particular TCRs from its amino acid sequence has not yet been achieved. Here, we present our current results on predicting T cell fate – whether a cell becomes $CD4^+$ or $CD8^+$ – from TCR sequences alone using machine learning, an intermediate step towards the aforementioned goal. After assembling a data set of approximately 55,000 unique murine TCR sequences containing both α and β chains, we analyzed it to identify distinguishing features between $CD4^+$ and $CD8^+$ T cells. We then numerically encoded the gathered data and applied various machine learning and deep learning methods, including neural networks. Our current best model, Gradient Boosting, achieves a prediction accuracy of 74% when tested with 10-fold cross-validation. Using an external software, DeepTCR (Sidhom et al., 2021), yielded comparable results. These results indicate that machine learning can extract patterns from TCR sequences that allow for prediction of $CD4^+$ vs. $CD8^+$ T-cell fate to a certain degree. However, existing theories and the expansion of specific T-cell types in monoclonal mice, such as OT-I, suggest that the accuracy of such methods can be further improved. Moving forward, we aim to enhance the performance of our best-performing model through better parameterization and explore new models based on transformer neural networks. Additionally, to facilitate feature extraction by machine learning, we plan to refine our data encoding approach, which currently relies on dividing sequences into k-mers.

P.49

Mapping Partner-Discriminating Interaction Epitopes on HCMV UL141 Packaged into Virions

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Human cytomegalovirus (HCMV) uses multiple strategies to evade host immunity. Viral glycoprotein UL141 limits cell-surface availability of CD155/CD112 and the TRAIL death receptors (TRAIL-R1/R2) and may also contribute to virion composition and function. Here, we asked whether UL141 is exposed at the plasma membrane during infection and incorporated into virions in a receptor-binding, functionally competent state, while also delineating putative contact regions that could support interactions with several partners and enforce selectivity or exclusion between ligands.

UL141 levels at the infected-cell surface were monitored during infection. Its association with cells versus purified virions was evaluated by apparent molecular mass and Endoglycosidase H (EndoH) susceptibility. Binding of soluble receptors by intact virions was assessed using surface plasmon resonance (SPR), and predicted binding hotspots from ProMateus were projected onto the UL141–TRAIL-R2 structure, and tested for recognition by an engineered anti-TIGIT antibody using isothermal titration calorimetry (ITC).

Initial results indicate that UL141 is detectable at the surface of infected cells throughout infection. Cellular UL141 was mainly EndoH-sensitive, whereas virion-associated UL141 was full-length and EndoH-resistant, consistent with extensive glycan maturation prior to packaging. SPR measurements suggest that virion-displayed UL141 binds soluble receptors, but does so in a partner-selective fashion. Structure-informed interface mapping identified two major solvent-accessible patches, together with additional exposed regions that could plausibly mediate contacts with host ligands (e.g., CD155/CD112) and viral cofactors (e.g., UL116/US2). ITC showed binding of an engineered anti-TIGIT antibody, supporting the presence of partial TIGIT-like determinants while preserving a distinct interaction mode with TRAIL receptors.

Together, these findings position UL141 as a prominent surface antigen incorporated into virions as a mature, interaction-competent glycoprotein. The proposed interfaces provide a roadmap for identifying partner-specific binding sites and testing competition, overlap, and mutual exclusivity among UL141 ligands.

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P.50

Single-cell Profiling of Nasal Mucosa Lymphocytes in Convalescent Pertussis Patients and Upper Respiratory Tract Lymphocyte Atlas

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Bordetella pertussis, the causative agent of pertussis, remains a major public health challenge despite widespread acellular vaccination. In 2024, over 37,000 cases were reported in the Czech Republic. Early infection is marked by colonization of the nasal epithelium, where the bacterium first interacts with the host immune system. The adaptive immune response in the nasal mucosa is likely critical for preventing severe disease and limiting transmission. We performed single-cell RNA sequencing of lymphocytes from nasal swabs of convalescent pertussis patients and healthy controls and integrated these data with publicly available datasets to generate an upper respiratory tract lymphocyte atlas. This atlas revealed differences in T and B cell subsets across anatomical sites, which we validated by flow cytometry. In pertussis convalescent patients, CD8 T cells expressing Killer-cell Immunoglobulin-like Receptors (KIR), an innate-like subset enriched in the inferior nasal turbinate, were reduced. Several Toll-like receptor and interferon response genes were also downregulated. In acute COVID-19 patients, in contrast, nasal lymphocytes exhibited a strong type I interferon response, which was absent in convalescent COVID-19 samples. These findings highlight site-specific heterogeneity of upper respiratory lymphocytes and reveal how nasal mucosal immune profiles change following infections. Our results provide insights into protective immune signatures at a key mucosal barrier and may inform the design of more effective mucosal vaccines.

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Autochthonous Bee Probiotic Bacteria and Humic Substances as Immunomodulators in Honey Bees

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High mortality and morbidity of honey bees recorded worldwide are the result of multiple negative factors, among the most significant of which are climate change, the widespread use of pesticides in agriculture, and improper beekeeping practices. The impact of these adverse factors leads to a weakening of colony defense capacity, thereby increasing their susceptibility to diseases. Given the enormous economic as well as ecological importance of honey bees, scientific research is focused on identifying ways to improve colony health and enhance their immunity. We developed a probiotic preparation based on autochthonous bee lactic acid bacteria and pollen, which enhances the immunity of bee colonies and can therefore be used in the prevention of bee infections. The experimental findings consistently demonstrated beneficial effects on the gut microbiota composition, inhibitory activity against *Paenibacillus larvae*, and an overall improvement in the clinical condition of the bee colonies. These improvements were reflected in enhanced hygienic (cleansing) behavior, better colony fitness, increased honey production, and a reduced prevalence of *Varroa* mites. Furthermore, the immunostimulatory potential of the probiotics was confirmed by elevated relative expression levels of genes encoding selected antimicrobial peptides (abaecin, defensin-1) as well as some pattern recognition receptors. Modulation of the relative expression of regulatory genes involved in the Toll and Imd signaling pathways was also observed. To increase the efficacy and broaden the spectrum of action of probiotic preparation, we combined the probiotic bacteria with humic substances, which possess a wide range of biological effects, including detoxifying properties. However, they have not yet been studied in honey bees. We found that humic substances are capable of binding amitraz (the acaricide most commonly used to treat bees against *Varroa* mites), increase the richness of the bee gut microbiota, and do not significantly affect the immune response of healthy bees. In subsequent experiments, we plan to test this combination in weakened or infected bees as well.

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P.52

Peripheral cGAS–STING-Mediated Inflamm-Aging Drives Neurodegeneration

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Aging occurs universally, yet its progression varies across individuals, tissues, and cells. Neurodegeneration, a hallmark of aging, often manifests long after peripheral aging markers emerge. However, the genetic determinants linking systemic aging to neurodegeneration remain poorly understood. Mutations in leucine-rich repeat kinase 2 (LRRK2) are major genetic risk factors for Parkinson's disease (PD). The most prevalent and best studied is the G2019S mutation which increases LRRK2 kinase activity. However, the pathogenic mechanism behind LRRK2-driven PD progression and its link to aging remains unclear. By analyzing PD patients and mice with LRRK2 gain-of-function mutation (LRRK2GoF), we demonstrate that PD is an accelerated aging disease characterized by age-associated inflammation (inflamm-aging). This STING-dependent inflamm-aging first manifests in the periphery, then disrupts the blood-brain barrier and progresses to the brain, resulting in neurodegeneration as characterized by the loss of dopaminergic neurons. We further demonstrate that, mechanistically, a primary consequence of aging or LRRK2GoF is a decline in the endo-lysosomal system. This decline results in cytosolic build-up of extraneous self-DNA and subsequent increase in DNA-containing extracellular vesicles which trigger the cGAS-STING pathway. Taken together, this study unveils the cGAS-STING pathway and LRRK2GoF as key determinants and potential targets for preventive or therapeutic strategies against accelerated aging, inflamm-aging, and neurodegeneration.

P.53

A Human Immune Cell–Based Approach to Therapeutic Protein Immunogenicity

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Immunogenicity remains a major challenge in the development of protein-and peptide-based therapeutics, as it can trigger unwanted immune responses. These responses may result in the formation of anti-drug antibodies, which can compromise efficacy, alter pharmacokinetics, and provoke adverse reactions such as hypersensitivity. Consequently, assessing immunogenicity risk is essential to ensure safety and therapeutic performance during drug development.

Immune responses to biologics typically involve antigen-presenting cells (APC), such as dendritic cells and macrophages, which internalize and process therapeutic compounds and present derived peptides to T lymphocytes. Upon recognition of foreign epitopes, $CD4^+$ T cells become activated, orchestrating downstream immune responses including cytokine production and antibody formation by B cells.

In this work, we developed a panel of complementary *in vitro* human immune cell–based assays designed to capture the immunogenic potential of biologics across distinct aspects of immune activation. The Monocyte Activation Test (MAT) is routinely applied as a safety assay to assess pyrogenicity and innate immune stimulation. Antigen-specific T-cell responses are characterized using flow cytometry-based proliferation assays together with cytokine secretion profiling via FluoroSpot technology, enabling sensitive detection of effector cytokines. To more closely recapitulate physiological immune interactions, APC:T cell co-culture systems are employed, providing a functional model of antigen processing, presentation, and subsequent T-cell activation in response to therapeutic proteins.

Together, these validated approaches enable an integrated assessment of immune activation and support early-stage evaluation of immunogenicity risk, facilitating informed selection and development of safer biotherapeutic candidates.

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Divergence of Effector and Memory Fates in CD8⁺ T Cells Is Imprinted before the First Division

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Upon activation, naive CD8⁺ T cells differentiate into short-lived effector cells (SLECs), which provide immediate protection against infections, and long-lived memory cells, which retain immunological information long after pathogen clearance. Despite the crucial roles of these subsets in developing effective anti-cancer therapies and vaccines, the molecular mechanisms governing SLEC vs. memory cell commitment remain unclear.

To address this gap, we built a comprehensive single-cell multiomics atlas of CD8⁺ T cells, profiling them across multiple steady-state and infection conditions, including various pathogens and time points. Alongside conventional types of CD8⁺ T cells, this approach revealed a novel transient state in effector differentiation-dependent on the polyamine biosynthesis pathway and hence named “Tpam”.

To study Tpam cells *in vivo*, we developed an inducible OT-I TCR mouse model (TCR-switch), in which T cells initially develop with a polyclonal TCR repertoire but, upon induction, switch to express an OVA-reactive TCR. Using this system, we demonstrated that Tpam is required for the emergence of SLECs *in vivo*.

From our atlas, we also uncovered key regulatory factors of effector vs. memory cell differentiation, including Bach2, Myc, Odc1, Satb1, and Zbtb20, whose CRISPR-mediated knockouts significantly skewed T-cell fate. Moreover, single-cell RNA and ATAC multiome sequencing of the earliest T-cell divisions during infection revealed a pivotal requirement for TGF-β signaling in regulating Tpam and effector cell development. Disruption of TGF-β receptor subunits or downstream signaling components led to an enhanced effector phenotype and improved SLEC survival. Finally, spatial transcriptomics demonstrated Tpam state emergence in tissue, dependent on interactions with cDC1 dendritic cells.

Collectively, our findings indicate that effector vs. memory fate is determined remarkably early after T-cell activation, involves a newly defined Tpam transitional state, and can be manipulated through targeted genetic interventions, offering new avenues to optimize T cell-based immunotherapies.

P.55

Species- and Strain-Specific Growth Promotion by *Bifidobacterium* During Chronic Malnutrition in Gnotobiotic Mice

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The ability of *Lactiplantibacillus plantarum* WJL bacteria to support the growth of gnotobiotic invertebrate and vertebrate hosts in models of chronic undernutrition is well-established (Schwarzer et al., Science 2016). But the potential for other commensal genera to provide similar host support remains largely unexplored. In this study, we selected four bacterial strains from two *Bifidobacterium* species to evaluate their impact in a gnotobiotic mouse model of chronic undernutrition.

Germ-free C57BL/6J founders were monocolonized with *Bifidobacterium longum* ssp. *longum* (Bl367, Bl372) or *Bifidobacterium adolescentis* (Bad368, Bad373). Male offspring were weaned at postnatal day 21 onto an experimental low-protein, low-fat (MAL) diet. Growth was monitored weekly for five weeks via body length and weight measurements.

Among the tested strains, Bl372 strain significantly improved weight gain and longitudinal growth. This physiological improvement correlated with increased systemic IGF-1 levels. Histological analysis of the small intestine revealed significantly longer intestinal villi in Bl372 monocolonized mice. Bacterial load in the gut was reduced in all mice on MAL diet compared to breeding diet-fed controls. Bulk RNA sequencing of jejunal tissue revealed distinct transcriptomic shifts. Specifically, interferon gamma-response genes were significantly up-regulated upon Bl colonization.

Our results demonstrate that the Bl372 strain has the ability to support vertebrate host juvenile growth during nutritional challenge. The growth-promoting capability varies across the *Bifidobacterium* genus and is both species- and strain-specific. This highlights the importance of strain-level selection when developing microbial interventions for growth recovery in malnourished populations.

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Unravelling SARS-CoV-2 Immune Evasion: Virion-Associated Host Proteins Expand Viral Tropism and Subvert Innate Immunity

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The innate immune system constitutes the first line of defense against enveloped viruses, including SARS-CoV-2. Within the humoral arm of innate immunity, the complement system can be activated on viral surfaces, promoting the clearance of opsonized virions through direct lysis or through phagocytosis by innate immune cells recruited by complement-derived anaphylatoxins. In parallel, innate immune cells, particularly monocytes and macrophages, sense viral components via pattern recognition receptors, triggering the production of inflammatory mediators that initiate and amplify antiviral responses. Although many viruses have evolved strategies to evade these defenses, the mechanisms by which SARS-CoV-2 modulates early innate immune responses remain incompletely understood. Here we show that SARS-CoV-2 incorporates multiple host-derived cellular proteins into its virions during egress from infected cells. Through biochemical, immunological, and functional analyses, we identify several of these factors and examine their contribution to viral immune evasion and pathogenesis. We find that virion-associated regulators of complement activation CD55, CD59 and Factor H limit formation of the terminal complement complex, thereby protecting complement-opsonized virions from virolysis. In addition, host-derived cyclophilins incorporated into virions facilitate infection of ACE2-negative cells via interaction with the receptor CD147 (Basigin), revealing a non-canonical pathway of viral entry. SARS-CoV-2 infection of human blood monocytes via the cyclophilin–CD147 axis activates monocytes and induces production of pro-inflammatory cytokines including TNF, IL-1β, and IL-6, which drive the hyperinflammatory response in severe COVID-19. Ongoing work extends this analysis to additional host proteins detected in SARS-CoV-2 virions, aiming to define how virion-incorporated host factors shape viral infectivity, immune evasion, and inflammatory signaling. Together, these findings highlight a previously underappreciated role of hijacked host proteins in SARS-CoV-2 biology and provide new insight into mechanisms contributing to COVID-19 immunopathology.

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P.57

Harnessing Functional Omics to Decipher How Probiotics Reduce Antibiotic Resistance Gene Carriage in Infants' Gut

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Background: The human microbiome plays a crucial role in infection prevention, as the resident microorganisms can shield their host from harmful pathogens. However, the microbiome also harbors microbes that have pathogenic potential. The rise in antibiotic-resistant opportunistic pathogens is a major global challenge in the fight against invasive infections. This issue is particularly pressing for infants, who are vulnerable due to their developing immune systems and common exposure to hospitals during birth.

Methods: Our research explores the potential of probiotic bacteria of the Bifidobacterium species in reducing the colonization with antibiotic-resistant bacteria in the gut, specifically extended-spectrum beta-lactamase-producing Enterobacteriales (ESBL-E). The infant's gut exhibits a relatively higher carriage of antibiotic resistance genes (ARGs) than that of adults, even for infants never exposed to antibiotics. As a first step, we characterized the factors that influence the ARG carriage by analyzing metagenomic data from 14 cohorts, covering 3,981 stool samples of 1,270 infants. We identified factors that significantly influenced the gut resistome, including birth mode, gestational age, antibiotic exposure, and geographical location. A gradual decrease of Escherichia coli was a key factor contributing to the reduction in ARGs as the infants matured.

Results: Next, we examined metagenomic data from 800 Kenyan newborns who participated in a clinical trial investigating the potential of probiotic use for infection prevention (ProRIDE). Our initial results indicate that bifidobacteria-based probiotics reduce colonization by ESBL-E without raising any safety concerns. In parallel, our group has developed a method for quantitative detection of short-chain fatty acids (SCFA). SCFAs are products of microbial fermentation that are suggested to confer colonization resistance against pathogens. We are currently measuring SCFA levels in the ProRIDE samples. This work aims to clarify whether the observed decrease of drug-resistant bacteria is linked to altered fecal metabolome.

Conclusions: The results may support the use of probiotics as part of strategies to combat antimicrobial resistance and related infections in infants.

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RIG-I Regulates Hypoxia-Driven Microglial Responses

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Hypoxia is a key feature of neuroinflammatory microenvironments and critically shapes microglial activation states. In this study, we investigated the role of the antiviral sensor RIG-I in regulating microglial responses under hypoxic conditions using human HMC3 cells. Chemical hypoxia was induced with $CoCl_2$, resulting in robust stabilization of HIF-1α at the protein level, confirming successful hypoxia induction. Hypoxic stimulation significantly reduced pro-inflammatory cytokine secretion and M1 marker expression, indicating a shift toward an M2-like phenotype. In parallel, hypoxia decreased AKT and mTOR phosphorylation while increasing autophagy markers, consistent with cellular adaptation to stress conditions. Notably, RIG-I knockdown further enhanced HIF-1α expression under hypoxia and increased the suppression of pro-inflammatory cytokine production. Interestingly, silencing RIG-I reversed hypoxia-associated suppression of AKT/mTOR signalling and reduced autophagy marker expression. These findings suggest that RIG-I contributes to the fine-tuning of inflammatory signalling and stress-adaptive pathways in hypoxic microglia. Collectively, our results reveal a previously underappreciated role of RIG-I beyond its canonical antiviral function, identifying it as a key regulator of hypoxia-driven microglial phenotype and intracellular signalling pathways. Our findings suggest that RIG-I may present a promising target for modulating microglial responses in hypoxia-associated neuroinflammatory conditions.

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Cellular Mechanisms Driving Microbiota-specific T cells Differentiation into Intraepithelial Lymphocytes

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Segmented filamentous bacteria (SFB) are Gram-positive, clostridia-like commensal bacteria that have evolved a unique life strategy involving close interaction with the host epithelial cells of the small intestine. Under homeostatic conditions, SFB remain non-pathogenic and they are controlled by the immune system - primarily through Th17 cell response - which prevents them from penetrating the epithelial barrier and causing pathological inflammation. While the Th17-mediated immune response to SFB has been well characterized, other responses induced by SFB in the host intestine remain poorly understood. Our recent findings revealed the additional role of immune system in response to SFB, which involves the conversion of SFB-specific $CD4^+$ T cells into granzyme-expressing intraepithelial lymphocytes (IELs). Although the molecular pathways behind IEL differentiation are increasingly understood, the specific cell types and signals that drive this process remain largely elusive.

Our findings demonstrate that SFB-specific IELs originate predominantly from exTh1 cells, and that this process critically depends on $CD11c^+$ dendritic cells. Additionally, exploring mechanisms underlying tissue adaptation of SFB-specific IELs, we show that $CSF1R^+$ myeloid cells are required for acquisition of CD8αα, a key determinant of gut residency. Finally, we identify MHC II expression on intestinal epithelial cells and IFN-γ signaling as essential cues for SFB-specific IELs retention in the epithelium and their full tissue adaptation. Collectively, these findings substantially expand our understanding of how host–microbe interactions shape immune cell fate and function at the intestinal barrier.

P.60

Impact of Immunodeficiencies on Immunity Induced by SARS-CoV-2 Infection, mRNA BNT162b2 Vaccination, and Their Combination in Children and Young Adults

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Immunocompromised (IC) patients are more susceptible to severe COVID-19 and may exhibit reduced vaccine immunogenicity. Immune responses in children and adolescents differ from adults, yet IC pediatric population remains understudied. We assessed humoral and cellular immunity in IC children, adolescents, and young adults following SARS-CoV-2 infection, two doses of the mRNA BNT162b2 vaccine, or a combination of both (hybrid immunity).

The study included 102 IC patients (5–25 years) with primary immunodeficiencies (n=17), bronchial asthma/allergic rhinitis (n=39), rheumatoid diseases (n=21), or after hematopoietic stem cell transplantation (n=28), as well as 30 age-matched controls. Plasma IgG, IgA, IgM antibodies targeting S protein were quantified by ELISA. Antibody-mediated inhibition of S protein internalization was tested using ACE2-overexpressing HEK293T cell line. S-specific CD4 T-cells were examined by flow cytometry-based proliferation assay (FASCIA).

All but eight IC patients produced detectable levels of IgG antibodies. The IgG titres after vaccination (Geometric mean titre (GMTvac) = 205023, 95% CI: 116074-362136) and a combination of vaccination and infection (GMThyb = 172819, 95% CI: 33133-901403) were higher than after infection alone (GMTinf = 3323, 95% CI: 578-19109, Pvac/inf = .006 and Phyb/inf = .001). Hybrid immunity induced the highest IgA titres (GMThyb = 2672, 95% CI: 566-12623) compared to vaccination (GMPvac = 275, 95% CI: 97-777, Phyb/vac = .016) or infection (GMTinf = 60, 95% CI: 13-280, Phyb/inf = .002). The IgG titres in vaccinated and hybrid immunity groups strongly correlated (r = 0.86, P <.0001) with the levels of neutralizing antibodies, determined by the cell-based S protein internalization assay. Most IC patients (except five) also developed above-threshold S-specific CD4 T-cell responses comparable to controls.

Both infection and vaccination elicited protective humoral and cellular immunity against SARS-CoV-2 in young IC individuals. These findings contribute to a better understanding of immune responsiveness in this population and hold relevance for clinical practice.

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Development of an *In Vitro* Human CD8⁺ Tc17 Assay for IL-17 Linked Inflammatory Disease

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IL-17 is a key driver of pathology in inflammatory diseases such as psoriasis, inflammatory bowel disease and cancer, and inhibition of the IL-17 pathway has delivered strong clinical benefit. While IL-17 responses are primarily attributed to Th17 biology, cytotoxic T cells ($CD8^+$) can also adopt an IL-17 producing "Tc17" phenotype and are increasingly implicated in these conditions. Despite this, assays to quantify IL-17 production by $CD8^+$ cells remain limited, restricting consistent translational assessment of new compounds.

We developed a reproducible *in vitro* assay to induce and measure Tc17 differentiation in primary human $CD8^+$ memory T cells. $CD8^+$ memory T cells were isolated from leukocyte cones from healthy human donors and cultured for 4 days with a Tc17 polarising stimulation cocktail containing IL-17 promoting cytokines. Tc17 differentiation and activation were assessed by flow cytometry using extracellular and intracellular staining for markers including IL-17, RORγT, CD25, PD-1 and granzyme B. Cytokine secretion was quantified by HTRF, measuring IL-17, TNFα, IL-6 and IL-23.

Stimulation induced robust proliferation and increased expression of Tc17 associated markers and effector readouts, including elevated IL-17 alongside granzyme B, with corresponding cytokine release detected by HTRF. Tool compounds, including the JAK inhibitor tofacitinib, attenuated the inflammatory response of these cells, demonstrating the potential of this assay to test therapeutic compounds in development. Together, this assay provides a practical and scalable foundation for reliable evaluation of tool compounds targeting Tc17 biology and for broader compound testing in IL-17 linked inflammatory disease.

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Deciphering the Development of the Adaptive Immune System in Early Life

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The transition from passive maternal antibody protection to endogenous infant immunity represents a critical yet incompletely characterized process in early immune development. Here, we used phage immunoprecipitation sequencing (PhIP-Seq) with a library of 357,000 peptides to profile longitudinal immune responses in 128 mother–infant dyads across 9 timepoints, measuring antibody binding against viral, bacterial, food, and environmental antigens in maternal blood across pregnancy, breast milk, and infant blood in the first year of life. At birth, cord blood repertoires closely mirrored maternal profiles, confirming efficient transplacental IgG transfer and carrying sufficient individual specificity to distinguish familial relationships. Maternally derived antibodies against pathogens declined substantially by three months, marking a critical window of immunological vulnerability, while some of the earliest endogenous responses were directed against dietary proteins. Stratification by feeding mode revealed that timing of antigen introduction was the primary determinant of food-specific IgG. The transition to complementary foods elicited strong humoral responses in early life, including epitope-specific IgG against milk, cereal, and legume proteins. Infants also mounted robust responses against commensal bacterial antigens, suggesting active calibration of mucosal tolerance.
Breast milk profiles remained stable throughout lactation and were largely consistent with maternal blood. By 12 months, infant repertoires had diverged entirely from maternal profiles, with the magnitude of remodeling exceeding that observed in adults over a decade. These findings provide a comprehensive epitope-resolved map of early-life humoral immune maturation, establishing a framework for understanding how the interplay of maternal passive immunity, microbial exposure, and dietary antigens shapes humoral immunity during the most dynamic period of immune development.

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Development and Homeostasis of T cells in Inborn Errors of Immunity

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In 2024, a nation-wide TREC screening programme was implemented across Czech Republic in order to identify newborns with congenital immunodeficiencies. The screening reveals defects in thymus development caused by a deletion on chromosome 22 (22q11.2) or DNA repair defects such as ataxia telangiectasia (AT), Nijmegen breakage syndrome (NBS) and Schimke immuno-osseous dysplasia (SIOD). Patients with both thymus development and DNA repair defects are characterized by T cell lymphopenia for which no treatment is available yet.

Patients with defects of thymus development and DNA repair defects are the main focus group of the project for two reasons. First, in terms of T-cell phenotype they are similar, shown by T cell lymphopenia and a Th1-IFNγ bias in the few present T cells, leading to chronic inflammation and eventually T cell exhaustion. Second, no treatment is readily available for patients of these groups.

The aim of the project is to characterize development, phenotype and responses of T cells in patients with congenital immunodeficiency and investigate potential therapy approaches. We will investigate how T cell phenotype differs in thymus development and DNA repair defect induced lymphopenia. In collaboration with the clinical department of Motol University Hospital we will study T cell populations present in blood and respiratory epithelium of immunodeficient children by using single cell RNA sequencing. In order to assess potential T cell lymphopenia therapies, we will use a mouse model of 22q11.2 deletion syndrome from the Riken repository in Japan.

Prior to testing of the treatments of lymphopenia, we will conduct a detailed characterization of T cell development, homeostasis and populations in del22q11.2 mice in both steady state and infection. Alongside testing on mice, potential therapeutics will be examined on artificial thymic organoid culture model made from induced pluripotent stem cells derived from human peripheral blood cells. Coupling of *in vivo* testing on mice and *in vitro* testing on artificial thymic organoids will result in a selection of therapy which is able to effectively restore thymic development and enchance effector functions of T cells without triggering autoimmunity and/or allergy.

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Enhanced Mobilization of Peripheral Osteoclast Progenitors and Bone Resorption in Streptozotocin-Induced Type 1 Diabetes in Mice

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Background: Monocyte/macrophage lineage derived osteoclast progenitors (OCPs) reside in the bone marrow, spleen and circulation, from where they can be mobilized to bone surfaces under inflammatory conditions and differentiated into bone-resorbing osteoclasts. Type 1 diabetes mellitus (T1D) is associated with chronic inflammation (elevated cytokines, advanced glycation end-products) and affects the circulatory kinetics of monocyte-derived OCPs.

Purpose: To assess the migratory properties of OCP subsets and their contribution to bone resorption in a streptozotocin (STZ)-induced T1D mouse model.

Methods: T1D was induced in male mice (9-11 weeks) by STZ administration (50 mg/kg i.p.) for 5 days, and monitored by fasting glycemia, body mass, glucose tolerance test and pancreatic histology. $CX3CR1^+$ OCPs were sorted by flow cytometry, differentiated into osteoclasts with M-CSF and RANKL, or subjected to *in vitro* migration assays using *Transwell* system. Systemic markers (TRAcP5b, CTX-I) of bone resorption were measured by ELISA/EIA. *In vivo* migration of $CX3CR1^+$ OCPs was evaluated using CX3CR1GFP knock-in/knock-out mouse strain. Lineage tracing and effect of deletion of $CX3CR1^+$ OCPs were assessed using inducible CX3CR1CreERT2/Ai9(TdTom) and CX3CR1CreERT2/DTA mouse strains, after tamoxifen treatment.

Results: STZ-treated mice developed hyperglycemia, weight loss, impaired glucose tolerance, pancreatic damage and increased serum TRAcP5b. $CX3CR1^+$ OCPs ($CD45^+CD3^-B220^-NK1.1^-Ly6G^-CD11b^{low/+}CD115^+$) in bone marrow and spleen were expanded, displaying an activated profile (RANK, CCR2, CD64, TLR4, CD27) in T1D. *In vitro*, T1D-derived $CX3CR1^+$ OCPs exhibited enhanced migration toward CCL2, and increased osteoclastogenic potential. *In vivo*, CX3CR1-deficient mice showed fewer bone-associated OCPs than CX3CR1-expressing littermates. Labeled $CX3CR1^+$ OCPs were able to circulate in the parabiosis model, and adhere to bone surfaces, forming multinucleated osteoclasts, in the lineage tracing experiment.

Conclusions: Activation and enhanced migration of $CX3CR1^+$ OCPs drive their recruitment from the circulation to bone, likely causing the accelerated bone resorption in T1D.

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Expression Profiling of Endothelial Injury Markers in Systemic Sclerosis: An *In Vitro* Approach

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Vascular dysfunction following endothelial cell injury is common across several autoimmune conditions, including systemic sclerosis (SSc). The initial manifestation of the disease is associated with vascular abnormalities and endothelial damage, including a clinical presentation of recurrent ischemia-reperfusion (I/R) events, defective angiogenesis, impaired vessel wall regeneration, autoimmune processes, and abnormal extracellular matrix production and deposition. While the background of the vascular damage remains unknown, microvascular injuries are followed by inflammation and the appearance of anti-endothelial cell antibodies (AECA), which might induce vascular dysfunction.

In our research, sera from patients with SSc and healthy controls were collected. The presence of disease-specific autoantibodies, such as anti-Scl-70, anti-centromere along with AECA was determined, and IgG fractions were purified and were used to stimulate *in vitro* HUVEC cell cultures. Following the *in vitro* treatments, total RNA was isolated from the cells. After cDNA synthesis, we analyzed the gene expression levels of specific markers characteristic of endothelial damage—including VCAM-1, ICAM-1, endoglin, endothelin-1, etc.—using real-time PCR. Besides gene expression studies, we measured the above-mentioned activation markers by cell surface staining using flow cytometry.

During the course of our work, we successfully established the *in vitro* cultivation and serum treatment protocol for HUVEC cells. Our gene expression analysis and flow cytometry studies indicate that samples containing SSc-specific autoantibodies induce significant changes in endothelial cells, particularly regarding markers indicative of endothelial activation.

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Improved Uterine Structure and Function Following Combined Pirfenidone and MSC-Conditioned Medium Therapy in a Murine Model of Intrauterine Adhesions

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Background: Intrauterine adhesions (IUAs), resulting from endometrial injury or infection, are a major cause of infertility, recurrent pregnancy loss, and menstrual disorders. The condition is initiated by a hyper-inflammatory response ultimately progressing to fibrotic remodeling of the endometrium. Current treatments are invasive and associated with a risk of recurrence, highlighting the need for effective regenerative and anti-fibrotic strategies. This study evaluated pirfenidone (PFD), an anti-fibrotic and FDA-approved agent, alone or in combination with mesenchymal stem cell-conditioned medium (MSC-CM), in a murine model of IUAs to enhance therapeutic efficacy while potentially reducing drug dosage.

Methods: MSCs were isolated from human umbilical cord tissue, and conditioned medium was collected. IUAs were induced in NMRI mice via mechanical endometrial scraping. Animals were assigned to seven groups: normal, sham, IUAs + PBS, IUAs + MSC-CM, IUAs + PFD (100 or 200 mg/kg), and IUAs + PFD (100 mg/kg) + MSC-CM. Treatments were initiated five days post-injury and continued for one week. Endometrial repair was assessed by hematoxylin and eosin and Masson's trichrome staining, α-smooth muscle actin (α-SMA) immunohistochemistry (IHC), fibrosis-related gene expression (α-SMA, TGF-β), and pregnancy outcomes.

Results: High-dose PFD significantly improved endometrial thickness and gland density while reducing fibrotic area. Both PFD doses decreased fibrosis-related gene expression (α-SMA and TGF-β). Improved pregnancy outcomes were observed in the high-dose PFD and combination therapy groups. Notably, low-dose PFD combined with MSC-CM produced comparable functional recovery to high-dose PFD alone.

Conclusion: Pirfenidone combined with MSC-conditioned medium enhances endometrial repair and improves fertility outcomes in experimental IUAs. Notably, this study represents the first report demonstrating the effect of PFD on IUAs. The combination approach may permit effective anti-fibrotic therapy at lower drug doses, supporting its potential as a regenerative treatment strategy.

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Effect of Probiotic-Enriched Feed on Immune Response in Rainbow Trout under Aquaculture Conditions

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Probiotics are underused in fish farming despite their proven benefits: reduced bacterial load, better growth, digestion, reduction of stress, and improved water quality.

This study examined the effect of feeding rainbow trout with feed enriched with the probiotic bacteria *Lactobacillus plantarum* R2 and *Lactobacillus fermentum* R3. The experiment was conducted in field conditions in an aquaculture flow-through system on juvenile trout. During the two weeks when the fish were fed probiotic feed, intestinal tissue samples were taken on days 3, 7, and 14. The control group received the same feed without probiotics. The effect of probiotic feed on the immune system of trout was evaluated based on the analysis of relative gene expression using qPCR. The genes for *transforming growth factor-β*, *interleukin 8*, *interleukin 1β*, and *immunoglobulin M* were analyzed. The results showed that probiotic feed has the ability to modulate the immune response of fish. *G*ene expression of *igm* was increased after three days of probiotic feed administration compared to the control, and this trend persisted throughout the experiment. Increased antibody production in the body increases an individual's readiness for potential infection. The initial increase in the expression of pro-inflammatory *il-8*, which has a chemotactic function, probably indicates activation of the immune system, and gene expression returned to the control group level after the initial increase, which can be considered positive, as a prolonged increase in expression associated with stimulation of the immune system may not be beneficial for fish. The expression of the second pro-inflammatory cytokine i*l-1β* was not increased at the beginning of the experiment, and an increase was only observed in the sample taken after 14 days, which could be the result of secondary immune stimulation unrelated to probiotics. The regulatory cytokine *tgf-β* had lower expression in the probiotic group compared to the control, but only during the second sampling, suggesting a potential suppression of the immune response.

The results of this study suggest a beneficial immunomodulatory effect of the probiotic feed, with potential for use in large-scale fish farming during periods when fish are exposed to stress.

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Elevated Plasma Adiponectin Levels in Patients with Late-Onset Alzheimer's Disease

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Adiponectin is an adipokine secreted by adipose tissue, playing an important role in metabolic regulation, insulin sensitivity, and inflammation. Emerging evidence suggests that adiponectin may also be involved in the pathogenesis of Alzheimer's disease (AD), although the nature of this association remains unclear. This cross-sectional study aimed to analyse plasma adiponectin levels in patients with late-onset Alzheimer's disease (LOAD), compare them with those of control individuals, and investigate their association with selected clinical parameters. The patient group included 159 LOAD patients (mean age: 78.87 ± 5.93 years) and 101 age-matched controls (mean age: 71.15 ± 6.5 years) without cognitive impairment. Adipokine plasma levels were determined using the sandwich ELISA method, and APOE ε4 genotyping was performed by direct sequencing. Data analysis revealed that plasma adiponectin levels were statistically significantly higher in LOAD patients compared with control subjects ($p = 0.003$). In both the LOAD and control groups, women exhibited significantly higher adiponectin levels than men ($p=0.0006$ and $p < 0.0001$, respectively). A positive correlation between adiponectin levels and age was observed in the LOAD group but not in controls. In conclusion, our findings demonstrate elevated plasma adiponectin levels in patients with LOAD and support the hypothesis that adiponectin may be involved in AD pathophysiology. The observed associations with age and sex further highlight the complexity of adiponectin regulation in ageing and neurodegeneration. Additional studies are needed to clarify the biological significance of increased adiponectin levels in AD and to evaluate its potential utility as a biomarker of the disease.

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Monitoring of Low Affinity Allergen-Specific IgG Antibodies During Allergen Immunotherapy

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Introduction: Allergen immunotherapy (AIT) modifies allergic immune responses by inducing allergen-specific IgE-blocking antibodies (Abs) that can inhibit IgE-mediated effector cell activation, mainly IgG1 and IgG4. However, not all AIT-induced antibodies are functional blockers and their proportion remains unclear. Assuming that high affinity is crucial for functional blocking we hypothesized that affinity-based separation of polyclonal allergen-specific serum antibodies allows the monitoring of protective responses during AIT.

Methods: Monoclonal antibodies with varying affinities for the model allergen, the major birch pollen allergen (Betv1), as determined by surface plasmon resonance, were spiked into serum from a non-allergic individual to establish affinity-based separation using NHS-Sepharose beads loaded with recombinant Betv1. Subsequently, sera collected longitudinally during AIT from 8 patients were incubated with Betv1-loaded beads. After centrifugation, supernatants and original sera were compared for allergen-specific Ab levels and their net-binding force by ELISA and acidic dissociation ELISA, respectively. IgE-blocking was tested by reduction of Betv1-induced CD63 expression on human basophils and murine bone-marrow-derived mast cells expressing human FcεRIa by flow cytometry.

Results: Abs with KD<250 nM (designated high-affinity) bound stably to the allergen-loaded beads and were depleted from sera, generating a fraction of negatively selected low-affinity Abs. Compared to the original sera, they had a lower net-binding force and neither prevented allergen-induced activation of heterologous basophils nor of mast cells sensitized with autologous IgE. The proportion of low-affinity Abs varied individually, comprised <50% of allergen-specific antibodies at all time points, and consisted of IgG1-3, but not IgG4. Low-affinity IgG1 and IgG3 levels increased during AIT.

Conclusion: For the first time, the proportion of allergen-specific low-affinity Abs was monitored during AIT. Since they lacked protective function, our approach allows conclusions on the proportions of functional blocking Abs. Contradicting the expected affinity maturation in response to allergen administration during AIT, low-affinity IgG1 increased with continued therapy.

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Uncovering the Magic of Memory CD8$^+$ T Cells

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Cytotoxic CD8$^+$ T cells generate long-lived memory T cells following encounters with pathogens or vaccinations, thereby providing the host with long-lasting protection. After an infection peak, antigen-specific memory T cells outnumber naive T cells with the same antigen-specificity, providing a quantitative advantage during subsequent cognate challenges. In our study, we aimed to elucidate qualitative differences between memory and naive CD8$^+$ T cells following antigen-specific infection. Previous research indicated that memory T cells confer superior protection against Listeria compared to their naive counterparts on a per-cell basis; however, the underlying molecular mechanisms remain poorly understood. We hypothesized that the production of effector molecules such as granzyme B and IFNγ or the trafficking potential of memory cells to the infection site mediated by Core 2 O-glycosylation via GCNT1 could be implicated in this enhanced protection. Our findings point to the direct cytotoxicity of highly proliferative memory cells in the early stage of infection as the major mechanism of this rapid protection. Although memory cells proliferate sooner, naïve cells catch up at the peak of infection and undergo stronger contraction. Moreover, the type of the original cells influenced the predominant subset of memory CD8$^+$ T cells generated post-infection: naive CD8$^+$ T cells gave rise to more central memory CD8$^+$ T cells, whereas central memory cells evolved into long-lived effector memory T cells, which retained their effector phenotype. Our study describes the single-cell differences between memory and naive CD8$^+$ T cells on transcriptional, phenotypical, and functional levels during infection, which could be beneficial in the optimal T-cell-based vaccine strategy designs.

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Langerhans Cell Histiocytosis Cells Exhibit Regulatory Dendritic Cell Characteristics due to Aberrant Induced RelB and Bone Morphogenetic Protein Signaling

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Introduction: Langerhans cell histiocytosis (LCH) represents a focal or systemic myeloid malignancy, occurring in children and adults, and is frequently driven by the oncogenic BRAFV600E mutation in hematopoietic stem/progenitor cells (HSPCs). Systemic LCH can manifest as a life-threatening therapy-refractory disease, warranting the identification of new therapeutic strategies. Immune evasion by regulatory T cells (Tregs) might be targeted, given elevated frequencies of Treg cells in LCH, but how LCH cells may induce high Treg cell frequencies remained elusive. Methods: We here employed gene edited human $CD34^+$ hematopoietic stem/progenitor cells (HSPCs) carrying disease-relevant monoallelic expression levels of BRAFV600E to address this question. Results: Comparison of *BRAFV600E/WT* vs *BRAFWT/WT* HSPCs revealed the induction of the non-classical NFkB transcription factor RelB. RelB protein is expressed by lesional $CD207^+CD1a^+$ LCH cells, but not in normal skin or lung. CRISPR-Cas9-mediated deletion of RelB in BRAFV600E/WT progenitor cell-derived LCs abrogated their capacity to induce Treg cells from naïve CD4 T cells. Moreover, RelB was required for the expression of CD14 by LCH cells, a well-documented aberrant marker in LCH diagnosis. BRAFV600E generally interfered with ordered monocyte to LC re-programming, and RelB signaling intersected with aberrant high TGF-B1 autocrine signaling in LCH differentiation. Autocrine TGF-B1 is an upstream inducer of Notch pathway members during LCH cell differentiation and was functionally required for Notch signal-dependent LCH cell differentiation. Moreover, TGF-B1 utilized the BMPR1a signaling cascade for inducing LCH differentiation and E-cadherin expression. Their BMPR1a dependency assigns LCH closely to monocyte-derived LCs in psoriatic inflammatory lesions, previously shown to maintain Treg cells in a BMPR1a signal-dependent manner. Conclusions: In conclusion, we showed here that RelB is essential for Treg cell induction by LCH cells. Moreover, BMPR1a is functionally required for LCH differentiation and intersects with RelB in inducing LCH formation and Treg stimulatory capacity.

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Before Translation: Covering the Bases of Inter-Donor Immunomodulation Among Mesenchymal Stromal Cells

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Pluripotent mesenchymal stromal cells derived from bone marrow, adipose tissue or the umbilical cord are promising candidates for human cell therapy due to their high regenerative and immunomodulatory capabilities. Multiple clinical studies attempted transfer of autologous, allogeneic, or genetically modified MSCs to treat a broad range of diseases, including GVHD, OA, MS, Covid-19, diabetes, myocardial infarction, and prostate cancer.

Full translation and reproducibility, however, are complicated not only by the shortcomings of preclinical and *in vitro* models, but also by the variability of MSCs derived from different individuals. Pooling MSCs from several donors is an effective strategy to increase cellular yield and mitigate donor variability and standard deviation of read-outs. However, it is unclear how the full breadth of donor capabilities is reflected in the pooled homogenate over time.

In a recent study we analyzed metabolic activity, proliferation, differentiation, and migration of pooled versus individual cultures. High-fitness donors soon dominated even pools composed of individuals with similar fitness. More importantly, we found that collective read-outs often deviated from or performed worse than average individual outcomes. This points to emergent behavior arising from co-cultivation and dynamic juxta- and/or paracrine modulation between donors. We are currently employing qPCR, flow cytometry and fluorescence microscopy to establish individual profiles for cytokines, senescence, and functional immunosuppression during co-culture with immune cells. With subsequent time-course analysis of pooled cultures we hope to gain further insights into inter-donor communication, to better inform and streamline MSC pooling for therapy.

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In Vitro Study of the Interferon Response Induced by Zoonotic Tick-Borne Orbiviruses (Kemerovo Virus, Tribeč Virus)

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The Kemerovo virus (KEMV) and the Tribeč virus (TRBV) are zoonotic orbiviruses that have previously been associated with central nervous system diseases in humans. This study aimed to compare the induction of type I interferons (IFN-I: IFN-α and IFN-β) by these viruses in bovine cells, mouse fibroblasts, and human astrocytes. For the *in vitro* infection, the cells were incubated with KEMV or TRBV (multiplicity of infection (MOI) = 0.1) for one hour, after which the viral inoculum was removed. The infected cells were then further incubated in complete culture medium at 37 °C in a CO_2 atmosphere. For IFN-I gene expression analyses, the cells were lysed 24 and 48 hours post-infection (hpi). Higher mRNA levels for IFN-β were observed in KEMV-infected cells than in TRBV-infected cells in each of the studied models. This difference was most pronounced in human astrocytes (24 hpi) and mouse fibroblasts (48 hpi). In human astrocytes, the difference in IFN-β expression was as follows: KEMV 2700× vs. TRBV 0.7× at 24 hpi and KEMV 2000× vs. TRBV 220× at 48 hpi. In mouse fibroblasts, a tenfold difference in IFN-β mRNA levels (KEMV infection vs.TRBV infection) was observed at 48 hpi, with values of 2303.98 ± 62.26 and 214.06 ± 24.49 for KEMV and TRBV, respectively. Regarding IFN-α mRNA levels, downregulation was observed in TRBV-infected astrocytes, whereas the highest levels were observed in bovine cells following infection with either KEMV or TRBV. These differences in IFN-I induction when infecting bovine, mouse, and human cells highlight the need for studies focusing on the mechanisms by which KPO evades the innate immune response.

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Aging, Biological Sex and Infection Affect TCR Repertoire Richness, Clonal Skew, Spread in Sequence Space, Publicity and Generational Probability

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An individual's life history can profoundly reshape the adaptive immune system, yet the most relevant factors as well as the specific consequences for the T cell receptor (TCR) repertoire remain incompletely understood. Here, we analysed two large human cohort datasets with TCRβ sequence information to characterize age-, sex- and infection-associated effects on the individual TCR repertoire. In agreement with previous work, we observed a significant age-dependent decline in TCR repertoire richness, reflected in the number of unique clonotypes, which is accompanied by a more uneven clonality. Using downsampled repertoires weighted by clonal abundance to focus on the clonally dominant compartment of the TCR repertoire, we constructed TCR similarity networks and defined clusters of TCR clonotypes likely sharing antigen specificity. Across these networks, age and infection were associated with an increased number of TCR clusters and a decreased mean cluster size. Together, these findings indicate increased clonal skewing and broader spread within the TCR sequence space, consistent with the cumulative effects of lifelong antigen exposure.

Furthermore, we detected an age-dependent decrease in the TCR repertoire overlap, reflected by a loss of public clonotypes shared across individuals. In parallel, mean generational probability of TCR clonotypes declined with age, indicating enrichment for TCR sequences that are less likely to arise from V(D)J recombination and reflect antigen-driven expansion. Overall, our results thus support a model in which age-dependent thymic involution reduces repertoire richness, while lifelong antigen exposure drives clonal skew, spread in TCR sequence space, privatization, and specialization of the TCR repertoire. These findings provide a framework for better understanding individual immune aging.

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The Effect of Enriched Environment and Supplemented Diet on the R3m4 Tau Transgenic Mice

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by pathological accumulation of intracellular tau and extracellular amyloid-β proteins, accompanied by neuroinflammation and metabolic disturbances across the CNS. AD is the most prevalent form of diagnosed dementia cases, with the majority of cases occuring sporadically in the process of aging. Despite extensive research efforts, currently available pharmacological treatments remain largely symptomatic and show limited efficacy when used as monotherapy. In addition, since lifestyle and environmental factors are widely recognized as modifiable risk factors for AD, these limitations have contributed to the increasing interest in the development and implementation of non-pharmacological interventions, such as the concept of enriched environment. In this study we aimed to investigate the effects of such intervention on tau propagation and associated immunological and bioenergetic processes in a preclinical mouse model of AD.

Methods: R3m4 tau transgenic mice were inoculated with human tau isolates to induce tau propagation and subsequently subjected to a combined intervention consisting of environmental enrichment and dietary supplementation. Histological and biochemical analyses were performed to evaluate tau pathology, neuroinflammation, synaptic integrity, and disruptions in bioenergetic pathway in the CNS and blood plasma.

Results: Environmental enrichment significantly attenuated tau propagation compared to animals with AD housed in standard conditions. This effect was accompanied by modulation of pathways implicated in synaptic plasticity, neuroinflammatory signalling and energetic pathways. These findings demonstrate that lifestyle-related interventions effectively modulate tau-driven neurodegeneration and provide experimental support for multimodal non-pharmacological strategies in Alzheimer’s disease research.

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The Role of Epithelial Cell Adhesion Molecule on Thymic Dendritic Cells

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Epithelial cell adhesion molecule (EpCAM) is generally known as a marker of various carcinomas, also promoting survival and proliferation of cancer cells through its signaling. Under normal circumstances, the molecule is expressed across multiple types of epithelial cells, including those found in the thymus. Surprisingly, we detected a profound expression of EpCAM also on thymic dendritic cells (DCs) which have no developmental link to epithelial cells. Since there is no information on the function of EpCAM on DCs, we have deleted this marker on DCs to assess its impact on thymic DCs physiology.

We depleted EpCAM in thymic DCs type 1, which resulted in a marked decrease of their numbers, perturbed maturation and functionality, yet no thymic structural changes were observed. In this model, we also observed a similar phenotype in thymic DCs type 2, although not as pronounced. Our results suggest that thymic DCs are equipped with EpCAM to interact with thymic epithelial cells which enhances survival and function of DCs and supports their cooperative antigen transfer and antigen presentation crucial for proper thymic function.

This research will provide insight into the role of EpCAM expression on DCs, along with a better understanding of how cells in the thymus cooperate to ensure development of a functional T-cell repertoire.

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The Role of Gastrointestinal Homeostasis in Immunodeficiency – Design of a Prospective Study

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The gut microbiota plays a key role in shaping immune responses, maintaining mucosal barrier integrity, and controlling inflammation. In patients with inborn errors of immunity and secondary immunodeficiencies, disturbances of gastrointestinal homeostasis may lead to dysbiosis, increased susceptibility to infections, more frequent antibiotic use, and reduced quality of life. Importantly, immunodeficiency itself may promote dysbiosis, while dysbiosis may further aggravate the symptoms and complications of immunodeficiency, creating a clinical vicious circle. Additional rationale for this project comes from our center's experience, including the medical experiment conducted by Associate Professor Katarzyna Napiórkowska-Baran, in which fecal microbiota transplantation in a patient with chronic diarrhea and coexisting primary and secondary immunodeficiency resulted in clinical improvement and favorable changes in selected immunological and microbiological parameters.

The aim of this project is to prospectively evaluate the effect of an intervention supporting intestinal homeostasis on immunological parameters, inflammatory activity, and the clinical course of immunodeficiency. The study will include patients aged 18–75 years under the care of allergy, immunology, hematology, and oncology centers. The protocol will include assessment of CRP, IL-6, lymphocyte subpopulations, immunoglobulin levels, fecal calprotectin, stool cultures, and a breath test for small intestinal bacterial overgrowth. In addition, the frequency of infections, hospitalizations, the need for antibiotic therapy, and quality of life will be analyzed. Participants will be assigned either to the study group receiving preparations modulating the microbiota and supporting the intestinal barrier or to the control group. Assessments will be performed at baseline and after 3 and 6 months.

This project may provide a basis for new adjunctive therapeutic strategies in clinical immunology.

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The Role of Myeloid Cells in Thymic T Cell Tolerance in Health and Disease

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The incidence of autoimmune diseases has been increasing in recent decades, often manifesting in association with or following infections. Our primary focus revolves around central immune tolerance, the pivotal process occurring in the thymus that is fundamental for the formation of a functional and safe T cell repertoire. Previous findings indicate that the thymus harbors various subtypes of myeloid cells crucial for presenting self-antigens to developing T cells. However, the effect of peripheral inflammation on thymic myeloid cell composition and function, and the subsequent impact on T cell selection, remain unexplored. The proposed project aims to characterize the role of various systemic as well as tissue-specific infections on the heterogeneity and function of thymic myeloid cells, as well as the mechanism of thymic T cell tolerance. The project will utilize various mouse infection models, and the changes in thymic myeloid cells induced by different types of infections will be elucidated by single-cell RNA sequencing, complemented by multiparametric flow cytometric analysis, as well as microscopic analysis of thymic architecture and myeloid cell localization. The changes in T cell selection associated with infection will be assessed by measuring numbers and phenotype of developing T cells, as well as by RNA sequencing of the T cell repertoire. The project also aims to implement the “dirty” co-housing mouse model to assess the potential changes in T cell selection in more physiological conditions. Addressing this project will provide valuable insight into how inflammation affects T cell tolerance processes and enhances our understanding of autoimmunity development.

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Caffeine Alters NLRP11 Expressions in Hypoxia-induced Human Microglia-like Cells

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Hypoxia-driven purinergic signaling is a central regulator of neuroinflammation yet the role of NLRP11 within the adenosine axis remains poorly defined. Here, we investigated the regulatory interplay between caffeine-mediated adenosine receptor blockade and hypoxia-induced signaling in human microglia-like HMC3 cells.

Caffeine treatment led to a robust dose-dependent downregulation of both ADORA2A and NLRP11 mRNA expression indicating effective suppression of adenosine signaling. Notably, these molecular alterations occurred without measurable changes in cell viability, demonstrating that caffeine selectively reprograms transcriptional responses rather than inducing cytotoxicity.

To model hypoxic stress, cells were treated with 125 μM $CoCl_2$ for 4 hours which efficiently mimicked hypoxia and significantly altered key components of the adenosine pathway. Under these circumstances, downstream signaling networks including MAPK and Akt pathways were differentially modulated suggesting that hypoxia reshapes purinergic signaling dynamics in human microglial-like cells. The combined effects of hypoxia and adenosine pathway modulation point to a context-dependent regulatory mechanism governing NLRP11 expression.

Collectively, our findings identify NLRP11 as a previously underappreciated component of the hypoxia-responsive adenosine axis and demonstrate that caffeine acts as a potent transcriptional modulator of this pathway without compromising cell viability. These results position the ADORA2A–NLRP11 axis as a potential immunometabolic checkpoint in microglia under hypoxic stress with implications for neuroinflammatory and ischemic conditions.

Keywords: Adenosine signaling, NLRP11, ADORA2A, hypoxia, caffeine, microglia, immunometabolism

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Repeated mRNA Vaccination Drives IgG1-to-IgG4 Class Switching and Functional Remodeling of the Antibody Response

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Recent research has substantially improved our understanding of immune responses induced by SARS-CoV-2 infection and COVID-19 vaccines, including activation of both cellular and humoral arms of adaptive immunity. Building on this framework, we performed longitudinal monitoring (18 months) of immune responses in an adult cohort following COVID-19 vaccination, SARS-CoV-2 infection, or hybrid immunity resulting from vaccination and infection in any sequence. A total of 433 participants were enrolled, including individuals vaccinated with Pfizer/BioNTech (n=173), Moderna (n=20), or AstraZeneca (n=36); individuals vaccinated and subsequently infected (Pfizer n=60, Moderna n=22, AstraZeneca n=17); and individuals infected before receiving Pfizer/BioNTech or Moderna vaccination (n=40) and individuals with SARS-CoV-2 infection only (n=65).

Antibody analysis included determination of Spike-specific antibody titers (IgG, IgM, IgA, including IgG subclasses IgG1, IgG2, IgG3, and IgG4), assessment of neutralizing capacity using both Spike-ACE2 binding inhibition and pseudovirus neutralization assays, and correlation analysis between binding and neutralizing antibody titers. Subclass profiling demonstrated IgG1 dominance across responses. IgG4 antibodies were detected exclusively after vaccination and hybrid immune stimulation, but not after natural infection alone. We observed that repeated stimuli, such as administration of two vaccine doses or vaccination combined with infection, were linked to isotype switching toward IgG4.

The functional impact of increased IgG4 after vaccination was tested using an antibody-dependent phagocytosis assay with the THP-1 monocytic cell line and plasma samples with distinct IgG1/IgG4 profiles. The results demonstrated a time-dependent qualitative shift in the antibody response toward increased IgG4 representation, accompanied by a partial reduction in effector activity. These findings suggest that IgG1-to-IgG4 switching may reflect functional remodeling of the humoral response and adaptive regulation of the immune system following repeated antigenic stimulation.

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Development and Diversity of RORγt⁺ eTACs

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While the Autoimmune Regulator (AIRE) is primarily recognized for driving central immune tolerance in the thymus, its role extends to peripheral tissues through RORγt⁺ extrathymic AIRE-expressing cells (eTACs). These cells reside in lymph nodes and are indispensable for mounting effective T cell responses against C. albicans. Despite their critical role in immune activation, the developmental relationship between AIRE⁺ subsets and other members of the RORγt⁺ antigen-presenting cell (APC) lineage remains poorly defined.

We generated an AIRE-ZsGreen reporter mouse model to faithfully track endogenous AIRE protein expression. Using this model, we constructed a comprehensive atlas of mouse lymph node APCs to characterize eTAC heterogeneity across different immunological contexts. We identified two major subpopulations of RORγt⁺ eTACs with divergent responses to inflammatory stimuli: Ncam1⁺ subset that proportionally increases upon stimulation, and Epcam⁺ subset that decreases. We identified human counterparts to these murine eTAC subsets that exhibit similar inflammatory dynamics. By mapping developmental trajectory programs, we identified a specific IL7Ra⁺ α_4 β_7⁺ Siglecg⁺ Flt3⁺ c-kit⁺ bone marrow and fetal liver progenitor populations with a transcriptional signature predictive of the RORγt⁺ eTAC state. This developmental potential was further validated through *in vitro* and *in vivo* differentiation assays.

Our study provides a high-resolution characterization of RORγt⁺ eTACs, revealing distinct functional subsets and a dedicated progenitor-to-effector cell pathway. These findings clarify the diversity of peripheral AIRE expression and offer a framework for understanding how these cells contribute to immune homeostasis and pathogen defense in both mice and humans.

P.82

SARS-CoV-2 Nucleocapsid Protein Modulates Complement Activation on Virions

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The involvement of complement system in pathogenesis of COVID19 is well known, thanks to international research effort during pandemic. The hyperactivation of complement cascade has been extensively studied and several mechanisms of hyperactivation have been proposed. However, complement evasion by SARS-CoV-2 received less attention. Incorporation of host membrane-bound complement regulatory proteins (CRPs) CD55 and CD59 and involvement of ORF8 in complement evasion has been demonstrated by others. Our previous results demonstrated that SARS-CoV-2 nucleocapsid (N) protein, which is present in blood circulation during infection, binds soluble CRPs (C1 inhibitor, C4-binding protein, factor H and vitronectin) and can form CRP-N-virion complexes. That led us to hypothesize that N protein might act as a complement evasion/modulation protein extending its immunomodulatory role during infection. Here we present our most recent findings obtained using proteomic analysis employing LS-MS/MS. Briefly, virions purified by gradient ultracentrifugation were incubated in normal human serum (NHS) with or without N protein. After incubation, samples were ultracentrifuged again and virions with deposited complement were analyzed. We demonstrate that N protein in normal human serum influences the complement activation as seen by decrease in deposition of complement components C3-C9 on virions. Decreased deposition of complement was confirmed on the same samples using dot blot and ELISA. We report that N protein influences complement activation and deposition on SARS-CoV-2 virions and could eventually protect the virus from complement attack during severe infection.

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P.83

Human Mast Cells Developed in NSG-Tg(hu-mSCF) Mice Represent Tools to Imitate the Immediate Allergic Reaction

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Background: Mast cells (MC) are key effector cells in IgE-mediated allergies as they bind allergen-specific IgE antibodies through the high-affinity Fc epsilon receptor and release preformed mediators, e.g. histamine, upon cross-linking by allergens. Thus, MC represent relevant tools to study the allergic reaction as well as approaches to block it. However, as tissue resident cells, human MC are difficult to assess. Here, we tested whether NSG-Tg(hu-mSCF) mice engrafted with human stem cells (HSC) may serve as a source for human MC, which are functional in mast cell activation assays (MAT).

Methods: HSC were isolated from cord blood mononuclear cells and injected intravenously into NSG-Tg(hu-mSCF) mice. Blood samples were monitored for huCD45$^+$ cells at monthly intervals. Bone-marrow cells were isolated and expanded *in vitro* by using human IL-3 and stem cell factor (SCF). The maturation of MC was monitored by flow cytometry. After 5-7 weeks, bone marrow-derived MC (BMC) were incubated overnight with sera from birch pollen-allergic individuals containing Bet v 1-specific IgE ranging from 10-55 ng/ml. After incubation with titrated amounts of major birch pollen allergen, Betv1, degranulation was detected via CD63 and CD107a expression by flow cytometry.

Results: Sixteen weeks after engraftment with HSC (0.5-1 x 105 /mouse) with a purity of 90.7±9%, an average of 55 ± 6.3 x 10^6 (SEM) bone-marrow cells was obtained, of which 14 ± 1.6% were huCD45$^+$FcεRI$^+$CD117$^+$ MC. Five to seven weeks of *in vitro* cultivation resulted in an average of 0.44 x 10^6 BMC per mouse. After passive sensitization with Bet v 1-specific IgE, incubation with different concentrations of rBet v 1 (1-100 ng/mL) resulted in 20-84% CD63$^+$ cells and 13-73% CD107a$^+$ cells. The percentage of CD63$^+$ and CD107a$^+$ cells was higher in BMC that had been sensitized and then stimulated with higher concentrations of rBet v 1.

Conclusion: NSG-Tg(hu-mSCF) mice engrafted with HSC represent a source for human MC that respond to allergen after sensitization with allergen-specific IgE. Our approach allowed expanding a high number of functional human MC, which can be used to study agents that block anaphylactic reactions and MC mechanisms.

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P.84

PD-L1⁺ Macrophages Impair Immune Organization by Disrupting Tertiary Lymphoid Structures in Ovarian Cancer Metastases

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Tumor-associated tertiary lymphoid structures (TLSs) have emerged as prognostic and predictive biomarkers in various solid malignancies by promoting local anti-tumor immune responses through the expansion of antigen-specific B and T cells. However, the immune contexture of tumors differs across cancer types and even among individuals with the same disease. High-grade serous ovarian carcinoma (HGSOC) remains largely resistant to current immunotherapeutic approaches, and many patients experience relapse following standard treatment. Defined as immunologically “cold”, HGSOC tumors exhibit low frequencies of functional, mature TLSs, resulting in a lack of T cells responsive to immunotherapy and highlighting the need for detailed molecular characterization of the tumor microenvironment to elucidate its low immunogenicity. In this study, using digital pathology and spatial transcriptomics, we show that immature TLSs in HGSOC are infiltrated with and surrounded by PD-L1⁺ tumor- associated macrophages (TAMs) and immunosuppressive regulatory T (Treg) cells, in contrast to non-small cell lung cancer (NSCLC), an immunologically “hot” carcinoma. Through transcriptomic analysis and functional characterization of ovarian TAMs, we demonstrate that antigen-presenting PD-L1⁺ TAMs promote the activation of PD-1⁺ cells, potentially leading to their terminal exhaustion. Furthermore, in a co-culture system using a mouse model of ovarian cancer, we show that PD-L1⁺ TAMs induce the differentiation of naïve

$CD4^+$ T cells into $FOXP3^+$ Treg cells through a mechanism at least partially dependent on the PD-1/PD-L1 interaction. Finally, Treg cell expansion suppressed TLS development in mice, while blockade of PD-L1 led to an increase in intra-tumoral TLS densities. Together, our findings indicate that $PD\text{-}L1^+$ TAMs are involved in the recruitment or direct induction of Treg cells within immature TLSs, which subsequently impair the generation and maturation of TLSs in immunologically cold tumors. This study strengthens the rationale for targeting TAMs as part of combination immunotherapeutic strategies for cancer patients resistant to standalone conventional immune checkpoint inhibitors and underscores the importance of precise tumor niche characterization in the development of effective treatments.

P.85

Diet Induced Metabolic Remodelling Modulates Tau Pathology and Neuroimmune Response in a Tau Transgenic Mouse Model

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Nearly half of Alzheimer's disease cases worldwide are attributable to modifiable risk factors, among which diet is increasingly recognized as a key modulator of neurodegenerative disease progression. However, the interaction between diet and neurofibrillary pathology remains incompletely understood. Here, we investigated the effects of a Western-style diet on tau tangle pathology and associated molecular and cellular alterations in the brain. Tau transgenic R3m4 mice, which express sporadic human tau and develop neurofibrillary tangles in the brainstem, were divided into four groups: transgenic and wild-type mice fed either a Western or control diet for 10 weeks. AT8-defined tau burden was assessed using immunohistochemistry. Plasma lipidomic profiling was performed by mass spectrometry to identify systemic lipid signatures associated with diet and genotype. Cerebral lipid deposition was examined histologically, and region-specific proteomic analyses were conducted in the cortex, hippocampus, and brainstem. Neuroimmune responses were assessed with a focus on microglial abundance and morphology. Western diet–fed transgenic mice exhibited increased brainstem tangle pathology. Lipidomic analyses revealed pronounced alterations in circulating lipid profiles, indicating significant diet-induced peripheral metabolic remodeling. Consistent with these changes, lipid droplets were detected in cortical and hippocampal regions. Proteomic analyses demonstrated distinct, region-specific inflammatory protein expression signatures associated with Western diet consumption. Although overall microglial abundance was unchanged, microglial morphology was markedly altered in Western diet–fed animals, consistent with a functionally distinct activation state. Activation of the TLR4 and NLRP3 inflammasome pathways was also observed. Together, these findings indicate that dietary context drives coordinated peripheral metabolic changes, brain proteomic remodeling, and microglial phenotypic alterations, potentially via inflammasome activation. This study highlights diet as a critical modifier of neuroimmune responses in Alzheimer's disease progression.

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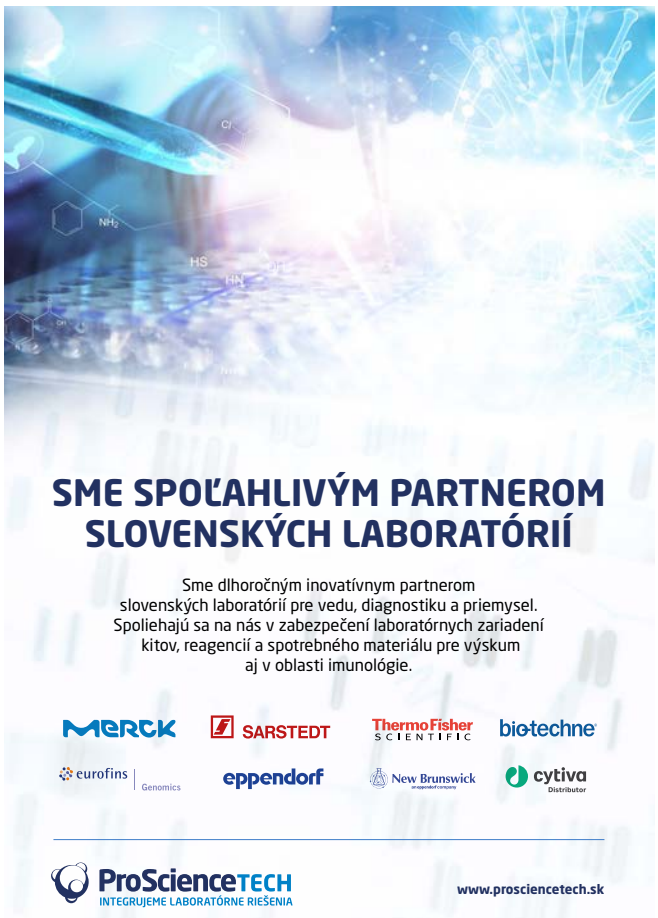

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