

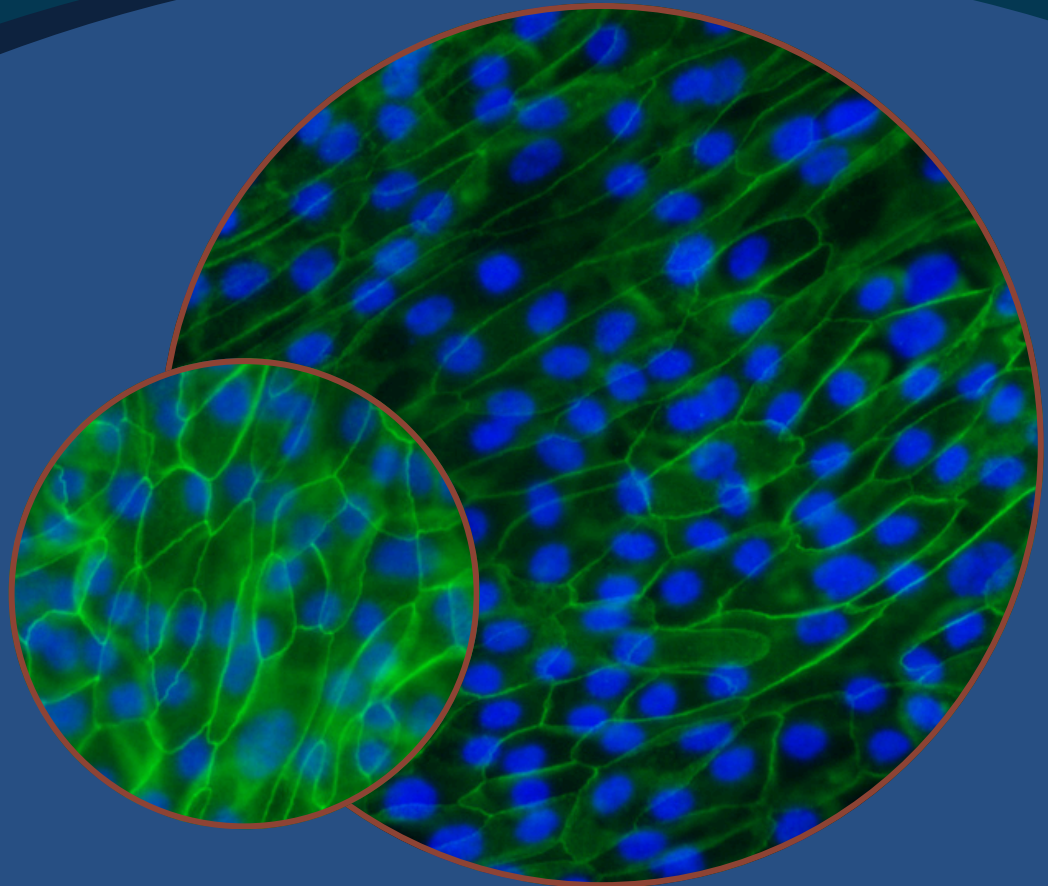


MESIA
SLOVENIA 2026

ABSTRACT BOOK

MESIA 2026

6th Meeting of Middle European Societies
of Immunology and Allergology



15 – 17 April 2026
Grand Hotel Union
Ljubljana, Slovenia

MESIA 2026, 15-17 April, 2026

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Organisers



Immunology Society of Slovenia

Slovenian Association for Allergology and Clinical Immunology



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Croatian Immunology Society



Croatian Society for Allergology and Clinical Immunology



Czech Immunological Society



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Congress programme

6th Meeting of Middle European Societies of Immunology and Allergology				
DAY 1 WEDNESDAY, 15.4.2026				
11:00		Registration		
12:15		Rising star <i>Chair: Hafner Bratkovič Iva (SLO) / Polič Bojan (CRO)</i>		
12:15 – 14:00	RS.01 20 min	Unveiling the Immune System's Surprising Role: Mastering Glucose Metabolism in Health and Disease	M Šestan	Croatia
	RS.02 20 min	Revealing vesicular communication pathways in 2D and 3D tumor models	T Böröczky	Hungary
	RS.03 20 min	Identification of antibodies suitable for a receptor-mediated transcytosis delivery system for the CNS	K Pichlerová	Slovakia
	RS.04 20 min	How Rapid Can We Be? Experience with Our One-Day Ultra-Rush VIT Initiation Protocol Using Depot Preparations	T Balner	Czech Republic
	RS.05 20 min	Tumor microenvironment in rare skin neoplasms	J Strobl	Austria
14:00		Coffee break		
14:30		Symposium in Memory of Prof. Ihan <i>Chair: Kopitar Andreja Nataša (SLO) / Vukman Krisztina (HUN)</i>		
14:30 – 15:35	YI.1.01 8 min	Metabolic modulation with 2-deoxy-D-glucose alters T lymphocyte functions	T Frlic	Slovenia
	YI.1.02 8 min	Decreased serum levels of neutralising anti-IL-6 autoantibodies in idiopathic inflammatory myopathies	A Srpčič	Slovenia
	YI.1.03 8 min	Irradiation-induced trained immunity of natural killer cells in mouse tumors	T Božič	Slovenia
	YI.1.04 8 min	The CAR-Cytotox score: a unified framework for measuring CAR T cell cytotoxicity	L Sršen	Slovenia
	YI.1.05 8 min	Expression of inducible damage-associated molecular patterns after interleukin-12 gene electrotransfer in cancer cell lines	A Medved	Slovenia
	YI.1.06 8 min	Immunogenicity of Luciferase suppresses tumor formation in an orthotopic K7M2luc osteosarcoma model	S Kupčič	Slovenia
	YI.1.07 8 min	Governing immunogenic cell death through transcriptional and translational control	T Železnik Ramuta	Slovenia
	YI.1.08 8 min	Proteome Analysis of Minor Salivary Glands Identifies Clinically and Molecularly Distinct Patient Subgroups in Sjögren's Disease	N Štucin	Slovenia
15:35		Coffee break		
15:55		Symposium in Memory of Prof. Ihan <i>Chair: Gašper Markelj (SLO) / Altrichter Sabine (AUT)</i>		
15:55 – 17:00	YI.2.01 8 min	Identification of IgE epitope-like peptides of peanut allergen Ara h 1	A Potočnik	Slovenia
	YI.2.02 8 min	Hereditary α -tryptasemia in children with peanut-induced anaphylaxis	T Pungertnik	Slovenia
	YI.2.03 8 min	Systemic immunomodulation as the new paradigm for severe atopic dermatitis in the young paediatric population	M Škulj	Slovenia
	YI.2.04 8 min	Comparison of ImmunoCAP ISAC and ALEX2 ALLERGY XPLOER	I Hasanović	Slovenia
	YI.2.05 8 min	Candidate variants associated with Hereditary Angioedema with normal C1-Inhibitor	H Jakopič	Slovenia
	YI.2.06 8 min	Diagnostic Comparison of Basophil Activation Test and Specific IgE to Venom Components in Hymenoptera-Allergic Patients with Low or Negative Venom-Specific IgE	L Dejanović	Slovenia
	YI.2.07 8 min	Diverse cellular scaffolds induce NLRP3 clustering toward inflammasome formation	E Boršič Mlinarič	Slovenia
	YI.2.08 8 min	Development of a platform for production of monoclonal antibodies against membrane proteins on the model of BCMA	P Kern	Slovenia
17:30 – 17:50		Welcome address and formal opening of the congress		
17:50– 18:40		Plenary lecture <i>Chair: Čurin Šerbec Vladka (SLO) / Kopač Peter (SLO)</i>		
	P.1 50 min	Living Cures: the Next Evolution in Cellular Medicine	Daniel J. Powell Jr.	USA
18:40 – 21:00		50th anniversary of Immunology Society of Slovenia and formal opening of the congress		
Reception				

DAY 2 – MORNING THURSDAY, 16.4.2026				
SESSION 1: From Mast Cells to Memory T Cells: New Frontiers in Allergy and Asthma <i>Chair: Kopač Peter (SLO) / Vannucci Luca (CZ)</i>				
08:00	S1.01.P 30 min	Mast cells and their progenitors in asthma – My perspective	J Hallgren Martinsson	Sweden
08:00 – 09:45	S1.02.P 20 min	Epigenetic regulation of pathogenic T cells driving allergic airway inflammation	N Boucheron	Austria
	S1.03.P 20 min	Mast cell degranulation and vesiculation	K Vukman	Hungary
	15 min	Discussion		
	S1.04.S 20 min	The epithelial alarmin axis in severe asthma and why TSLP matters: From Pathway to Patients	P Korošec / A Ilovar Bežjak	ASTRA ZENECA
09:45	Coffee break			
SESSION 2: Type 2 Inflammation in Asthma and Allergic Disease <i>Chair: Hallgren Martinsson Jenny (SWE) / Boucheron Nicole (AUT)</i>				
10:15	S2.01.I 15 min	Resident memory T cells in atopic dermatitis	Z Dajnoki	Hungary
	S2.02.I 15 min	Autoreactive IgE in Dermatological Diseases	S Altrichter	Austria
10:15 – 12:10	S2.03.I 10 min	Bronchial hyperreactivity is associated with T2 inflammation and loss of asthma control: evidence from a cross-sectional study in pediatric patients	I Topalušić	Croatia
	S2.04.I 10 min	Linking Gastroesophageal Reflux Characteristics to Airway Inflammation in Severe Preschool Wheeze	I Topalušić / I Pavić	Croatia
	20 min	Discussion		
	S2.05.S 20 min	Top-down approach and Ratio analysis in Allergy Diagnostics	O Luengo	LKB
	S2.06.S 15 min	Towards a Next-Gen and Streamlined Basophil Activation Test	J-M Busnel	HERMES - ANALITICA
12:10	Coffee break			
SESSION 3: Immune Regulation in Health and Disease: Mechanisms, Monitoring and Targets <i>Chair: Kovač Valerija (SLO) / Antica Mariastefania (CRO)</i>				
12:30 – 13:55	S3.01.P 20 min	Inhibition of homeostatic regulators in a tissue elicits an underhand inflammation that drives the microenvironment toward pathologic evolution	L Vannucci	Czech Republic
	S3.02.I 15 min	Human thymic epithelial stem cells for lymphocyte development	M Antica	Croatia
	S3.03.I 15 min	Exposure to micro and nanoplastics on markers of inflammatory immune response in allergic children	M Turkalj	Croatia
	S3.04.I 15 min	Expanding testing to monitor the immunological status of patients after heart transplantation	S Montanič	Slovenia
	S3.05.I 10 min	Values of salivary cortisol and interleukin-1β, perceived stress and clinical features of patients with dermographic urticaria, patients with chronic spontaneous urticaria and healthy individuals	L Lugović-Mihic	Croatia
	10 min	Discussion		
13:55 – 15:00	Lunch			

DAY 2 – AFTERNOON		THURSDAY, 16.4.2026				
15:00	SESSION 4: Harnessing Immunity in Cancer: Novel Therapeutic Platforms and Tumor Microenvironments <i>Chair: Janžič Urška (SLO) / Gácser Attila (HUN)</i>					
	S4.01.S 20 min	How has immunotherapy changed oncological treatment: from theory to prolonged survival	U Janžič	MSD		
	S4.02.P 20 min	Therapeutic application of antibody–drug conjugates and vaccines in solid tumor patients	S Borštnar	Slovenia		
15:00 – 16:40	S4.03.P 20 min	From GVL to CAR-T therapy	P Novak	Slovenia		
	S4.04.I 15 min	Spatial immune niches as determinants of anti-tumor immunity in ovarian cancer	J Fucikova	Czech Republic		
	10 min	Discussion				
	S4.05.S 15 min	Redefining lung cancer care: the immunotherapy era	M Unk	MEDISON		
16:40 - 17:50		Coffee break				
16:40 - 17:50		Poster session P1.01 - P1.20				
17:50	SESSION 5: Cancer–Immune Crosstalk: Microbial Interactions, Cell Death and Immune Profiling <i>Chair: Jesenko Tanja (SLO) / Jitka Palich Fučíková (CZ)</i>					
	S5.01.S 15 min	Rybrevant – innovative targeted therapy for patients with EGFR positive non-small cell lung cancer	U Janžič	Johnson & Johnson		
	S5.02.P 20 min	Candida species on oral cancer: insights into pathogenesis and interaction	A Gácser	Hungary		
17:50 – 19:15	S5.03.P 20 min	Inflammasome-inspired induction of immunogenic cancer cell death	I Hafner Bratkovič	Slovenia		
	S5.04.I 15 min	Immunoprofiling of Gamma Delta T cells and their potential for the development of cancer immunotherapies	A Smole	Slovenia		
	15 min	Discussion				
20:00	Formal congress dinner					

DAY 3 FRIDAY, 17.4.2026				
8:00 SESSION 6: Autoimmunity and Immune Dysregulation: From Mechanisms to Clinical Innovation				
<i>Chair: Rijavec Matija (SLO) / Ellmeier Wilfried (AUT)</i>				
	S6.01.P 20 min	Systemic vasculitides through the lens of a rheumatologist – IgA vasculitis in adults	A Hočevar	Slovenia
	S6.02.P 20 min	The role of histone deacetylases in the control of T cell-mediated immunity	W Ellmeier	Austria
	S6.03.I 15 min	Organ-on-a-chip platform of a human joint for evaluating response to treatment of inflammatory arthritides	A Sucur	Croatia
08:00	S6.04.I 15 min	The role of microRNAs in the development of systemic autoimmune diseases	G Papp	Hungary
–	S6.05.I 15 min	New trends in laboratory transplantology	A Čereš	Slovakia
10:00	S6.06.I 10 min	The brain's Trojan war: Local brain immunity may modulate Alzheimer's disease neurodegeneration	N Basheer	Slovakia
	S6.07.I 10 min	Neuroimmune effects of environmental and dietary enrichment in an AD model	V Stuchlikova	Slovakia
	15 min	Discussion		
10:00 – 11:00 Coffee break				
10:00 – 11:00 Poster session (P2.01 - P2.20)				
11:00 SESSION 7: Inborn Errors of Immunity: From Genetics to Clinical Complexity				
<i>Chair: Kačar Mark (SLO) / Jeseňák Miloš (SK)</i>				
	S7.01.P 20 min	Autoinflammatory disorders in Slovakia - unexpected results from awareness campaigns	M Jeseňák	Slovakia
	S7.02.P 20 min	The Immunological Basis of Lung Disease in Primary Antibody Deficiencies: Exploring B Cell Dysfunction	T Milota	Czech Republic
	S7.03.I 15 min	Role of genetics in IEI	G Markelj	Slovenia
11:00	S7.04.I 15 min	Beyond enteroviral meningitis: chronic neurological sequelae of XLA	M Bizjak	Slovenia
–	S7.05.I 10 min	IEI screening in Slovakia - first experiences	P Čižnár	Slovakia
13:15	S7.06.I 10 min	Complexity of IEI in adults - one centre experience	A Bobčáková	Slovakia
	15 min	Discussion		
	S7.06.S 15 min	Translating biomarkers into personalized care for hereditary angioedema	M Rijavec	TAKEDA
	S7.07.S 15 min	Redefining the trajectory of allergic rhinitis	M Kačar	Ewopharma
13:15 – 14:30 Lunch				
14:30 SESSION 8: Innate Immunity in Infection, Tissue Remodeling and Adaptive Crosstalk				
<i>Chair: Eiwegger Thomas (AUT) / Milota Tomas (CZ)</i>				
	S8.01.I 15 min	Immunological mechanisms of oral immunotherapy	T Eiwegger	Austria
14:30	S8.02.P 20 min	Mechanistic insights into the protective role of lactoferricin in SARS-CoV-2 infection	V Leksa	Slovakia
–	S8.03.P 20 min	Inflammation and bone remodeling	D Grcevic	Croatia
15:35	S8.04.I 15 min	How Signaling Pathways Shape T-Cell Diversity in Phenotype and Function	O Štěpánek	Czech Republic
	20 min	Discussion		
16:00 - 16:30 Coffee break				
16:30 SESSION 9: Innate Immunity in Viral Defense, Neuroinflammation and Reproductive Health				
<i>Chair: Božič Borut (SLO) / Lekša Vladimír (SK)</i>				
	S9.01.P 20 min	Innate immune responses against cytomegalovirus infection in the ovary	V Juranić Lisnić	Croatia
16:30	S9.02.I 15 min	Microglial priming balances latent virus control and synaptic loss	I Brzić	Croatia
–	S9.03.I 15 min	IFN γ is necessary for <i>Lactiplantibacillus plantarum</i> -mediated growth promotion in undernourished mice	M Schwarzer	Czech Republic
17:35	S9.04.I 15 min	Discussion		
17:35 End of Congress MESIA 2026				
–	25 min	Best poster presentation award winners announcement		
18:00		Closing remarks		

Rising Star

RS.01**UNVEILING THE IMMUNE SYSTEM'S SURPRISING ROLE: MASTERING GLUCOSE METABOLISM IN HEALTH AND DISEASE**

Marko Šestan

Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Presenting author e-mail: marko.sestan@medri.uniri.hr

The immune and endocrine systems play crucial roles in the body. The immune system defends against lethal pathogens, while the endocrine system maintains proper metabolic function in peripheral organs by regulating systemic metabolic homeostasis. Traditionally, these systems were thought to operate independently, with the immune system using cytokines and immune receptors, and the endocrine system using hormones to regulate metabolism. However, our recent findings reveal a close interaction between the immune and endocrine systems, particularly in the regulation of glucose metabolism during both homeostasis and viral infections. In summary, new findings on immune-induced changes in systemic metabolism during homeostasis and following viral infections, with a focus on glucose metabolism regulation, will be discussed.

RS.02**REVEALING VESICULAR COMMUNICATION PATHWAYS IN 2D AND 3D TUMOUR MODELS**

Tímea Böröczky^{1,2,3}, Gabriella Dobra^{1,3}, Mátyás Bukva^{1,3}, Edina Gyukity-Sebestyén³, Árpád Szöör⁴, Péter Horváth³, Krisztina Buzás^{1,3}

¹Department of Immunology, Albert Szent-Györgyi Medical School, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary; ²Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary; ³HUN-REN Biological Research Centre, Szeged, Hungary; ⁴Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Presenting author e-mail: boroczky.timea@brc.hu

Background: Understanding resistance to HER2-targeted immunotherapy (trastuzumab) in HER2-positive cancers remains a major clinical challenge, demanding models that better reflect the *in vivo* tumour environment. Emerging evidence suggests that both mucin expression and extracellular vesicle (EV) composition may contribute to therapeutic evasion, yet conventional 2D and static 3D cultures do not fully recapitulate key features like drug diffusion, mucin layering, and EV signalling. To minimise animal use and improve translational relevance, we compared 2D, 3D spheroid, and dynamic perfusion-based cultures using the MIVO system, which mimics physiological flow and shear stress experienced by solid tumours *in vivo*.

Methods: We used three human cancer cell lines: N87 (HER2+, trastuzumab-sensitive), JIMT-1 (HER2+, trastuzumab-resistant), and MDA-MB-468 (HER2-, control). Cells were cultured under 2D, 3D spheroid and dynamic conditions. Mucin was visualised by mucicarmine staining. EVs were isolated by ultracentrifugation and analysed for MUC4 and CD44 surface expression by flow cytometry. Protein content and particle concentration were measured by BCA assay and NTA, respectively.

Results: N87 cells showed strong mucicarmine staining in both 2D and 3D, suggesting gel-forming mucins, while JIMT-1 displayed a more pericellular mucin pattern. MDA-MB-468 displayed minimal mucin signal, consistent with its HER2-negative phenotype. JIMT-1 EVs displayed the highest MUC4 levels, while CD44 was unexpectedly elevated in N87 EVs. Importantly, the dynamic MIVO system induced a shift towards a different mucin architecture, especially in JIMT-1. This suggests that perfusion-based conditions alter the membrane-bound mucin expression and may further reduce trastuzumab accessibility via HER2 masking.

Conclusions: Mucin composition and EV surface markers are cell line-specific and influenced by culture architecture. The MIVO system better mimics *in vivo* conditions and reveals subtle but relevant differences in mucin expression and EV phenotype linked to resistance. Our findings highlight mucin architecture as a functional barrier to trastuzumab efficacy.

Future directions: We aim to explore whether EVs derived from HER2-targeted CAR-NK cells possess direct cytotoxic potential against HER2-positive cancer cells under static and dynamic 3D conditions. This may offer a vesicle-based immunotherapeutic strategy to bypass mucin-related resistance mechanisms.

Funding: TKP-EGA-09; OTKA-K143255.

RS.03**IDENTIFICATION OF ANTIBODIES SUITABLE FOR A RECEPTOR-MEDIATED TRANSCYTOSIS DELIVERY SYSTEM FOR THE CNS**

Karoline Pichlerová¹, Kevin James^{1,2}, Petra Majerová¹, Michaela Škrabanová¹, Ľubica Fialová¹, Krutika Khiratkar^{1,2}, Jozef Hanes¹

¹Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia; ²University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

Presenting author e-mail: karoline.pichlerova@savba.sk

Background: The group of tauopathies, which includes Alzheimer's disease (AD), is a part of neurodegenerative diseases. Tauopathies are mainly characterized by neuroinflammation, cerebral atrophy, and the presence of insoluble neurofibrillary tangles, which consist of hyperphosphorylated tau proteins. To combat neurodegeneration, several therapeutic monoclonal antibodies (mAb) are in development or clinical use. For an effective treatment is the penetration of mAb into the central nervous system, through the blood-brain barrier (BBB), crucial. The permeability of compounds through the BBB is selectively enabled by tight junctions and receptors. Our aim is to develop a novel drug delivery system, which facilitates crossing through the BBB.

Methods: The pipeline of animal immunization, creation of hybridomas, screening with ELISA, production and purification of chosen mAbs was utilized. The binding specificity of antibodies was determined via Western blot and fluorescent co-localization. The binding capacity of novel antibodies was quantified with fluorescence-activated cell sorting (FACS). Subsequently, individual fragment antigen-binding (Fab) regions were generated by enzymatic cleavage. A permeability assay for the Fab region was carried out in an in vitro model of the BBB, consisting of primary rat endothelial and glial cells.

Results: We produced novel mAbs targeting the lipolysis-stimulated lipoprotein receptor (LRP8) of rat endothelial cells. The binding specificity to the target receptor of rat endothelial cells was confirmed, enabling a receptor-mediated transcytosis (RMT) for improved BBB penetration. The binding capacity of the mAb was determined, and its size was decreased by generating Fab regions. The LRP8 Fab region specificity was confirmed by its ability to recognize LRP8 receptors in rat brain capillaries. Additionally, is the LRP8 Fab region capable of crossing through the in vitro BBB model.

Conclusion: Novel produced antibodies against the LRP8 receptor were capable of specific recognition of rat endothelial cells. The Fab region was similarly capable of antigen recognition and penetration of an in vitro model of the BBB. Our results show that the specific antibodies may represent a novel delivery system for the central nervous system.

Funding: APVV-21-0254, APVV-22-313, 09I03-03-V03- 00086, VEGA 2/0075/2.

RS.04**HOW RAPID CAN WE BE? EXPERIENCE WITH OUR ONE-DAY ULTRA-RUSH VIT INITIATION PROTOCOL USING DEPOT PREPARATIONS**T. Balner^{1,2}, J. Bystron^{1,2}, M. Balner¹, P. Adámková¹, J. Kačorová¹¹Department of Allergology and Clinical Immunology, University Hospital Ostrava, Ostrava, Czech Republic; ²Institute of Laboratory Medicine, University of Ostrava, Ostrava, Czech Republic

Presenting author e-mail: tomas.balner@fno.cz

Background: Venom immunotherapy (VIT) is highly effective and the recommended treatment for patients with a history of systemic anaphylactic reactions to Hymenoptera stings. Achieving a maintenance dose of immunotherapy in the shortest possible time is desirable in some situations. This study aims to report the almost 6-year experience with our half - day ultra - rush venom immunotherapy protocol in outpatients and to assess the safety of this protocol.

Methods: This is a retrospective single-center study of 45 VIT inductions using half-day ultra-rush protocols conducted from 2020 to 2025 in 44 outpatients aged 16 to 69 years (25 males [57%], 19 females [43%]) with confirmed bee or wasp venom allergy, using depot venom preparations. The patients experienced 3 systemic reactions (SR) grade 1, 27 SR grade 2, 11 SR grade 3, and 4 SR grade 4 according to the Ring and Messmer classification. On day 1 of our protocol, an initial venom dose of 0.2 ml at 100 SQ-U/ml was followed by doses of 0.1 ml at 1000 SQ-U/ml, 0.1 ml at 10,000 SQ-U/ml, 0.1 ml at 100,000 SQ-U/ml, 0.3 ml at 100,000 SQ-U/ml, and 0.6 ml at 100,000 SQ-U/ml, administered at 30-minute intervals. Patients received one booster injection of 1 ml at 100,000 SQ-U/ml on day 15. Ultra-rush VIT protocols were performed with honeybee (20 protocols) and wasp (25 protocols) venom, with one patient receiving injections of both venoms. All documented side effects were classified into local reactions and SR using the Ring and Messmer classification.

Results: Small local reactions on day 1 were observed during 23 (51%) VIT induction procedures. Three (7%) patients experienced large local reactions. Two (4%) patients experienced delayed systemic urticaria after the day 15 booster injection. The maintenance dose was temporarily reduced in 3 (7%) patients. There was no need to interrupt the treatment. The bee venom group showed a non-significant trend towards a higher incidence of reactions, resulting in more frequent antiallergic therapy. There were no immediate SRs on day 1 or day 15, despite the presence of patients with arrhythmias, antihypertensives, autoimmune diseases, and mastocytosis. Epinephrine as rescue medication was never necessary.

Conclusions: The half-day ultra-rush protocol is a safe therapeutic option for Hymenoptera venom-allergic outpatients, displaying no SR in our study. The protocol appears to be safe even in high-risk and elderly patients, but further data are needed to confirm these findings.

RS.05**THE TUMOR MICROENVIRONMENT IN RARE SKIN NEOPLASMS**

Johanna Strobl

Department of Dermatology, Medical University of Vienna, Vienna, Austria

Presenting author e-mail: johanna.strobl@meduniwien.ac.at

Rare cutaneous malignancies such as Kaposi sarcoma (KS) and cutaneous T cell lymphoma (CTCL) may progress depending on their tumor microenvironment (TME). To delineate shared and disease-specific microenvironmental programs, we performed single-cell and spatial transcriptomic analyses of KS and mycosis fungoides–type CTCL skin lesions.

In KS, a vascular neoplasm driven by Kaposi sarcoma–associated herpesvirus and prevalent in people living with HIV, spatial profiling identified distinct tumor and TME compartments. Tumor regions exhibited an endothelial–fibroblastic signature, whereas the TME was enriched for T cells, B cells, and cancer-associated fibroblasts. Notably, tissue-resident memory T cells (T_{RM}), particularly $CXCR3^+$ and $IFN-\gamma$ –producing $CD8^+ T_{RM}$, were enriched in HIV-negative individuals but depleted in people living with HIV, indicating impaired local immune surveillance. PD-1 expression was predominantly localized within the TME, highlighting immune checkpoint pathways as potential therapeutic targets.

In CTCL, malignant T cells co-opted T helper 2 (TH2) gene programs and actively remodeled their microenvironment. We identified $MHC-II^+$ fibroblasts and dendritic cells capable of sustaining TH2-like tumor cells. CTCL lesions demonstrated spatial association of tumor cells with B cells, forming tertiary lymphoid structure–like aggregates. Across independent cohorts, B cell enrichment correlated with disease progression, underscoring their functional relevance.

Collectively, these findings establish the TME as a central hub of cellular interaction in rare skin neoplasms. While KS progression is shaped by impaired T_{RM} immunity, CTCL is characterized by tumor-driven TH2 polarization and B cell–rich niches. Spatially resolved analyses provide mechanistic insight and identify immunologic vulnerabilities that may inform targeted therapeutic strategies.

**Young Immunologists:
Symposium in Memory of Prof. Ihan**

YI.1.01

METABOLIC MODULATION WITH 2-DEOXY-D-GLUCOSE ALTERS T LYMPHOCYTE FUNCTIONS

Tjaša Frlic^{1,*}, Tadeja Snedec¹, Mojca Pavlin¹¹Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana

Presenting author e-mail: tjasa.frlic@mf.uni-lj.si

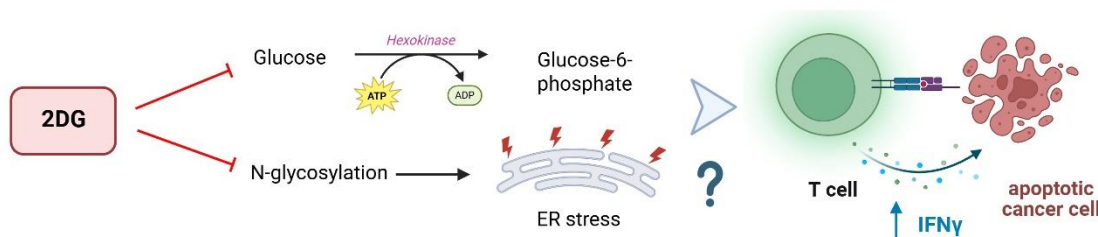
Background: Immunotherapies have transformed oncology; however, poor clinical outcomes in some patients necessitate innovative strategies to augment anti-tumor T cell responses. T cell effector functions are intrinsically linked to metabolic programming; thus, metabolic modulation represents a promising avenue for therapeutic enhancement. 2-deoxy-D-glucose (2DG) acts as both a glycolytic inhibitor and disruptor of protein N-glycosylation.

Ex vivo 2DG treatment of T cells before adoptive transfer can enhance the efficacy of adoptively transferred T cells [1]. Specifically, T cells exposed to 2DG could switch metabolic reprogramming from glycolysis to oxidative phosphorylation, a metabolic state that favors memory T cell differentiation. Additionally, 2DG could increase cytotoxicity and IFN- γ secretion [2, 3]. This study explored the potential of 2DG to prime T cells for enhanced cytotoxicity.

Methods: PBMCs from healthy donors were activated and treated with varying concentrations of 2DG prior to transcriptomic sequencing. Bioenergetic profiles (OCR/ECAR) were assessed using Seahorse XFe analysis. T cell differentiation and activation markers were quantified using flow cytometry, and effector functions were evaluated by measuring the secretion of IFN- γ and interleukin 2 (IL-2).

Results: We identified a significant hormetic effect of 2DG on T cell function in pre-activated T lymphocytes. Low-dose 2DG (0.6 mM) significantly enhanced IFN- γ secretion, whereas higher concentrations (>2.4 mM) were inhibitory. This stimulatory low-dose window, which is clinically achievable *in vivo*, was associated with a more potent immune phenotype characterized by reduced PD-1 expression and elevated CD69 levels. Transcriptomic profiling revealed an increase in the mRNA levels of genes associated with ER stress and the unfolded protein response (UPR). In addition, transcriptomic analysis provided enhanced insights into the effects of 2DG on T cells, elucidating its impact on key metabolic pathways.

Conclusions: Altogether, 2DG presents a promising drug that could be explored for further development of adjuvant therapies that could improve cancer adoptive immunotherapy.



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YI.1.02

DECREASED SERUM LEVELS OF NEUTRALISING ANTI-IL-6 AUTOANTIBODIES IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Anja Srpčič^{1,2,*}, Manca Ogrič¹, Felicita Urzi³, Saša Čučnik^{1,4}, Sergej Pirkmajer², Katja Lakota¹, Katja Perdan Pirkmajer^{1,5}

¹Department of Rheumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia; ²Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia; ⁴Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia; ⁵Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: Anja.Srpcic@kclj.si

Background: Idiopathic inflammatory myopathies (IIM) are heterogeneous systemic auto-inflammatory diseases that primarily affect skeletal muscle tissue, but often manifest in extramuscular symptoms as well. The majority of IIM patients present with myositis-specific or myositis-associated autoantibodies with well established prognostic and diagnostic value. Anti-cytokine autoantibodies (ACAAs) have previously been reported in both healthy and diseased individuals, however, they were not studied in IIM. Their function is not yet entirely clear.

Methods: Serum samples were collected from 13 treatment-naïve and 31 previously treated IIM patients at the Department of Rheumatology, University Medical Centre Ljubljana, and compared with serum from 31 healthy controls (HCs). Serum levels of ACAAs targeting interleukin-6 (anti-IL-6) were measured by an in-house ELISA. Their effect on IL-6 signalling was analysed using an IL-6-responsive reporter gene assay (RGA). Anti-IL-6 autoantibodies were isolated from HC serum using tosyl activated magnetic beads and assessed for neutralising activity on RGA. Additionally, the associations between patients RGA levels and markers of muscle damage (serum creatine kinase and manual muscle testing score) were evaluated.

Results: Regardless of treatment status, serum of IIM patients had lower anti-IL-6 levels ($p=0.04$) and reduced neutralisation of IL-6 signalling ($p<0,0001$) on RGA compared to HCs. An inverse trend between anti-IL-6 and RGA levels was observed. Isolated anti-IL-6 autoantibodies abolished IL-6 signalling on RGA, confirming their neutralising capacity. Patients with lower neutralisation showed higher levels of muscle damage markers.

Conclusions: Our data suggests the presence of anti-IL-6 autoantibodies in HCs and their decreased levels in IIM patients. RGA results indicate these autoantibodies neutralise IL-6 signalling, which is otherwise associated with pathological immune-mediated muscle damage. Their potential protective function suggests that reducing IL-6 signalling may be a promising therapeutic approach in IIM.

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YI.1.03

IRRADIATION-INDUCED TRAINED IMMUNITY OF NATURAL KILLER CELLS IN MOUSE TUMORS

Tim Bozic¹, Bostjan Markelc^{1,2}, Barbara Liseč¹, Iva Santek^{1,3}, Simona Kranjc Brezar^{1,3}, Ziva Pisljar¹, Tanja Jesenko^{1,3}, Maja Cemazar^{1,4}

¹Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia; ³University of Ljubljana, Faculty of Medicine, Slovenia; ⁴Faculty of Health Sciences, University of Primorska, Izola, Slovenia

Presenting author e-mail: tbozic@onko-i.si

Trained immunity, where innate immune cells develop memory-like properties, plays a key role in cancer [1]. Among these, memory-like natural killer (mNK) cells show promise as therapeutic targets, either alone or combined with treatments like radiotherapy [2]. However, the effects of irradiation (IR) on NK cells remain unclear, particularly with respect to different IR doses and regimens [3]. While fractionated radiotherapy appears to enhance NK cell function, a deeper understanding of IR's impact on NK cells in tumors is essential for advancing NK cell-based therapies. The aim of our study was to determine the effects of IR of murine tumors on: a) tumor growth delay, b) NK cell infiltration into the tumor microenvironment, c) cytokine expression profile associated with NK cell infiltration, d) formation of mNK cells in lymph nodes, and e) to evaluate cytotoxic function of IR-induced mNK cells.

Using murine breast cancer 4T1 and colorectal cancer CT26 models, tumors were treated with either single (3, 5, 10, 15 Gy) or fractionated (3x 1, 3x 2, 3x 5, 3x 7 Gy) IR regimens, resulting in a dose-dependent tumor growth delay. Immunofluorescence showed increased infiltration of granzyme B-positive NK cells in CT26 tumors following 3x 5 Gy IR at both day 3 and day 7 compared to control, whereas 4T1 tumors exhibited limited NK cell infiltration after IR. Cytokine profiles of irradiated tumors assessed via qRT-PCR showed increased expression of proinflammatory cytokines *Ccl5*, *Cxcl9*, *Cxcl10* and *Cxcl16* associated with NK cell infiltration into tumors. Flow cytometry analysis of murine lymph nodes at day 3 after single-dose IR (10 Gy) and at day 7 after fractionated IR (3x 5 Gy) demonstrated the induction of diverse NK cell subpopulations, including highly mature mNK cells defined by six investigated surface markers: NKp46, KLRG1, CD122, CD244, CD49b, and Ly49C.

The results showed that tumor IR modulates NK cell populations in a dose- and regimen-dependent manner. Further experiments involving multiomic single-cell sequencing will identify specific subpopulations of tumor IR-induced mNK cells and elucidate their epigenetic regulation, thereby providing mechanistic insight into how IR affects NK cell populations in murine tumors. Together, these results will provide a critical basis for evaluating the potential of IR to enhance NK cell-based cancer therapies.

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YI.1.04

THE CAR-CYTOTOX SCORE: A UNIFIED FRAMEWORK FOR MEASURING CAR T CELL CYTOTOXICITY

Lucija Sršen¹, Larisa Janžič¹, Irena Auersperger², Samo Zver^{2,3}, Matjaž Sever^{2,3}, Alojz Ihan¹, Andreja Nataša Kopitar¹

¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ²Department of Hematology, University Medical Centre Ljubljana, Ljubljana, Slovenia; ³Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

Presenting author e-mail: lucija.srsen@mf.uni-lj.si

Background: CAR T cell therapy represents a breakthrough in cancer treatment, yet its cytotoxic potency varies markedly across donors, manufacturing runs, and patients. Current product release assays confirm cell identity and viability but do not measure functional strength. A standardized approach for quantifying CAR T cytotoxicity is needed.

Methods: We developed a unified framework integrating complementary functional readouts after 48-hour CD19-bead stimulation. CD107a degranulation was assessed by flow cytometry, transcriptional responses by RT-qPCR for nine cytotoxicity-related genes (*BCL2*, *DOCK8*, *FHL3*, *GZMB*, *ITGB1*, *NKG7*, *PRF1*, *STX11*, *UNC13D*), and cytokine and effector secretion by multiplex immunoassay (IL-4, IL-6, IL-10, IL-17A, TNF- α , IFN- γ , soluble Fas, FasL, granzyme A, granzyme B, perforin, granulysin). Marker-specific fold changes were normalized to the healthy-donor (n=13) 90th percentile and integrated into a weighted 0–10 CAR-Cytotox score, classifying samples as low (0–3), moderate (3–6), or high (6–10) in cytotoxicity.

Results: In CAR T cell products generated from 13 healthy donors and three B cell acute lymphoblastic leukemia patients, stimulation consistently enhanced CD107a expression, upregulated *GZMB* and *BCL2*, and increased IFN- γ , IL-6, IL-4, and FasL release. The mean donor score was 4.53 ± 0.66 , while patient scores were 2.14, 6.70, and 7.38, corresponding to low or high cytotoxicity bands.

Conclusions: The CAR-Cytotox score provides a standardized, quantitative measure of CAR T cell cytotoxicity by integrating multiple functional assays into a single interpretable metric. The scoring system and calculator are freely accessible at <https://lsrsen.github.io/CAR-Cytotox/>.

YI.1.05**EXPRESSION OF INDUCIBLE DAMAGE-ASSOCIATED MOLECULAR PATTERNS AFTER INTERLEUKIN-12 GENE ELECTROTRANSFER IN CANCER CELL LINES**Ajda Medved^{1,2}, Tanja Jesenko^{1,2}, Gregor Sersa^{1,3}, Maša Bošnjak¹, Maja Cemazar^{1,4}

¹Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; ³University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia; ⁴Faculty of Health Sciences, University of Primorska, Izola, Slovenia

Presenting author e-mail: amedved@onko-i.si

Interleukin-12 (IL-12) is a potent immunostimulatory cytokine with well-documented antitumor activity through the activation of natural killer (NK) cells and T lymphocytes, induction of interferon-gamma (IFN- γ), and modulation of the tumor microenvironment. Despite its therapeutic promise, systemic administration of IL-12 has been limited in clinical use due to severe toxicity. To overcome this challenge, gene electrotransfer (GET) has emerged as a localized delivery approach that enables efficient expression of therapeutic genes within tumors, thereby maximizing antitumor effects while minimizing systemic exposure. In this study, we explored the effects of IL-12 GET on the expression of cytosolic nucleic acid sensors, damage-associated molecular patterns (DAMPs), and cytokine production in two murine tumor cell lines, melanoma B16F10 and colorectal carcinoma CT26. Using a plasmid encoding IL-12 (pmIL12) and a noncoding control plasmid (pScramble), we compared transfection efficiency, cell viability, transcriptional activation of DNA and RNA sensors, and subsequent cytokine responses. Our results demonstrated successful IL-12 expression at both the mRNA and protein levels, peaking 24 hours post-treatment. Notably, IL-12 GET significantly reduced cell viability, with melanoma cells showing greater sensitivity than carcinoma cells. We observed that GET activated cytosolic sensors and induced cytokine production in both cell lines. Importantly, the upregulation of IL-6 and TNF- α was detected exclusively after IL-12 GET, but not after control plasmid delivery. These cytokines are classified as inducible DAMPs (iDAMPs) that may contribute to the immunostimulatory potential of IL-12 gene therapy. Furthermore, differential responses between B16F10 and CT26 cells highlight the importance of tumor type-specific mechanisms in shaping outcomes of gene-based immunotherapies. Taken together, our findings provide new insights into the multifaceted immune activation triggered by IL-12 GET. By demonstrating the involvement of iDAMPs alongside classical cytokine pathways, this work emphasizes the dual role of IL-12 as both a therapeutic cytokine and an inducer of innate immune activation. These results form a basis for further *in vivo* studies aimed at optimizing IL-12-based gene therapies and harnessing iDAMP signaling to enhance antitumor immunity.

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YI.1.06**IMMUNOGENICITY OF LUCIFERASE SUPPRESSES TUMOR FORMATION IN AN ORTHOTOPIC K7M2LUC OSTEOSARCOMA MODEL**

Saša Kupčič^{1,2}, Urška Kamenšek^{1,3}, Maja Čemažar^{1,4}, Urša Lampreht Tratar^{1,5}

¹Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;

³Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; ⁴Faculty of Health Sciences, University of Primorska, Izola, Slovenia; ⁵Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: skupcic@onko-i.si

Background: Osteosarcoma (OSA) is a rare, aggressive primary bone tumor that mainly affects adolescents and young adults and typically arises at metaphyses of long bones within a complex bone microenvironment. Because this niche strongly influences tumor behavior, orthotopic mouse models are preferred for translational research. Non-invasive bioluminescence imaging (BLI) using luciferase reporters has become a widely adopted approach for preclinical evaluation of deep-seated tumors. However, growing evidence suggests that luciferase can trigger immune responses that interfere with tumor establishment. The objective of this study was to investigate immune mechanisms underlying failure of an orthotopic luciferase-expressing OSA model.

Methods: K7M2 wild type (wt) cells were used, which are known to form orthotopic OSA tumors in tibiae of BALB/c mice. K7M2 wt cells were transduced with luciferase to generate stable luciferase-expressing line K7M2luc. After confirming comparable in vitro proliferation between wt and luciferase-expressing cells, both cell lines were orthotopically implanted into BALB/c mice (Approval no. U34401-17/2023/1).

Results: After intratibial implantation, x-ray imaging demonstrated successful tumor growth with K7M2 wt, whereas K7M2luc cells, monitored with BLI, consistently failed to establish tumors. ELISpot for granzyme B (GrB) detection revealed modest but significant activation of cytotoxic lymphocytes, reflected by increased GrB secretion from splenocytes, while serum IFN- γ , measured with ELISA assay, remained undetectable. In contrast, immunohistochemical analysis of the tumor microenvironment revealed pronounced local immune activation. Immunohistochemistry of tibiae after K7M2luc tumor regression demonstrated increased GrB staining and infiltration of CD8⁺ cells, indicating localized activation of cytotoxic T lymphocytes as the primary mechanism of tumor suppression. To determine the involvement of adaptive immunity, K7M2luc cells were implanted into NUDE mice lacking T cells. In these hosts, tumors formed reliably and displayed typical OSA histopathology, confirming that CD8⁺ T cells are essential for rejection of luciferase-expressing tumors.

Conclusions: Overall, this study demonstrates that luciferase acts as a strong immunogenic driver, capable of activating adaptive cytotoxic responses within the OSA microenvironment, ultimately preventing orthotopic tumor establishment in immunocompetent mice.

YI.1.07**GOVERNING IMMUNOGENIC CELL DEATH THROUGH TRANSCRIPTIONAL CONTROL**

Taja Železnik Ramuta^{1,*}, Sara Orehek^{1,5}, Borna Bacinger^{1,5}, Lucija Kadunc Polajnar¹, Roman Jerala^{1,2,4}, Iva Hafner Bratkovič^{1,2,3,*}

¹Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia; ²EN-FIST Centre of Excellence, Ljubljana, Slovenia; ³University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; ⁴CTGCT, Center for Technologies of the Gene and Cell Therapy, National Institute of Chemistry, Ljubljana, Slovenia; ⁵Interdisciplinary Doctoral Study of Biomedicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: taja.zeleznik@ki.si

Background: To improve cancer treatment outcomes, emerging therapies seek not only to eliminate malignant cells directly but also to stimulate antitumor immunity to clear residual disease. Conventional chemotherapy induces predominantly immunologically silent apoptosis, whereas immunogenic cell death modalities such as pyroptosis and necroptosis offer the potential to enhance anticancer efficacy.

Methods: We engineered synthetic biology tools to enable precise transcriptional control of immunogenic cell death. Using a luciferase reporter system, we screened stress-responsive promoters and a panel of inducers, including reactive oxygen species (ROS)-generating agents, chemotherapeutics, and hypoxia-mimicking compounds, aiming to identify combinations that yielded robust and specific promoter activation. Top-performing promoters were then used to regulate expression of the pyroptosis effector gasdermin D (GSDMD) in both *in vitro* and *in vivo* models.

Results: A synthetic minimal promoter containing NF-κB binding sites exhibited the strongest stress responsiveness, followed by the CCL20 and IP10 promoters, particularly when paired with doxorubicin. To induce pyroptotic cell death, the N-terminal fragment of GSDMD was expressed under the control of selected promoters and activated with doxorubicin. The system was first established in HEK293T cells and subsequently validated in additional cancer cell lines, including HeLa (human ovarian cancer), N2A (human neuroblastoma), and B16F10 (murine melanoma). The best-performing construct was further evaluated *in vivo*, where it enabled stringent transcriptional control of cancer cell death, significantly reduced tumor burden, and prolonged survival in a murine melanoma model. In parallel, we developed a transcriptionally inducible necroptosis effector (MLKL), demonstrating the generalizability of this strategy across distinct immunogenic cell death pathways.

Conclusion: Leveraging transcriptional control enables highly specific and adaptable induction of immunogenic cell death in cancer, highlighting its potential as an innovative therapeutic modality.

YI.1.08**PROTEOME ANALYSIS OF MINOR SALIVARY GLANDS IDENTIFIES CLINICALLY AND MOLECULARLY DISTINCT PATIENT SUBGROUPS IN SJÖGREN'S DISEASE**

Neža Štucin^{1,2}, Katja Perdan Pirkmajer^{1,3}, Alojzija Hočevar^{1,3}, Britta Maurer MD^{4,5}, Saša Čučnik^{1,2}, Polona Žigon^{1,6}, Kerstin Klein^{4,5}

¹University Medical Centre Ljubljana, Department of Rheumatology, Ljubljana, Slovenia; ²University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia; ³University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; ⁴Department of Rheumatology and Immunology, Bern University Hospital, University of Bern, Inselspital, Bern, Switzerland; ⁵Department for BioMedical Research, University of Bern, Bern, Switzerland; ⁶University of Primorska, FAMNIT, Koper, Slovenia
Presenting author e-mail: neza.stucin@kclj.si

Background: Sjögren's disease (SjD) is a systemic autoimmune disorder with marked clinical and biological heterogeneity. Identifying SjD subgroups with distinct pathobiological features may clarify disease mechanisms and support targeted therapy development. Yet little is known about clustering patients based on tissue-specific traits, particularly in minor salivary glands (MSG). We therefore performed an exploratory study to identify potential SjD subgroups using MSG proteomics.

Methods: Flash-frozen MSG from 18 SjD patients meeting the 2016 ACR/EULAR criteria were analyzed along with their clinical data. MSG proteomes were analyzed by mass spectrometry. PCA was performed on all detected proteins, differentially expressed proteins were identified between subgroups, and pathway enrichment analysis was conducted.

Results: Proteome analysis of MSG tissues detected 8192 proteins and PCA identified three separate subgroups of SjD patients. These subgroups differed in clinical characteristics—patients in cluster 1 (n=3) demonstrated increased lymphocytic infiltration, a higher number of germinal centers in their MSG, and reduced salivary flow relative to clusters 2 (n=9) and 3 (n=6). Furthermore, cluster 1 exhibited a greater proportion of individuals positive for rheumatoid factor and cryoglobulins. Cluster 1 also displayed elevated IFN signatures, including upregulated CXCL10 and CXCL13 in MSG. PEA showed that IFN signaling, antigen presentation, and T cell activation were key dysregulated pathways in cluster 1 compared with clusters 2 and 3.

Conclusion: In conclusion, SjD patients in our cohort clustered in 3 subgroups based on MSG proteomes, with the first group showing the most pronounced glandular damage and immune dysregulation.

YI.2.01**IDENTIFICATION OF IGE EPITOPE-LIKE PEPTIDES OF PEANUT ALLERGEN ARA H 1**Ana Potočnik^{1,2}, Ana Zupančič², Zala Celan², Mojca Lunder², and Peter Korošec^{1,2}¹University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; ²Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: ana.potocnik@klinika-golnik.si

Background: Peanuts and other tree nuts are a leading cause of severe food-induced allergic reactions, with Ara h 1 being a major allergen and important predictor of clinical reactivity in both adult and pediatric patients. Aside from oral immunotherapy with limited safety due to side effects, there is no approved safe medication to prevent peanut sensitization. The binding of allergens to IgE antibodies, bound to FcεRI on mast cells and basophils, leads to receptor cross-linking and degranulation, releasing mediators of immediate hypersensitivity reactions. The interaction between peanut allergen epitopes and IgE paratopes therefore represents a key step leading to an anaphylactic reaction. This interaction could be inhibited by IgE epitope-like peptides, which would bind to the paratopes of IgE antibodies instead of the allergens, thereby preventing allergic reaction.

Methods: Epitope-like peptides (mimotopes) were selected by using bacteriophage display libraries. The phage display technique enables the presentation of foreign peptides on the surface of bacteriophages, generating libraries of up to 10¹² unique peptide-displaying clones. Phage libraries displaying linear and cyclic peptides were used. The binding target was polyclonal antibodies specific to the Ara h 1 allergen, which were isolated from the serum of immunized animals. After three rounds of affinity selection, we sequenced the phagemids of the eluted phages using NGS and obtained the amino acid sequences of the mimotopes through bioinformatic analysis. A preliminary phage ELISA was carried out to assess the binding of the most abundant individual clones to polyclonal anti-Ara h 1 antibodies.

Results: We identified IgE epitope-like peptides of peanut allergen Ara h 1 and aligned some motifs to the linear amino acid sequence of the protein. Binding of the most frequently occurring individual phage clones, displaying the identified peptides, to polyclonal anti-Ara h 1 antibodies was confirmed.

Conclusions: Using phage display technology, next-generation sequencing, and bioinformatic analysis, we successfully identified IgE epitope-like peptides of the peanut allergen Ara h 1. The next step in this research will be inhibition assays to test whether synthetic IgE epitope-like peptides of Ara h 1 can block the binding of the allergen to IgE antibody paratopes.

YI.2.02

HEREDITARY α -TRYPTASEMIA IN CHILDREN WITH PEANUT-INDUCED ANAPHYLAXIS

Tadej Pungertnik^{1,2}, Tina Vesel Tanjšek^{1,2}, Tadej Avčin^{1,2}, Peter Korošec^{3,4}

¹University Children's Hospital Ljubljana, University Clinical Center, Ljubljana, Slovenia; ²Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia ; ³University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia; ⁴Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: tadej.pungertnik@kclj.si

Background: Hereditary α -tryptasemia (H α T) is a genetic trait caused by an increased number of α -tryptase-encoding genes, characterised by elevated basal serum tryptase (BST) and distinct α/β -tryptase heterotetramers with distinct biochemical properties, affecting PAR-2 and EMR2 signalling. The trait is present in 5.5% of the general population and is associated with a higher likelihood of venom, drug, and idiopathic anaphylaxis. Food allergy affects up to 10% of children, but there is a lack of reliable predictive biomarkers. We aimed to determine the impact of H α T on food-induced anaphylaxis, as suggested by recent studies.

Methods: A prospective cohort of children with food-induced anaphylaxis (n = 129) and a group of healthy controls (n = 59) were recruited from a tertiary centre in Slovenia and underwent tryptase genotyping. Allergen causes, clinical and laboratory findings were subsequently assessed.

Results: We noted a higher prevalence of H α T in food allergic children of 8.5% (n = 11 of 129), which was statistically significant in the group of peanut allergic children (16.1%, n = 5 of 31) both in comparison to the general population (p = 0.026, binomial test) and to our health controls (p = 0.045, Fisher's exact test). All children with an increased basal tryptase level had H α T. Moreover, allergen tolerance development was observed in children with H α T.

Conclusions: The risk of food-induced anaphylaxis is associated with H α T in children with peanut-induced anaphylaxis. These findings provide further insight into a novel genetic biomarker for personalised risk stratification in peanut-induced anaphylaxis in children.

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YI.2.03**SYSTEMIC IMMUNOMODULATION AS THE NEW PARADIGM FOR SEVERE ATOPIC DERMATITIS IN THE YOUNG PAEDIATRIC POPULATION**Manca Škulj¹, Tina Vesel Tajnšek², Tadej Avčin²¹Faculty of Medicine, University of Ljubljana, Slovenia; ²Department of Allergology, Rheumatology and Clinical Immunology, Children's hospital, University Medical Centre Ljubljana, Slovenia

Presenting author e-mail: manca.skulj@gmail.com

Background: Severe atopic dermatitis (AD) in infancy and early childhood is a chronic, highly inflammatory condition causing significant morbidity (quality of life, sleep, and development). Systemic treatment is warranted for disease refractory to maximal topical therapy and dietary exclusion. Dupilumab, an established targeted therapy, is a human monoclonal antibody inhibiting the IL-4R α subunit to block IL-4/IL-13 signaling. This case series evaluates the clinical utility and safety profile of dupilumab in very young paediatric patients with severe, refractory AD [1].

Methods: A retrospective review analyzed one infant and two toddlers (age 5 months – 3,9 years) with severe, generalized AD who were refractory or intolerant to conventional management. Analyzed data included disease history, prior treatments, dupilumab dosing, clinical response, and adverse events.

Results: Case 1: A 6-month-old male (severe, impetiginised, generalised AD, multiple food allergies) commenced dupilumab (200 mg/4 weeks) due to recalcitrant disease despite maximal topical therapy and dietary exclusion. Partial remission of AD was achieved, indicating successful dupilumab therapy. Case 2: A 15-month-old male (severe generalised AD, hyper IgE, multiple food allergies) was introduced to dupilumab (200 mg/4 weeks) after failing standard therapy. Partial remission of AD was achieved, demonstrating successful dupilumab therapy. Case 3: A 3,9-year-old male (severe AD, multiple allergies), partially controlled with cyclosporine, demonstrated excellent efficacy when dupilumab (200 mg/4 weeks) was added as combination therapy.

Conclusions: Dupilumab is a valuable, targeted therapeutic option for severe, refractory AD in the very young paediatric population, demonstrating clinical benefit both as monotherapy and as an adjunct to conventional systemic agents. These findings support the early consideration of targeted biologic therapy with dupilumab in this challenging patient demographic when conventional approaches fail to achieve adequate disease control.

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YI.2.04**COMPARISON OF IMMUNOCAP ISAC AND ALEX² ALLERGY XPLOERER**Ines Hasanović¹, Kaja Zevnik¹, Urška Bidovec-Stojkovič¹ and Peter Korošec¹¹University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia

Presenting author e-mail: ines.hasanovic@klinika-golnik.si

Background: ImmunoCAP ISAC E112i (ISAC) is a multiplex assay that detects specific immunoglobulin E (sIgE) reactivity against 112 molecular allergen components. It is the most clinically used and studied multiplex array to date. Another multiplex platform, ALEX² Allergy Explorer (ALEX²), has recently become available, that detects specific immunoglobulin E (sIgE) reactivity against 295 molecular allergen components (178) and extracts (117). This study aimed to validate the ALEX² assay in our diagnostic routine setting by comparing the performance of ISAC and ALEX².

Methods: We compared the ALEX² multiplex assay with the results of serum samples of 50 individuals routinely tested with ISAC between 2020 and 2024. Positive cut-off values for ISAC is 0.30 ISU-E and 0.30 kUA/L for ALEX².

Results: Overall, we found a high concordance of the results between both assays (80-100%).

When comparing 100 allergen components presented in both assays, a concordance above 90% was found for 95 (95%) allergen components. A concordance below 90% was observed only for 5 (5%) allergen components: Cry j 1 (sugi - *Cryptomeria japonica*), Cyn d 1 (bermuda grass - *Cynodon dactylon*), Act d 2 (kiwi - *Actinidia deliciosa*), Ani s 1 (*Anisakis simplex*) and Der f 1 (American house dust mite - *Dermatophagoides farina*).

Furthermore, when comparing 164 allergen components sorted in 46 groups of allergens, a concordance between assays above 90% was found for 39 (85%) groups of allergens. A concordance below 90% was found for 7 (15%) groups of allergens: sugi (*Cryptomeria japonica*), bermuda grass (*Cynodon dactylon*), herring worm (*Anisakis simplex*), kiwi (*Actinidia deliciosa*), apple (*Malus domestica*), celery (*Apium graveolens*) and german cockroach (*Blatella germanica*).

Conclusion: We showed a high concordance between assays ALEX² and ISAC. ALEX² enables the detection of specific IgE (sIgE) reactivity to a broader spectrum of allergen components compared to ISAC. Moreover, including allergen extracts in ALEX² adds additional data that may affect the understanding individual sensitization profiles. ALEX²'s expanded analytical scope may improve the potential of multiplex sIgE diagnostics.

YI.2.05

CANDIDATE VARIANTS ASSOCIATED WITH HEREDITARY ANGIOEDEMA WITH NORMAL C1-INHIBITOR

Helena Jakopič^{1,3}, Mitja Košnik^{1,3}, Mihaela Zidarn^{1,3}, Julij Šelb^{1,3}, Slađana Andrejević⁴, Marko Barešić⁵, Peter Korošec^{1,6}, Matija Rijavec^{1,2}

¹University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; ²Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; ³Medical Faculty Ljubljana, Ljubljana, Slovenia; ⁴Clinic of Allergology and Immunology, University Clinical Center of Serbia, Belgrade, Serbia; ⁵University Hospital Center Zagreb, Zagreb, Croatia; ⁶Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: helena.jakopic@klinika-golnik.si

Background: Hereditary angioedema with normal C1 inhibitor (HAE-nC1INH) is a rare disorder characterized by recurrent swelling episodes and significant clinical variability. While pathogenic variants in *F12*, *PLG*, *KNG1*, *ANGPT1*, *MYOF*, *HS3ST6*, *CPNI*, and *DAB2IP* have been identified in some patients, the genetic basis remains unknown in many cases, suggesting additional contributing factors.

This study aimed to identify new candidate variants associated with HAE-nC1INH through whole exome sequencing (WES) and analysis of 180 angioedema-related genes, focusing on rare variants.

Materials and Methods: We performed WES on 34 HAE-nC1INH patients without known pathogenic/likely pathogenic variants in causing genes, focusing on 180 angioedema-related genes (GeneCards). Using an in-house bioinformatics pipeline, we aligned sequencing data to the hg19 reference genome and filtered variants, excluding common variants (1000 Genomes), retaining only rare variants with predicted high or moderate functional impact and excluding *HLA-DBR* gene. Further filtering with gnomAD v2.1.1 (hg19) retained variants with a frequency of ≤ 0.01 in the European (non-Finnish) population. Variant reliability was verified using IGV to exclude sequencing artefacts and false positives. We calculated variant frequencies in our cohort, compared them with gnomAD, and applied a binomial test (Python) to assess statistical significance (p -value < 0.05).

Results: Our analysis identified rare variants in 18 genes, including *XPNPEP2*, *BDKRB1*, *CFH*, *CFHR2*, *F5*, and *ANPEP*, as potential contributors to HAE-nC1INH, being more common among our patients than in the general population (GnomAD2.1.1). *XPNPEP2* encodes X-prolyl aminopeptidase 2, an enzyme that degrades bradykinin, a key mediator of vascular permeability, while *BDKRB1* encodes the bradykinin B1 receptor, which regulates inflammation and vasodilation. Dysregulated bradykinin metabolism is strongly implicated in HAE. *CFH* and *CFHR2* regulate the complement system, and their disruption can lead to excessive inflammation and increased vascular permeability. *F5* (coagulation factor V) plays a central role in blood clotting, while *ANPEP*, a peptidase, influences the breakdown of bioactive peptides. These findings highlight the interplay between bradykinin metabolism, complement regulation, and coagulation in HAE-nC1INH pathogenesis.

Conclusions: Our findings suggest that these variants may play a role in disease development and add new pieces to the genetic puzzle of HAE-nC1INH. Larger studies and laboratory experiments will be needed to confirm their importance.

YI.2.06**DIAGNOSTIC COMPARISON OF BASOPHIL ACTIVATION TEST AND SPECIFIC IGE TO VENOM COMPONENTS IN HYMENOPTERA-ALLERGIC PATIENTS WITH LOW OR NEGATIVE VENOM-SPECIFIC IGE**

Luka Dejanović¹, Urška Bidovec Stojković¹, Ana Koren¹, Mitja Košnik^{1,2}, Peter Kopač^{1,2}, Peter Korošec^{1,3}

¹University Clinic of Respiratory and Allergic Diseases Golnik; ²University of Ljubljana Faculty of Medicine; ³ University of Ljubljana Faculty of Pharmacy

Presenting author e-mail: luka.dejanovic@klinika-golnik.si

Background: The diagnosis of Hymenoptera venom allergy (HVA) relies on an IgE-mediated mechanism, with the specific IgE (sIgE) to venom components and basophil activation test (BAT) currently providing the most accurate diagnostic value. However, it remains unclear whether venom components can replace BAT in confirming sensitization in patients with negative or very low sIgE to whole venom extracts. This study aims to evaluate the utility of sIgE to venom components in patients with low or negative sIgE to whole venom extracts and to compare it with BAT results.

Methods: We prospectively selected 25 patients who required venom immunotherapy (VIT) but had negative (<0.10 kIU/L) or very low (<0.35 kIU/L) sIgE to whole venom extracts. These patients were referred to the University Clinic of Respiratory and Allergic Diseases Golnik between 2021 and 2024. All sIgE measurements (extracts, Api m 1, 2, 3, 5, 10, and Ves v 1, 5) were done on ImmunoCAP Phadia 250 system. BAT was done from fresh heparinized blood with a stimulation buffer containing IL-3. Samples were incubated with 0.001-1 mg/mL of whole venom extract (HBV and/or YJV), stained with CD123-PE/HLA-DR-PerCP/CD63-FITC-labeled antibodies, and analyzed by flow cytometry.

Results: All the 25 patients had a history of severe allergic sting reactions (12 Mueller grade III and 13 grade IV), and only 7 patients had positive intradermal tests for HBV or YJV. In 8 patients, the suspected culprit was HBV, in 13 it was YJV, and in 4 the culprit was unknown. Median of serum sIgE levels to whole venom extracts (HBV and YJV) was <0.10 kIU/L (>0.10-0.30), with 25 measurements showing negative results (<0.10 kIU/L). Among the 8 patients with HBV history, only one had positive sIgE to HBV components (Api m 3 and 10); however, all 8 had a positive HBV BAT. Of the 13 patients with YJV history, 11 had positive sIgE to YJV components (Ves v 1: 3, Ves v 5: 7, and both 1), and all 13 had a positive YJV BAT. Of the 4 patients with an unknown culprit, one had a positive YJV BAT and positive Ves v 5 sIgE, while the remaining 3 had positive HBV BAT, but none had positive sIgE to HBV components.

Conclusion: Our data showed that in patients with very low or negative sIgE to venom extracts, component testing is only useful for confirming YJV sensitization, but not HBV sensitization. Therefore, in HBV allergy, BAT remains the first choice to confirm sensitization in cases with low or negative sIgE to venom extracts and is essential for facilitating HBV VIT in such patients.

YI.2.07**DIVERSE CELLULAR SCAFFOLDS INDUCE NLRP3 CLUSTERING TOWARD INFLAMMASOME FORMATION**

Elvira Boršič Mlinarič^{1,2}, Taja Železnik Ramuta¹, Sara Orehek¹, Mateja Erdani Kreft³, Matthias Geyer⁴, Roman Jerala^{1,5} and Iva Hafner Bratkovič^{1,6,7}

¹Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia; ²Interdisciplinary Doctoral Study of Biomedicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ³Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ⁴Institute of Structural Biology, University Clinics Bonn, University of Bonn, Bonn, Germany; ⁵Centre for the Technologies of Gene and Cell Therapy, National Institute of Chemistry, Ljubljana, Slovenia; ⁶EN-FIST Centre of Excellence, Ljubljana, Slovenia; ⁷Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: elvira.borsic@ki.si

Background: Inflammasomes are multiprotein complexes that initiate early immune responses against invading pathogens and cellular stress. NLRP3 is one of the most studied inflammasome-forming proteins and responds to a wide range of pathogen- and self-derived triggers, while its dysregulated activity contributes to the progression of common noncommunicable diseases, including neurodegenerative and cardiovascular diseases. Despite significant advances in understanding the structural mechanism of NLRP3 inflammasome assembly, it remains unclear how such diverse triggers and cellular locations facilitate its activation.

Methods: NLRP3 variants targeted to distinct subcellular locations, including the endoplasmic reticulum, Golgi apparatus, plasma membrane, centrosome, lysosomes, mitochondria and peroxisomes, were introduced into murine NLRP3-deficient macrophages. Subcellular localization of the prepared variants was verified by confocal microscopy. Canonical NLRP3 activators were used to stimulate inflammasome assembly, while NLRP3-specific inhibitors were applied to dissect the activation process. Inflammasome activation was quantified by measuring cytokine release and cell death.

Results: NLRP3 variants localized to different organelles responded to canonical activators comparably to the wild-type NLRP3 (WT). Although WT NLRP3 requires a basic membrane-binding segment (K127–K130) for activation, organelle-targeted variants retained inflammasome activity despite mutation of this segment, indicating that association with a lipid scaffold, rather than a specific membrane interaction, is essential for inflammasome formation. Fusion of NLRP3 to protein-based scaffolds substituted for membranes and revealed that NLRP3 clustering on distinct scaffolds unlocks the inactive NACHT domain conformation and initiates inflammasome assembly.

Conclusions: These findings demonstrate that NLRP3 does not rely on a single cellular compartment and identify clustering at membrane or protein scaffolds as an important step in its activation, enabling NLRP3 to sense distinct activator-induced cellular imbalances.

YI.2.08**DEVELOPMENT OF A PLATFORM FOR PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST MEMBRANE PROTEINS ON THE MODEL OF BCMA**

Petra Kern^{1,2}, Valerija Kovač¹, Maja Svetličič¹, Duško Lainšček³, Vladka Čurin Šerbec¹

¹Slovenian Institute for Transfusion Medicine, Ljubljana, Slovenia; ²University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; ³National Institute of Chemistry, Ljubljana, Slovenia

Presenting author e-mail: petra.kern@ztm.si

Background: Monoclonal antibodies (mAbs) against membrane proteins are essential diagnostic tools, widely used therapeutics and are part of targeted cell therapies like CAR-T cell therapy. They are still mostly produced using hybridoma technology or phage display, generating large numbers of antibody candidates that need to be tested in a short period of time. As membrane proteins do not retain their three-dimensional structure in soluble form, cell-based high-throughput assays are needed for the primary screening to ensure that the selection process yields useful mAbs. The aim of our research was thus to produce original mAbs against human B-cell maturation antigen (BCMA) as a model membrane protein.

Methods: We developed a simple high-throughput cell-based ELISA using seven established cell lines (suspension and adherent cells), to optimize a protocol that enabled detection of antibodies at low concentrations. We compared this test to conventional ELISA and imaging flow cytometry and concluded that the cell ELISA has comparable specificity and sensitivity. Antibodies were produced using hybridoma technology. After immunization of Balb/c mice with recombinant extracellular domain of BCMA or chosen peptides, antibody candidates were selected using cell ELISA. The most promising mAb was chosen for further characterisation, large scale production, enzymatic degradation, and recombinant expression in humanized IgG form.

Results: The best candidate mAb was determined to be IgM class. It was produced on a larger scale and purified by FPLC affinity chromatography. Variable regions of the antibody were sequenced and recombinantly expressed in human IgG vectors in HEK293 cell line (the latter work is still in progress). Enzymatic degradation of IgM mAb was also performed and smaller fragments were tested by Western blot to visualize their size and identity. The preservation of specificity of our recombinant IgG mAbs and IgM fragments will be confirmed using cell ELISA and flow cytometry.

Conclusions: We have developed a versatile cell ELISA that can easily be translated to diverse antibody-antigen-cell line systems and integrated as a screening test in laboratories with limited access to equipment. Using cell ELISA, we were able to successfully select antibody candidates and showed that this protocol is suitable to produce antibodies against membrane proteins.

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Lectures

S1.01.P**MAST CELLS AND THEIR PROGENITORS IN ASTHMA – MY PERSPECTIVE**

Jenny Hallgren Martinsson

Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

Presenting author e-mail: jenny.hallgren@imbim.uu.se

Mast cells (MCs) not only become activated in allergic asthma but also accumulate in specific regions of the lung. Experimental models indicate that this expansion is preceded by the recruitment of bone marrow–derived MC progenitors (MCps). Although rare, circulating human MCps can be quantified by flow cytometry. In birch pollen–sensitized individuals with asthma, MCp frequencies increase during pollen season, and higher levels correlate with worsening symptoms and loss of asthma control. A larger fraction of MCps in allergic asthma than in healthy subjects expresses the interleukin-5 (IL-5) receptor, and IL-5 promotes their survival and/or proliferation *in vitro*. Consistent with this, biologics targeting IL-5 signalling improve asthma control in severe or uncontrolled asthma and reduce circulating MCp frequencies.

To better understand MC biology during the resolution of airway inflammation, we recently examined MC dynamics in a mouse model of allergic inflammation using CITE-seq. We observed that MC populations show unexpected persistence and remodelling at a stage when most other inflammatory cells have already resolved. For example, mucosal MCs located within the airway epithelium remain present but undergo notable phenotypic and transcriptional adaptations. In addition, we identified a novel MC subset with distinguishing features compared with previously described MC populations. Together, these findings reveal previously underappreciated MC heterogeneity during the return to lung homeostasis and raise new questions about how MCs may contribute to the restoration or stabilization of airway function.

S1.02.P**EPIGENETIC REGULATION OF PATHOGENIC T CELLS DRIVING ALLERGIC AIRWAY INFLAMMATION**

Nicole Boucheron

Division of Immunobiology, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Presenting author e-mail: nicole.boucheron@meduniwien.ac.at

Allergic asthma is a growing global health concern with steadily increasing prevalence. Lung pathogenic T helper type 2 (pTh2) cells are key drivers of disease; however, the early events governing their differentiation, their heterogeneity, and particularly their epigenetic regulation remain poorly understood. Histone deacetylase 1 (HDAC1), a major epigenetic regulator, is thought to control T cell function, but its role in pTh2 biology is unclear. We investigated pTh2 cell emergence and regulation using single-cell RNA sequencing in a murine house dust mite (HDM) model with and without HDAC1 function. We identified two distinct, highly proinflammatory pTh2 subsets and demonstrate that alarmins and tumor necrosis factor receptor superfamily (TNFRSF) signals are key drivers of pTh2 differentiation. Guided by these data, we established an *in vitro* differentiation system to dissect the signals driving pTh2 development. Using our *in vitro* model, we show that these pathways regulate chromatin accessibility at type 2 cytokine loci via modulation of HDAC1-mediated repression. Together, these findings provide new insights into pTh2 heterogeneity and epigenetic regulation and establish a robust platform for mechanistic studies and therapeutic target discovery in allergic asthma.

S1.03.P**MAST CELL DEGRANULATION AND VESICULATION**

Kelsey Fletcher¹, Tamás Visnovitz^{1,2}, Péter Lőrincz³, Dorina Lenzinger¹, Edit I. Buzás^{1,4,5}, Krisztina V. Vukman¹

¹Semmelweis University, Institute of Genetics, Cell- and Immunobiology, Hungary; ²ELTE, Department of Plant Physiology and Molecular Plant Biology, Hungary; ³ELTE, Department of Anatomy, Cell and Developmental Biology, Hungary; ⁴HUN-REN-SU Translational Extracellular Vesicle Research Group, Hungary; ⁵HCEMM-SU Extracellular Vesicle Research Group, Hungary

Presenting author e-mail: vukman.krisztina@semmelweis.hu

Mast cells (MCs) are traditionally recognized for their role in allergic inflammation, where IgE-dependent activation triggers the release of granules enriched in bioactive mediators. Recent evidence, however, highlights that MCs also secrete extracellular vesicles (EVs) carrying proteins and nucleic acids, suggesting additional immunoregulatory functions beyond classical degranulation. Despite these advances, the interplay between extracellular granules (EGs) and EVs remains poorly understood.

In this study, we systematically characterized the extracellular particle (EP) spectrum released by murine bone marrow-derived MCs under distinct immunological stimuli: IgE–antigen complexes (type 1 activation), lipopolysaccharide (LPS; type 2 mimic), and calcium ionophore A23187. EPs were isolated using differential centrifugation and filtration and analyzed by high-resolution flow cytometry, and advanced imaging. Real-time monitoring combined with pharmacological inhibition provided mechanistic insights into EP secretion.

Our findings reveal stimulus-specific EP profiles: IgE-mediated activation predominantly induced EG release, whereas LPS stimulation favored EV secretion. Ca²⁺ ionophore elicited a mixed response, underscoring the need for caution when interpreting data from ionophore-based models. Furthermore, we identified histamine as a reliable marker to discriminate EGs from EVs.

These results demonstrate that MCs deploy distinct extracellular pathways depending on the immune context, with EGs dominating allergic responses and EVs prevailing in Th1-type inflammation. Understanding these differential mechanisms may uncover novel targets for modulating MC-driven pathology in asthma, autoimmunity, and chronic vascular disease.

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S2.01.I

RESIDENT MEMORY T CELLS IN ATOPIC DERMATITIS

László Sajtos¹, Zsolt Czimmerer³, Anikó Kapitány^{1,2}, Viktória Nagy¹, Eszter Anna Janka^{1,2}, Attila Gábor Szöllősi⁴, Gábor Koncz⁴, Andrea Szegedi^{1,2}, Zsolt Dajnoki^{1,2}

¹Department of Dermatology, MTA Centre of Excellence, Faculty of Medicine, University of Debrecen, Hungary; ²HUN-REN-UD Allergology Research Group, Debrecen, Hungary; ³Biological Research Centre, Institute of Genetics, Hungarian Research Network, Szeged, Hungary; ⁴Department of Immunology, Faculty of Medicine, University of Debrecen, Hungary
Presenting author e-mail: zsolt.dajnoki@gmail.com

Background: Atopic dermatitis (AD) and psoriasis (PSO) are common chronic inflammatory skin diseases where tissue-resident memory T cells (Trms) are key factors in the pathogenesis. However, Trm cell subpopulations driving AD and PSO remain incompletely characterized.

Objective: Using single-cell multi-omics, we aimed to characterize and compare memory T cells, especially Trms in AD and PSO, to uncover their detailed functional properties beyond cytokine production.

Methods: We analyzed publicly available single-cell CITE-Seq data on CD45⁺ cells from the skin of severe AD (n=7) and PSO (n=7) patients, and healthy controls (n=7). We performed deep characterization and comparison of key pathogenic Trm populations within both the CD4 and CD8 compartments using functional profiling to assess their cytokine, co-signaling, migration, and survival programs.

Results: Our analysis revealed a prominent expansion of Trms in both AD and PSO, identifying them as the primary producers of pathogenic cytokines. Focused re-clustering identified six distinct CD4⁺ and CD8⁺ Trm clusters, including disease-specific and inflammation-associated populations. The AD-specific clusters expressed IL-13, IL-16, IL-32, and lymphotoxin-beta, showed a high recirculatory Tcm-like phenotype, with a prominent co-activation signature. In PSO, the central pathogenic clusters exhibited a potent Th1/Th17 profile, a classical tissue-resident phenotype, high coactivation with signs of exhaustion, and survival supported by TLE1. Inflammation-associated clusters also displayed disease-specific functional divergences.

Conclusions: The differences identified in the migratory features of Trms in AD and PSO may explain certain clinical distinctions between these two immune-mediated diseases. Furthermore, targeting the revealed co-signaling and survival programs, as well as the TF networks in Trms, could contribute to identifying new disease-specific therapeutic targets that aim to prevent chronicity.

S2.02.I**AUTOREACTIVE IGE IN DERMATOLOGICAL DISEASES**Sabine Altrichter^{1,2,3,4}

¹Department of Dermatology and Venerology, Kepler University Hospital, Linz, Austria; ²Clinical Research Institute for Inflammation Medicine, Medical Faculty, University Linz, Austria; ³Institute of Allergology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, and Humboldt-Universität zu Berlin, Berlin, Germany; ⁴Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology and Allergology, Berlin, Germany

Presenting author e-mail: Sabine.Altrichter@kepleruniklinikum.at

IgE crosslinking via foreign molecules on mast cells and basophil granulocytes are the key mechanisms in allergic reactions. More recent the pathophysiological concept of autoallergy involving IgE autoantibodies targeting self-antigens has been established. These are found in allergy-like conditions like chronic spontaneous urticaria and atopic dermatitis, but also in diseases that are currently classified as classical autoimmune diseases like bullous pemphigoid or systemic lupus.

Autoallergy is emerging as a distinct IgE-mediated mechanism that may contribute to chronic inflammation in various immune-mediated diseases. Common pathophysiological mechanisms and new treatment approaches are presented.

S2.03.I**BRONCHIAL HYPERREACTIVITY IS ASSOCIATED WITH T2 INFLAMMATION AND LOSS OF ASTHMA CONTROL: EVIDENCE FROM A CROSS-SECTIONAL STUDY IN PEDIATRIC PATIENTS**

Iva Topalušić¹, Ozana Hofmann Jaeger², Petar Prcela¹, Tamara Poljičanin³, Asja Stipić Marković⁴, Ivan Pavić^{1,5}

¹Department of Pulmonology, Allergology, Immunology and Rheumatology, Children's Hospital Zagreb, Croatia; ²Health resort Veli Lošinj, Veli Lošinj, Croatia; ³Zagreb County Health Center, Croatia; ⁴University Hospital for Infectious Diseases "Fran Mihaljević", Zagreb; ⁵University of Split, School of Medicine, Split, Croatia

Presenting author e-mail: iva.topalusic89@gmail.com

Background: Bronchial hyperreactivity (BHR) is an important feature of asthma, associated with disease severity and persistence over time. The aim of the study was to investigate a relationship between BHR and parameters of type 2 (T2) inflammation, lung function, and asthma control in children with asthma.

Methods: This retrospective, cross-sectional study included schoolchildren aged 7-18 years, diagnosed with asthma. The children underwent clinical evaluation, which included assessment of asthma control and peripheral blood sampling to determine peripheral eosinophil count, total and specific immunoglobulin E (IgE), and eosinophilic cationic protein (ECP). Lung function tests included spirometry, fractional exhaled nitric oxide (FENO) and a dosimetric bronchial methacholine challenge test.

Results: A total of 152 children with asthma were included in the study, 85 (56%) boys and 67 (44%) girls, with a mean age of 12.6±3.2 years. BHR was detected in 140 (92.10%) children. Patients with BHR were significantly younger ($p=0.001$) and used asthma medication for a shorter period ($p=0.002$). Patients with BHR had significantly higher FENO ($p=0.036$) and peripheral eosinophilia ($p=0.006$). BHR was significantly associated with male gender ($p=0.046$) and uncontrolled asthma ($p=0.015$). The provocative dose of methacholine that caused a decrease of -20% in FEV1 was significantly lower in children with uncontrolled asthma ($p=0.038$). Lung function parameters, total or specific IgE levels, and ECP were not significantly associated with BHR.

Conclusion: Our study showed a high prevalence of BHR in pediatric patients with asthma. BHR was associated with higher peripheral eosinophilia and FENO levels, and with poorer asthma control.

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S2.04.I**LINKING GASTROESOPHAGEAL REFLUX CHARACTERISTICS TO AIRWAY INFLAMMATION IN SEVERE PRESCHOOL WHEEZE**

Ivan Pavić^{1,2}, Iva Topalušić^{1,*}, Ana Močić Pavić³, Roberta Šarkanji Golub⁴, Ozana Hofman Jaeger¹, Iva Hojsak^{3,5,6}

¹Department of Pulmonology, Allergology and Immunology, Children's Hospital Zagreb, Zagreb, Croatia; ²School of Medicine, University of Split, Split, Croatia; ³Referral Center for Pediatric Gastroenterology and Nutrition, Children's Hospital Zagreb, Zagreb, Croatia; ⁴Department of Cytology, Children's Hospital Zagreb, Zagreb, Croatia; ⁵School of Medicine, University of Zagreb, Zagreb, Croatia; ⁶School of Medicine, University Josip Juraj Strossmayer, Osijek, Croatia
Presenting author e-mail: iva.topalusic89@gmail.com

Background: Severe, recurrent preschool wheeze is still an unresolved problem in pediatric respiratory medicine. It is often refractory to inhaled corticosteroids and the underlying mechanisms are not well understood. Gastroesophageal reflux disease (GERD) has been implicated in recurrent wheezing as one of the mechanisms leading to severe preschool wheeze. This study aims to examine the relationship between reflux characteristics, bronchoalveolar lavage (BAL) cytology and clinical outcomes in preschool children with severe recurrent wheeze.

Methods: Preschool children aged 12-60 months with severe recurrent wheeze, who were unsuccessfully treated with inhaled corticosteroids and/or montelukast, underwent combined multichannel intraluminal impedance-pH (MII-pH) and bronchoscopy. BAL samples were assessed for lipid-laden macrophages (LLM). Associations between reflux parameters, BAL cytology and response to antireflux treatment were analysed. Patients were followed up for 6 and 12 months, after which the outcome of GERD treatment as an add-on therapy of severe preschool wheeze was evaluated.

Results: GERD was identified in 70% of participants with severe preschool wheeze. Children with GERD showed significantly higher percentages of LLM compared with those without GERD (12% vs. 1%, $p < 0.001$). LLM percentage correlated with multiple reflux characteristics, including weakly acidic, liquid and proximal reflux ($p < 0.047$; $p < 0.047$ and $p < 0.047$, respectively), as well as symptom indices ($p < 0.001$). After antireflux therapy, wheezing episodes were substantially reduced during a follow-up period.

Conclusions: Microaspiration following GERD is an important mechanism in severe preschool wheeze. Our results show the importance of considering reflux as a potential contributor to refractory respiratory symptoms and support using MII-pH monitoring and BAL cytology in the diagnostic work-up of difficult-to-treat preschool wheezing. Antireflux therapy may improve symptoms of severe preschool wheeze.

S3.01.P**INHIBITION OF HOMEOSTATIC REGULATORS IN A TISSUE ELICITS AN UNDERHAND INFLAMMATION THAT DRIVES THE MICROENVIRONMENT TOWARD PATHOLOGIC EVOLUTION**

Luca Vannucci¹ Dmitry Stakheev^{1,8}, Pavol Lukác^{1,2}, Lenka Rajsiglova¹, Paolo Tenti¹, Daniel Hadraba³, David Vondrašek³, Gianluca Mucciolo⁴, Paola Cappello⁴, Renata Štěpánková⁵, Peter Makovický⁶, Pavol Makovický⁷, Tomáš Hudcovic⁵, Petr Šima¹, Fabián Čaja¹, Daniel Smrz^{1,8}

¹Institute of Microbiology of the CAS, Prague, Czech Republic; ²Faculty of Science, Charles University, Prague, Czech Republic; ³Institute of Physiology of the CAS, Prague, Czech Republic; ⁴University of Torino, Torino, Italy; ⁵Institute of Microbiology of the CAS, Nový Hrádek, Czech Republic; ⁶University of Ostrava, Ostrava, Czech Republic; ⁷J. Selye University, Komárno, Slovakia

Presenting author e-mail: vannucci@biomed.cas.cz

Chronic activation of immune responses in a tissue, with unregulated pro-inflammatory signaling, drives remodeling of microenvironmental structures, with significant effects on extracellular matrix organization, particularly collagen scaffold reorganization. Studying changes of collagen structure in tissues under induced chronic inflammation (DSS-induced colitis) and during experimentally AOM-induced carcinogenesis, we have shown increased collagen deposition and alterations in the collagen scaffold's stereometry, attributable to the local immune environment. Moreover, we have, for the first time, demonstrated an inflammatory pattern attributable to reduced regulatory control of inflammatory cytokines, even at apparently normal or near-normal levels. This kind of smoldering inflammation can explain the pathologic background that may sustain not only tumor preparation niche and following development but also many pathological conditions (chronic inflammatory bowel diseases, nervous system illnesses, lupus) that, if investigated using the current parameters, remain scarcely explained in their onset, evolution, and recurrences, many times classified as “borderline”. Additionally, the expression of LAIR-1, a checkpoint receptor on immune cells that binds collagen, links alterations in the scaffold to the inhibition of immune cell activity, migration, and exhaustion, as observed in the tumor microenvironment. We conclude that the active interplay between local immunity and collagen scaffold reciprocally affects their functions, influencing the development of pathological microenvironments. Collagen's microscopic analysis in confocal SHG can reveal the underlying smoldering inflammation.

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S3.02.I

HUMAN THYMIC EPITHELIAL STEM CELLS FOR LYMPHOCYTE DEVELOPMENT

Mariastefania Antica

Faculty of Science, Zagreb University, Zagreb, Croatia

Presenting author e-mail: antica216@outlook.com

The thymus is indispensable for the establishment of a T cell repertoire during postnatal life. Although thymic activity declines with age, it retains a remarkable regenerative capacity. In infants undergoing corrective surgery for congenital cardiac malformations, the thymus is frequently removed to facilitate surgical access. Growing clinical evidence indicates that early thymectomy compromises long-term immune competence, predisposing individuals to increased susceptibility to infections, allergic, autoimmune disorders, as well as increasing the malignancies and mortality risk in adulthood. These observations underscore the need to preserve the thymus in infants and to regenerate it in adults. Essential to thymus regeneration is the identification of epithelial progenitor or stem cells capable of generating both cortical and medullary thymic epithelial compartments required for T cell selection. Using a substantial cohort of postnatal human thymic samples, including tissue obtained during pediatric cardiac surgery, we have identified and characterized a rare population of human thymic epithelial stem/progenitor cells (TESCs). Under 3D ultra-low attachment culture conditions, individual stromal cells form spheroids demonstrating self-renewal and differentiation capacity. Notably, neonatal and adult thymic tissues generate comparable numbers of organoids, indicating that epithelial progenitor potential persists across age groups. These cells express genes associated with early thymic epithelial development alongside markers of mature epithelial lineages, supporting their bipotent differentiation potential.

Building on these findings, we further explore the re-aggregation of thymic cell cultures, developing the “thymus-in-a-dish” as a preparative platform for constructing functional mini-thymic organs. Our long-term objective is thymic reconstitution either *in vivo*, including xenogeneic settings, or *in vitro* through organ reassembly. However, TESCs derived from healthy tissue exhibit limited proliferative capacity compared to malignant counterparts. By incorporating defined CD34⁺ hematopoietic progenitors and common HLA haplotypes into engineered systems, we aim to establish controlled platforms for efficient T cell selection and the generation of personalized T lymphocyte populations.

Together, our work uncovers previously unrecognized characteristics of human thymic epithelial stem cells and establishes a translational framework for immune system regeneration. These advances hold significant promise for patients with congenital thymic loss, immune deficiency, or immune damage, and open new avenues toward personalized thymus bioengineering and restorative immunotherapy.

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S3.03.I

EXPOSURE TO MICRO AND NANOPLASTICS (MNP) IS ASSOCIATED WITH THE ADAPTIVE IMMUNE RESPONSE REMODELLING IN CHILDREN

Ana Kujavec¹, Ivana Banić^{1,2}, Manuela Oroz¹, Jan Pantlik¹, Marcel Lipej³, Mirjana Turkalj^{4,5,6}

¹Department of medical research, Srebrnjak children`s Hospital, Zagreb, Croatia; ²Department of innovative diagnostics, Srebrnjak children`s Hospital, Zagreb, Croatia; ³IT department, Srebrnjak children`s Hospital, Zagreb, Croatia; ⁴Department of allergy, clinical immunology and rheumatology, Srebrnjak children`s Hospital, Zagreb, Croatia; ⁵Faculty of medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia; ⁶Catholic university of Croatia, Zagreb, Croatia
Presenting author e-mail: mturkalj@bolnica-srebrnjak.hr

Background and aims: Micro- and nanoplastic particles (MNPs) are emerging environmental pollutants, and it is estimated that the human exposure to MNP is increasing rapidly, primarily through diet, inhalation, and consumer products. Although existing research indicates that MNPs may trigger oxidative stress, cytotoxic effects, and inflammatory responses, their impact on the human immune system remains poorly understood. Clarifying how MNP exposure affects immune cell viability and activation is essential for evaluating potential immunotoxicological risks, particularly in vulnerable pediatric populations.

This study aimed to assess the effects of estimated MNP exposure on immune cell viability and activation in children.

Methods: As part of the H2020 IMPTOX project, 1155 children were recruited into an observational study. Clinical information was collected from medical records, and estimated MNP exposure was evaluated using questionnaires, including a food frequency questionnaire (FFQ) and an MNP exposure survey. Participants were categorized into exposure clusters through a hierarchical cluster analysis. In a subset of 40 participants, peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood samples. Immune phenotyping was conducted using polychromatic flow cytometry on a DXFlex flow cytometer (Beckman Coulter, USA).

Results: Higher MNP exposure via ingestion (FFQ clusters) was associated with lower circulating CD19⁺ B-cell proportions ($r = -0.322$, $p = 0.043$). Higher estimated exposure to MNPs was positively correlated with the proportion of CD19⁺CD23⁺ B cells ($r = 0.400$, $p = 0.031$).

Conclusion: Higher estimated exposure to micro- and nanoplastics in children was associated with selective alterations in adaptive immunity, including reduced total CD19⁺ B-cell proportions and increased CD19⁺CD23⁺ B cells. This pattern suggests a shift toward activated or regulatory B-cell subsets, indicating potential skewing of the adaptive immune response. These findings imply that environmental MNP exposure may modulate immune homeostasis and activation, highlighting the need for further studies to clarify the immunological consequences of chronic exposure in pediatric populations.

S3.04.I**EXPANDING TESTING TO MONITOR THE IMMUNOLOGICAL STATUS OF PATIENTS AFTER HEART TRANSPLANTATION**

Sendi Montanič

Slovenian Institute for Transfusion Medicine, Ljubljana, Slovenia

Presenting author e-mail: sendi.montanich@ztm.si

Anti-HLA antibodies can develop in response to transfusions, pregnancy or organ transplantation. One of the main causes of graft loss is antibody mediated rejection (AMR). In suspected AMR or Cardiac Allograft Vasculopathy (CAV), the presence of Donor Specific Antibodies (DSA) must be determined. For this purpose, patients' sera are tested using Luminex technology with the OL-LabScreen SA method (SAB), which allows semi-quantitative determination of anti-HLA antibodies. The method is highly sensitive and specific, but has certain limitations, as the clinical significance of all the determined antibodies is not yet fully explained. To determine which of these antibodies are also cytotoxic due to their ability to activate complement, the serum can also be analyzed using the C1qScreen test (SAB-C1q). Regular monitoring of the patient's immune status for at least one year after transplantation is crucial, as newly formed antibodies, the so-called 'de novo' DSA, play an important role in the long-term survival of the transplant. However, if they are also cytotoxic, they can be a good predictor of AMR or CAV. Newer studies are providing more and more evidence of the connection between the rejection of a transplanted heart and the presence of donor-derived cell-free DNA (dd-cfDNA) in the recipient's plasma, as episodes of rejection cause damage to the graft, resulting in larger amounts of dd-cfDNA being released into the recipient's circulation, which can be quantified. Therefore, determination of dd-cfDNA is incorporated into the International Society of Heart and Lung Transplantation guidelines as a non-invasive biomarker for monitoring graft rejection. For patients involved in the study, sera have been tested with SAB, SAB-C1q and CDC (Complement Dependent Lymphocytotoxic) tests and dd-cfDNA was determined in the patient's plasma. All test results were compared with clinical data. Comparisons of the results of the SAB-C1q test with the results of the SAB and CDC tests showed the expected findings. The SAB-C1q test demonstrated the specificity of all those antibodies that are positive in the CDC test, and only some of the specificities that were demonstrated with the SAB test. In the presence of DSA dd-cfDNA was always determined. Moreover, obtained results correlated with clinical data. Although endomyocardial biopsy remains the gold standard, combination of several new methods could yield successful alternative way of heart transplant rejection monitoring, with the distinct advantage of avoiding procedural complications.

S3.05.I**VALUES OF SALIVARY CORTISOL AND INTERLEUKIN-1 β , PERCEIVED STRESS AND CLINICAL FEATURES OF PATIENTS WITH DERMOGRAPHIC URTICARIA, PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA AND HEALTHY INDIVIDUALS**

Liborija Lugović-Mihić^{1,2}, Novak-Hlebar^{1,2}, Dajana Smoljan^{1,2}, Ines Vukasović^{3,4}, Ema Barac⁵, Maja Vilibić^{4,6}

¹Department of Dermatovenereology, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia; ²School of Dental Medicine, University of Zagreb, Zagreb, Croatia; ³Department of Clinical Chemistry, University Hospital Center Sestre milosrdnice, Zagreb, Croatia; ⁴School of Medicine, Catholic University of Croatia, Zagreb, Croatia; ⁵Family Physician Office, Zagreb, Croatia; ⁶Department of Psychiatry, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia
Presenting author e-mail: liborija@gmail.com

Background: Dermographic urticaria is the most frequent form of chronic inducible urticaria, but little is known about its etiopathogenesis and associated factors such as psychological stress.

Methods: This cross-sectional study included a total of 100 examinees, and compared the data/findings of 34 dermographic urticaria patients to 33 chronic spontaneous urticaria (CSU) patients, and 33 healthy controls. We looked at perceived psychological stress (Perceived Stress Scale/PSS), salivary cortisol and IL-1 β levels, clinical characteristics [dermographism activity/severity (on a scale from 1 to 4), disease control (Urticaria Control Test/UCT) and disease activity of CSU patients (Urticaria Activity Score/UAS)], and demographic data [1].

Results: In patients with dermographic urticaria and CSU patients, no significant correlations were observed between salivary cortisol, IL-1 β , and PSS, but urticaria severity positively, linearly correlated with perceived stress ($p=0.004$) [1]. Males had significantly higher salivary cortisol (median 1.1 vs. 0.9; $p=0.028$) and higher IL-1 β levels than females (687.6 vs. 408.9 pg/mL; $p=0.029$), while females had significantly higher perceived stress levels ($p=0.006$). CSU patients had significantly higher disease control than the dermographic urticaria group (moderate effect size) ($p=0.001$). In dermographic urticaria patients with concomitant allergies, lower cortisol values were recorded (moderate effect size; $p=0.032$) and disease lasted longer than in those with no allergies (a large effect size) (48 vs. 18 months; $p=0.002$). Duration of dermographic urticaria was negatively linearly related to salivary cortisol ($r=-0.369$; $p=0.032$), but not to IL-1 β . In patients with dermographic urticaria with associated conditions, their urticaria lasted significantly longer than for those without comorbidities (60 vs. 24 months; $p=0.017$) [1].

Conclusions: Since stress is often perceived by patients with dermographic urticaria and affects dermographic urticaria activity/severity, psychological support and related measures could be beneficial for these patients.

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S4.02.P**THERAPEUTIC APPLICATION OF ANTIBODY–DRUG CONJUGATES AND VACCINES IN SOLID TUMOUR PATIENTS**

Simona Borštnar

Institute of Oncology, Ljubljana, Slovenia; Faculty of Medicine, University of Ljubljana, Ljubljana Slovenia

Presenting author e-mail: Sborstnar@onko-i.si

Background: Antibody–drug conjugates (ADCs) and therapeutic cancer vaccines represent rapidly evolving strategies designed to enhance tumour specificity while minimising systemic toxicity. ADCs deliver highly potent cytotoxic payloads directly to antigen-expressing cancer cells, whereas vaccines aim to stimulate durable antitumor immunity. This abstract summarises current evidence on their clinical efficacy, safety profiles, and therapeutic applications in patients with advanced solid malignancies.

Methods: A comprehensive review of randomised controlled trials, systematic reviews, and meta-analyses evaluating ADCs and therapeutic vaccines in solid tumours was conducted. Primary outcomes included overall survival (OS), progression-free survival (PFS), objective response rate (ORR), and treatment-related adverse events (AEs).

Results: Recent ADCs have demonstrated clinically meaningful improvements in survival across several tumour types, supported by mechanisms such as bystander killing and improved linker stability. Multiple ADCs have demonstrated significant clinical benefit in solid tumours. Trastuzumab deruxtecan and trastuzumab emtansine showed substantial improvements in PFS and OS in HER2-positive breast and gastric cancers. Sacituzumab govitecan prolonged survival in triple-negative and hormone receptor-positive, HER2-negative breast cancer. Meta-analyses demonstrated that ADCs significantly improved PFS (HR 0.69, 95% CI 0.56-0.82) and OS (HR 0.76, 95% CI 0.61-0.92) compared with chemotherapy. Enfortumab vedotin, tisotumab vedotin, and mirvetuximab soravtansine achieved regulatory approval for urothelial, cervical, and ovarian cancers, respectively. Common treatment-related AEs included myelosuppression (neutropenia 43.7%, thrombocytopenia 22.6%), peripheral neuropathy (39.6%), and nausea (44.1%), with grade ≥ 3 AEs occurring in 46.1% of patients. Interstitial lung disease emerged as a notable toxicity, particularly with topoisomerase inhibitor payloads.

Therapeutic cancer vaccines demonstrated more modest clinical outcomes. In advanced non-small cell lung cancer, most vaccines showed no significant OS benefit compared with standard therapy. Contemporary neoantigen-based vaccines combined with immune checkpoint inhibitors showed promising immunogenicity and early clinical activity, particularly in early-stage disease and minimal residual disease settings.

Conclusions: ADCs represent an established therapeutic option for biomarker-selected patients with solid tumours, demonstrating significant survival benefits with manageable toxicity. Therapeutic vaccines show limited efficacy as monotherapy in advanced disease but demonstrate potential when combined with checkpoint inhibitors in early-stage settings. Future research should focus on optimising patient selection through predictive biomarkers, refining combination strategies, and advancing personalised vaccine platforms.

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S4.03.P**FROM GVL TO CART – AND BACK**

Polona Novak

Hematology department, UMC Ljubljana

Presenting author e-mail: Polona.novak@kclj.si

Allogeneic stem cell transplantation (allo-SCT) has long represented a cornerstone of curative therapy for patients with high-risk hematologic malignancies, primarily through the graft-versus-leukemia (GVL) effect. GVL is mediated by a complex, polyclonal donor-derived immune response targeting a broad repertoire of alloantigens and tumor-associated antigens, involving coordinated activity of T cells, NK cells, and antigen-presenting cells. This immunological breadth underlies its durability but is intrinsically linked to graft-versus-host disease (GVHD), reflecting the fine balance between beneficial and deleterious alloreactivity.

In contrast, chimeric antigen receptor T-cell (CAR-T) therapy represents a highly specific, engineered immune response directed against a single or limited set of tumor antigens, independent of HLA presentation. While this precision enables potent cytotoxicity with reduced off-target alloreactivity, it also introduces vulnerability to immune escape mechanisms, including antigen loss, lineage switching, and limited CAR-T persistence driven by T-cell exhaustion and an immunosuppressive tumor microenvironment.

Relapse following CAR-T therapy thus highlights a fundamental immunological limitation of targeted cellular therapy: the lack of antigenic diversity and adaptive immune evolution characteristic of the GVL effect. Conversely, the broad and dynamic nature of GVL provides sustained immune surveillance but at the cost of systemic immune activation and toxicity.

In this context, allo-SCT and CAR-T therapy should be viewed as complementary rather than competing modalities. CAR-T therapy may induce deep remissions and reshape tumor burden, creating an optimal immunological landscape for subsequent allo-SCT, while allo-SCT can restore a diversified and self-renewing immune repertoire capable of long-term disease control. The use of allo-SCT as consolidation following CAR-T is therefore increasingly supported, particularly in patients with high-risk features or early signs of CAR-T failure.

Emerging approaches aim to bridge the gap between these paradigms, including donor-derived CAR-T cells, dual-targeting CAR constructs, and post-transplant immune modulation strategies designed to enhance GVL while mitigating GVHD. A deeper understanding of immune dynamics—spanning antigen recognition, T-cell fitness, and microenvironmental interactions—will be critical to optimizing the sequencing and integration of these therapies.

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S4.04.I**SPATIAL IMMUNE NICHE AS DETERMINANTS OF ANTI-TUMOR IMMUNITY IN OVARIAN CANCER**

Jitka Fuciková

Sotio Biotech, Prague, Czech Republic; Department of Immunology, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic

Presenting author e-mail: fucikova@sotio.com

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 are formed only in 16% of HGSOC. Limited TLS maturation was associated with a CD8⁺ T-cell compartment dominated by CPI-resistant PD-1⁺TIM3⁺ effector cells, whereas CPI-responsive TCF1⁺PD-1⁺ progenitor CD8⁺ T cells were rarely observed. A similar pattern of functional restraint characterized the NK cell compartment. HGSOC tumors were enriched in CD56^{bright} 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.

S5.03.P**INFLAMMASOME-INSPIRED INDUCTION OF IMMUNOGENIC CANCER CELL DEATH**

Taja Železnik Ramuta, Elvira Boršič Mlinarič, Sara Orehek, Borna Bacinger, Duško Lainšček, Roman Jerala, Iva Hafner Bratkovič

Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia

Presenting author e-mail: iva.hafner@ki.si

Inflammasomes are central components of early immune responses to pathogens. Upon sensing infection or disrupted tissue homeostasis, inflammasome sensors lead to the assembly of inflammasomes and activation of inflammatory caspases. Caspase-1 subsequently proteolytically activates proinflammatory cytokines IL-1 β and IL-18 and gasdermin D into its N-terminal pore-forming domain that causes pyroptosis. Proinflammatory cytokine release combined with immunogenic cell death (ICD) induces potent inflammatory responses. Cancer is an example of a disease where the immune system fails and several types of tumors downregulate different cell death pathways. Using the synthetic biology combined with the mechanistic insight into inflammasome assembly, we are currently developing inflammasome-inspired approaches to provide efficient priming of immune responses in cancer immunotherapy.

One of the approaches we developed is cytokine-armed pyroptosis [1]. To induce pyroptosis, we utilized designed tightly regulated gasdermin D variants with different pore-forming capabilities and diverse modes of activation, representing a toolbox of immunogenic cell death (ICD) inducers. We demonstrated that the electrogenic transfer of ICD effector-encoding plasmids into mouse melanoma tumors, when combined with intratumoral expression of cytokines IL-1 β , IL-12, or IL-18, enhanced anti-tumor immune responses. Careful selection of immunostimulatory molecules is, however, imperative as a combination of IL-1 β and IL-18 antagonized the protective effect of pyroptosis by IFN γ -mediated upregulation of several immunosuppressive pathways. Additionally, we show that the intratumoral introduction of armed pyroptosis provides protection against distant tumors and proves effective across various tumor types without inducing systemic inflammation in preclinical mouse models.

Inflammasome-mimicking approaches are tunable and tumor-agnostic strategies to enhance antitumor response, even against the most resilient types of tumors.

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S5.04.I**IMMUNOPROFILING OF GAMMA DELTA T CELLS AND THEIR POTENTIAL FOR THE DEVELOPMENT OF CANCER IMMUNOTHERAPIES**

Pia Bertonecjl¹, Urška Rupnik¹, Katja L. Zupet¹, Gašper Markelj², Tina Vesel Tajnšek², Barbara Jenko Bizjan³, Jernej Kovač³, Alenka Trampuš Bakija³, Neja Šamec², Valerija Kovač⁴, Vladka Čurin Šerbec⁴, Tadej Avčin², Jelka Pohar¹, Anže Smole^{1,5}

¹Immunology and Cellular Immunotherapy (ICI) Group, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia; ²Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Center Ljubljana and Department of Pediatrics, Faculty of Medicine, University of Ljubljana, Slovenia; ³Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; ⁴Centre for Immunology and Development, Slovenian Institute for Transfusion Medicine, Ljubljana, Slovenia; ⁵Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: anze.smole@nib.si

Background: $\gamma\delta$ T cells form a distinct T cell lineage defined by $\gamma\delta$ rather than $\alpha\beta$ TCR chains. They display unique antiviral, antibacterial, and antitumor properties, acting independently of classical MHC-restricted antigen presentation [1]. $\gamma\delta$ T cells combine features of T cells, NK cells, and antigen-presenting cells, and include heterogeneous subsets with diverse TCR repertoires and tissue-tropic distributions [2]. Their potent effector functions and ability to respond to tissue stress offer attractive opportunities for next-generation cellular immunotherapies, while also suggesting potential roles in immune-mediated pathology.

Methods To investigate $\gamma\delta$ T cell immunobiology, we established a comprehensive spectral flow cytometry platform to enable immunoprofiling of $\gamma\delta$ T cells within the broader immune landscape. A multiparameter panel was designed, optimized, and validated using PBMCs isolated from healthy donor buffy coats and peripheral blood from healthy adult volunteers. In parallel, we developed *ex vivo* strategies for $\gamma\delta$ T cell activation and expansion to support future efforts aimed at generating $\gamma\delta$ T cell-based cellular products.

Results The validated flow cytometry platform enables identification of major immune populations and $\gamma\delta$ T cell subsets. *Ex vivo* approaches reliably induce $\gamma\delta$ T cell activation and expansion, establishing feasibility for subsequent functional and translational studies.

Conclusions Together, these platforms provide a comprehensive basis for studying $\gamma\delta$ T cell immunobiology in health and disease and establish foundation to support the development of $\gamma\delta$ T cell-based immunotherapies.

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S6.01.P**SYSTEMIC VASCULITIDES THROUGH THE LENS OF A RHEUMATOLOGIST – IGA VASCULITIS IN ADULTS**Alojzija Hočevar^{1,2}¹Department of Rheumatology UMC Ljubljana, Ljubljana, Slovenia; ²Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: alojzija.hocevar@kclj.si

Background: IgA vasculitis (IgAV) is a small vessel vasculitis characterized by a heterogeneity of clinical manifestations. With an incidence of 5 cases per 100,000 adults, IgAV represents the second most frequent systemic vasculitis in our population [1]. The long-term prognosis depends mainly on visceral organ involvement, especially kidneys [2]. Recently, with the aim to improve disease management and outcome, clustering of IgAV patients into 3 prognostically different groups was proposed by a French research group (cluster 1 representing younger men with gastrointestinal and articular involvement and low rate of renal insufficiency; cluster 2 representing middle-aged patients without gastrointestinal involvement; and cluster 3 with older patients manifesting with necrotic skin lesions and abnormal kidney function) [3]. Our IgAV cohort served as a validation cohort of the study.

Methods: 208 IgAV patients diagnosed and followed at our rheumatological department with a histologically proven disease were included in a validation clustering study. The agglomerative hierarchical clustering was applied as in the original cohort, using k-means and Ward's method based on 7 parameters (sex, age, constitutional symptoms, skin necrosis, articular and gastrointestinal tract involvement, abnormal kidney function).

Results: The mean (SD) age of patient in our cohort was 57.0 (19.9) years, and 122 (58.7%) patients were males. Constitutional symptoms, skin necrosis, articular, gastrointestinal tract and kidney involvement were present in 37 (18%), 88 (43%), 111 (53%), 84 (40%) and 126 (61%) patients at baseline, respectively. The distribution of patients based on three proposed clusters is presented in the Table 1.

Conclusions: The presence of three distinct groups of adult IgAV identified by clustering analysis was confirmed also in our cohort. This approach represents an important step toward personalized management of adult IgAV.

Table 1. Adult IgAV clusters

Characteristics	Cluster 1 (60)	Cluster 2 (78)	Cluster 3 (70)	P value
Age (mean (SD))	42.9 (15.3)	50,7 (17.0)	76.1 (8.8)	<0.001
Male sex	45 (75%)	45 (58%)	32 (46%)	0.003
Constitutional symptoms	18 (30%)	9 (12%)	10 (14%)	0.012
Articular involvement	39 (65%)	63 (81%)	9 (13%)	<0.001
Skin necroses	16 (27%)	26 (33%)	46 (66%)	<0.001
GIT involvement	60 (100%)	0	24 (34%)	<0.001
Abnormal kidney function	1 (1.7)	1 (1.3%)	42 (60%)	<0.001
Relapses	12 (20%)	13 (17%)	3 (4.3%)	0.019
Residual CKD	4 (6.7%)	8 (10%)	45 (64%)	<0.001

Legend: GIT gastrointestinal tract. CKD chronic kidney disease

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S6.03.I**ORGAN-ON-A-CHIP PLATFORM OF A HUMAN JOINT FOR EVALUATING RESPONSE TO TREATMENT OF INFLAMMATORY ARTHRITIDES**

Alan Šućur^{1,3}, Darja Flegar^{1,3}, Sara Priselac^{2,3}, Dominik Lukičić³, Tomislav Balen^{2,3}, Ivo Krešić^{3,5}, Amir Šećerkadić², Marko Perić², Nataša Kovačić^{2,3}

¹Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; ²Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia; ³Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; ⁴Izit d.o.o., Sveta Nedelja, Croatia; ⁵Department of Physiology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina
Presenting author e-mail: alan.sucur@mef.hr

Background: Pathogenesis of inflammatory arthritides involves complex multicellular interactions within bone, cartilage, and synovium. Traditional 2D cultures and animal models often fail to recapitulate the human 3D microenvironment, limiting predictive value for therapeutic efficacy. We aim to develop a modular joint-on-a-chip platform (CHIPART) integrating 3D-printed biocompatible supports with bioprinted tissues to study inflammatory cell infiltration and drug responses.

Methods: Biocompatible resins (MED610, MED620) were 3D-printed and optimised via post-processing. Cell-laden (1×10^6 cells/mL) constructs were bioprinted using the BioX6 system with decellularised extracellular matrix (adECM)-, GelMA-, and collagen-based bioinks. Human mesenchymal (hBMSC, HS27a), synovial (SW982), endothelial (HUVEC) lines, and primary cells from osteoarthritis patients were used. Viability (annexin V/7-AAD) and phenotype (CD73, PDPN, CD146, CD140a/b, CD105) were assessed by flow cytometry. Osteogenic differentiation was evaluated by alizarin red and RUNX2, ALP, BGLAP expression; chondrogenic by alcian blue and SOX9. Monocyte migration toward chemokine gradients (CCL2, CCL5, CXCL10) was assessed using Transwell-based adECM.

Results: MED610 and MED620 supported OchP survival, proliferation and osteogenic differentiation comparable to commercial plates. adECM outperformed commercial bone inks, showing higher OchP viability, altered RANKL/OPG ratio, and enhanced RUNX2 and SOX9 expression. Histology revealed lacuna-like structures in chondrogenic constructs resembling native cartilage. Primary cells exhibited greater heterogeneity and reduced differentiation versus immortalized lines. Synovial fibroblasts maintained viability but showed phenotypic shifts (CD146 loss, CD271 acquisition). HUVEC co-culture with mesenchymal constructs showed capillary-like cords. adECM density <2% allowed monocyte recruitment; 3% permitted chemokine gradients but restricted cell movement.

Conclusions: We identified adECM as a superior bioink for maintaining OchP function and provided a quantified threshold for modelling immune cell migration within the joint niche. Collectively, our work establishes a foundation for a functional multi-modular joint-on-a-chip system.

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S6.04.I**THE ROLE OF MICRORNAS IN THE DEVELOPMENT OF SYSTEMIC AUTOIMMUNE DISEASES**

Gábor Papp¹, Szilárd Póliska², Krisztina Szabó¹, Ádám Diós¹, Antónia Szántó¹, Tünde Tarr¹, Ágnes Gyetvai¹

¹Division of Clinical Immunology, Institute of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; ²Genomic Medicine and Bioinformatics Core Facility, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Presenting author e-mail: papp.gabor@med.unideb.hu

Background: The pathologic B-cell response and autoantibody production are key features in systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS). A subset of CD4⁺ T cells, termed follicular T helper (TFH) cells, are responsible for optimal B-cell selection and long-lived, high-affinity antibody-producing plasma cell generation in secondary lymphoid tissues. Based on the critical role of follicular T helper cells in B cell activation and antibody production, their failure to maintain self-tolerance potentially leads to the development of autoreactive immune processes. For better understanding of the molecular mechanisms and pathways behind the pathological changes of B and TFH cell functions in SLE and pSS, in our study, we focused on the role of the altered expression profiles of microRNAs (miRNAs).

Methods: Circulating CD19⁺ B and CD4⁺CXCR5⁺ TFH (cTFH) cells were magnetically isolated from the peripheral blood of 13 SLE patients, 9 pSS patients and 11 healthy individuals for the analysis of miRNA expression profiles using Illumina next-generation sequencing technique. Differentially expressed microRNAs (DEmiRs; FC>1.5, p<0.05) were identified using StrandNGS software. Experimentally validated miRNA-target interactions (MTIs) were determined based on the miRTarBase database. Functional enrichment analysis of the identified target genes was performed using ClueGO plug-in of Cytoscape.

Results: A total of 102 DEmiRs were identified in the cTFH cells, and 94 DEmiRs were found in the B cells of SLE patients, while 46 DEmiRs were identified in the cTFH cells, and 102 DEmiRs were found in the B cells of pSS patients, compared to healthy controls. In the case of cTFH cells, 28 DEmiRs were common in SLE and pSS patients, whereas in B cells, 53 DEmiRs were common to both diseases. Functional enrichment analysis showed that the target genes of DEmiRs were mainly involved in myeloid cell differentiation, complement activation, immunoglobulin mediated immune response, antimicrobial humoral immune response and B cell receptor signaling pathway.

Conclusions: The observed changes and differences in miRNA expression profiles in B and cTFH cells may help elucidate the molecular pathways contributing to the development of SLE and pSS; furthermore, individual miRNAs may serve as potential biomarkers or therapeutic targets in the future.

S6.05.I

NEW TRENDS IN LABORATORY TRANSPLANTOLOGY

Andrej Čeres¹, Michal Cibulka^{1,2}, Tatiana Košarišťanová¹, Martina Schniederová¹, Dana Slovákova¹, Jela Petrisková¹, Ivana Dedinská³, Miloš Jeseňák^{1,4,5}, Anna Ružinák Bobčáková^{1,4,5}

¹Institute of Clinical Immunology and Medical Genetics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Hospital in Martin, Martin, Slovakia; ²Department of Medical Biochemistry, Jessenius Faculty of Medicine of Comenius University in Bratislava, Mala Hora 4D, 036 01, Martin, Slovak Republic; ³Transplant-Nephrology Department and 1st Internal Department, Jessenius Faculty of Medicine of Comenius University in Bratislava and University Hospital Martin, Kollarova 2, 036 59, Martin, Slovak Republic; ⁴Outpatient Department of Allergy and Clinical immunology, Department of Pneumology and Phthisiology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin University Hospital, Martin, Slovakia; ⁵ Outpatient Department of Allergy and Clinical immunology, Department of Paediatrics and Adolescent Medicine, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin University Hospital, Martin, Slovakia

Presenting author e-mail: andrejceres@gmail.com

Background: Incompatibility in the human leukocyte antigen (HLA) between the donor and the recipient can lead to alloreactivity of the immune system caused by T-lymphocytes and B-lymphocytes after solid organ transplantation (SOT). One of the main manifestations of T and B-lymphocytes alloreactivity in case of HLA incompatibility is the synthesis of the *de novo* donor-specific antibodies (dnDSA). The synthesis of dnDSA can lead to ABMR after kidney transplantation. Studies from several transplant centres indicates that PIRCHE-T2 and the peptide differences between HLA alleles of the donor and the recipient, which are recognized by the α and β -domains of the TCR CD4⁺ T lymphocytes, as a predictive tool for the assessment of the immunological risk for synthesis of the dnDSA. The second part of the PIRCHE algorithm is PIRCHE-B, which predicts dnDSA synthesis based on degree of protrusion and exposure of the aminoacid residues on the surface of the HLA antigens.

Material and Methods: Our cohort consists of 80 patients who underwent kidney transplantation from 1998 to 2021 at Transplantat center at the University Hospital in Martin. In study we had performed testing of the anti-HLA, nonDSA, dnDSA against HLA alloantigens by Luminex® method. Subsequently, we have determined the immunocompatibility between HLA alleles of the recipient and donor by PIRCHE-T2 algorithm, based on different peptides presented by HLA-DRB1 to recipient's TCR of the CD4⁺ T-lymphocytes. PIRCHE-T2 and PIRCHE-B scores were determined using the PIRCHE-II® version 4 platform, incorporating the Frost-1.1.IMGT-3.54 database and the Snow algorithm version 1.1, including the Snowball module (protrusion rank = 0.66) and the Snowflake module (surface accessibility rank = 0.28) To determine the score of alloreactive aminoacid residues on HLA alloantigens that can be recognized by the BCR of recipients B cells we used the PIRCHE-B algorithm. High-resolution HLA typing was performed using Oxford Nanopore sequencing technology, followed by HLA allele calling and analysis with NanoTYPE™ software. The minimum post-transplant follow-up period for all patients was three years. Welch's unequal variances t-test, receiver operating characteristic (ROC) analysis with area under the curve (AUC) and Youden index calculation, and pairs plot analyses were performed using R (version 4.0.5) with relevant R packages, as well as jamovi medical statistical software. Random forest machine learning algorithms were applied where appropriate.

Results and conclusion: Analysis of the results of dnDSA synthesis against HLA-A, B, DRB1 and DQB1 in individual patient significantly correlates by statistics and by effect size with the increasing scores of the PIRCHE-T2 and PIRCHE-B. This applies primarily to HLA-A, B, DQB1 and scores of the PIRCHE-T2 and PIRCHE-B algorithms. For PIRCHE-B and HLA-A, a significant difference was

identified ($p = 0.019$ and $p = 0.013$), with a large effect size (effect size = 0.802). For PIRCHE-T2 and HLA-A, the difference was highly significant ($p < 0.001$ and $p = 0.002$), with a very large effect size (effect size = 1.186). For HLA-B, significant differences were observed using PIRCHE-B ($p = 0.002$ and $p < 0.001$; effect size = 1.697) and PIRCHE-T2 ($p = 0.002$ and $p < 0.001$; effect size = 1.740). Similarly, for HLA-DQB1, statistically significant differences were detected with PIRCHE-B ($p < 0.002$ and $p < 0.001$; effect size = 1.476) and PIRCHE-T2 ($p < 0.001$ and $p < 0.001$; effect size = 1.568). In the case of the HLA-DRB1 we demonstrated statistical and effect size significance only by PIRCHE-T2. Consistent discriminatory performance was observed in ROC analyses, including AUC and Youden index, combined non-DSA/de novo DSA, and de novo DSA-only groups based on pooled PIRCHE-T2 and PIRCHE-B scores across all anti-HLA antibodies against HLA-A, B, DRB1, DQB1 alloantigens. The results of our study showed that PIRCHE-T2 and PIRCHE-B algorithm are significant predictors for determining the immunological risk in case of *de novo* DSA synthesis against transplanted kidney.

S6.06.I**THE BRAIN'S TROJAN WAR: LOCAL BRAIN IMMUNITY AS A MODULATOR OF ALZHEIMER'S DISEASE NEURODEGENERATION**Norbert Zilka¹, Neha Basheer¹¹Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Presenting author e-mail: nilumabh@savba.sk

Alzheimer's disease (AD) represents a battleground where immune mechanisms act as both defenders and potential aggressors. Increasing evidence suggests that local brain immunity plays a decisive role in modulating tau pathology—the propagation and aggregation of neurofibrillary tangles that drive neurodegeneration and tightly correlate with the clinical decline. Microglial activation, cytokine signalling, and peripheral immune interactions appear to orchestrate a complex response that can either protect neural integrity or accelerate damage. Environmental factors, including diet and metabolic status, further influence this immune balance, shaping disease onset and progression. This duality positions the immune system as a crucial therapeutic target. Emerging immunotherapies, such as vaccines and antibodies, aim to recalibrate this immune network to counteract disease-driven neurodegeneration. Understanding how protective and propelling immune factors interact in this “Trojan war” of the brain may open new avenues for precision immunotherapy in AD.

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S6.07.I**NEUROIMMUNE EFFECTS OF ENVIRONMENTAL AND DIETARY ENRICHMENT IN AD MODEL**

Viola Stuchlíková¹, Muhammad Khalid Muhammadi¹, Neha Basheer¹, Natalia Hryntsova¹, Shahid Ullah Zadrán¹, Vanesa Cirbusová¹, Tomáš Smolek¹

¹Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

Presenting author e-mail: viola.stuchlikova@savba.sk

Background: 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. Despite extensive research efforts, currently available pharmacological treatments of AD 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. 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: R3m/4 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.

Conclusions: Our 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.

Funding: This work was supported by JPND MULTI-MEMO, APVV-23-0420 and VEGA 2/0155/25 grants.

S7.01.P**AUTOINFLAMMATORY DISORDERS IN SLOVAKIA – UNEXPECTED RESULTS FROM AWARENESS CAMPAIGNS**

Miloš Jeseňák^{1,2}, Katarína Hrubíšková³, Lenka Kapustová^{1,2}, Eva Malicherová Jurková¹, Branislav Šlenker^{1,2}, Otilia Petrovičová^{1,2}, Veronika Spurná^{1,2}, Dušana Genšor¹, Tomáš Dallos⁴, Anna Ružinák Bobčáková^{1,2}

¹National Centre for Periodic Fever Syndromes, Department of Paediatrics and Adolescent Medicine and Department of Pulmonology and Phthisiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Hospital Martin, Martin, Slovakia; ²Institute of Clinical Immunology and Medical Genetics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Hospital in Martin, Martin, Slovakia; ³Centre for Periodic Fever Syndromes, 5th Department of Internal Medicine, Faculty of Medicine, Comenius University in Bratislava, University Hospital Bratislava – Ružinov, Bratislava, Slovakia; ⁴Department of Paediatrics, Faculty of Medicine, Comenius University in Bratislava, National Institute of Children's Diseases, Bratislava, Slovakia
Presenting author e-mail: jesenak@gmail.com

Background: Autoinflammation refers to a pathophysiological process involved in a broad spectrum of immune-mediated diseases, encompassing both innate and primary disorders. This process is characterized by overactive and dysregulated activation of innate immunity and the inflammatory response. Today, monogenic autoinflammatory diseases (AIDs) represent a distinct category of inborn errors of immunity. In a broader sense, the spectrum of autoinflammatory diseases also includes mixed-pattern diseases (e.g., Still's disease), phenocopies of primary immunodeficiencies (e.g., VEXAS syndrome), and PFAPA—the most common polygenic autoinflammatory disease in clinical practice. SURF (syndrome of undifferentiated recurrent fever) denotes a group of conditions with defined criteria that cannot be classified into other nosological units within the AID group. Early recognition of AIDs enables adequate treatment and the prevention of complications and sequelae, including the avoidance of non-indicated examinations or inappropriate therapy. In Slovakia, AIDs are managed at the National Centre for Periodic Fever Syndromes in Martin and Bratislava.

Methods: Over the past 15 years, the National Centre for Periodic Fever Syndromes, operating at the University Hospitals in Martin and Bratislava, Slovakia, has been actively engaged in increasing the clinical awareness of autoinflammatory disorders nationwide. This outreach has been implemented through targeted lectures at local and national medical and scientific symposia, complemented by awareness campaigns disseminated via electronic mail, mass media, and professional social networks. Currently, the Centre serves as a nationwide referral and consultation hub for the entire territory of the Slovak Republic.

Results: Total number of the patients with AID followed-by the Centre is reaching 500 subjects. The most prevalent form of AID is familiar Mediterranean fever (146 patients, in the recent publication 113 [1]); PFAPA syndrome (over 250 children), rare forms of monogenic AID – mevalonate kinase deficiency (6), TRAPS (7 [2]), kryopyrinopathies (9), FCAS 2 and 3 (2), PAPA syndrome (2), Aicardi-Goutieres syndrome (3), RELA haploinsufficiency (1), VEXAS syndrome (1 [3]), morbus Blau (1), Yao syndrome (3), Majeed syndrome (1), DADA2 (2) and SURF (15). The management is provided according to the latest recommendations including biologics (anakinra, canakinumab, etanercept) and small molecule inhibitors (JAK-inhibitors) and the disease activity is monitored through the standardized questionnaires and a broad spectrum of inflammatory biomarkers (including TNF-alfa, serum amyloid A, serum calprotectin, neopterin). The transition process from childhood to adulthood is based on the national recommendations.

Conclusions: Currently, autoinflammatory diseases represent a fascinating and expanding group of immune-mediated disorders. Given the broad spectrum of clinical manifestations, AIDs can be identified across nearly every medical specialty; they frequently mimic or mask other conditions, such as rheumatic diseases. Contemporary advances, particularly in molecular genetics, enable the definitive diagnosis of AIDs, subsequently facilitating the selection of targeted therapeutic strategies.

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S7.02.P**THE IMMUNOLOGICAL BASIS OF LUNG DISEASE IN PRIMARY ANTIBODY DEFICIENCIES: EXPLORING B CELL DYSFUNCTION**

Tomas Milota

Department of Immunology, Second Faculty of Medicine Charles University and Motol and Homolka University Hospital, Prague, Czechia

Presenting author e-mail: tomas.milota@fnmotol.cz

Background: Common Variable Immunodeficiency (CVID)-associated chronic lung disease (CLD) is a frequent non-infectious complication that significantly contributes to patient morbidity and mortality. While increased levels of CD21^{low} B cells in peripheral blood and bronchoalveolar lavage fluid characterize this condition, their exact role in CLD pathogenesis remains poorly understood.

Methods: In a multicenter prospective study, we investigated the prevalence and characteristics of CLD in CVID patients. Evaluation included chest computed tomography (Baumann's score), body plethysmography, diffusion capacity, and the King's Brief Interstitial Lung Disease (K-BILD) questionnaire for patient-reported outcomes. In addition to routine humoral and cellular immunity parameters, we assessed the functional characteristics of B cell subpopulations, specifically CD21^{low} B cells, following BCR and TLR stimulation.

Results: The study enrolled 88 patients (59% female; mean age 52.8 years; mean disease duration 11.9 years) from six referral centers. Mean Baumann's scores (BS) for bronchial and parenchymal changes were 2.7 and 3.7, respectively. The most prevalent structural patterns were bronchiectasis (40%) and nodules (37.5%); Granulomatous and Lymphocytic Interstitial Lung Disease (GLILD) features were present in 15%. Only 17% of patients showed no CT-detected structural changes. BS correlated primarily with lung function tests (LFTs). Serum IgA levels, relative CD8⁺ T cell counts, and the presence of enteropathy emerged as the most significant predictors of lung involvement. Furthermore, patients displayed increased counts of naive-like CD21^{low} B cells, which exhibited distinct functional responses to TLR7 and TLR9 agonists compared to other B cell subsets.

Conclusions: These findings reveal a high prevalence of bronchial and parenchymal changes in CVID. K-BILD and LFTs represent viable alternatives to CT for longitudinal monitoring. Our results suggest that naive-like CD21^{low} B cells play a pivotal role in immune dysregulation and the pathogenesis of non-infectious complications in CVID.

S7.04.I**BEYOND ENTEROVIRAL MENINGITIS: CHRONIC NEUROLOGICAL SEQUELAE OF XLA**

M. Bizjak¹, T. Butenko², M. Kačar^{1,3}, M. Zajc Avramovič¹, S. Gomezelj⁴, G. Brecl Jakob⁴, T. Meško¹, A. Pikelj Pečnik⁵, T. Vipotnik Vesnaver⁶, T. Avčin¹, G. Markelj¹

¹Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; ²Department of Pediatric Neurology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; ³University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; ⁴The Division of Neurology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; ⁵Clinic for Infectious Diseases and Febrile Illnesses, University Medical Centre Ljubljana, Ljubljana, Slovenia; ⁶Institute of Radiology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: masa.bizjak@kclj.si

Background: Neurologic manifestations are increasingly recognised as an important comorbidity in X-linked agammaglobulinemia (XLA), but not well understood. Up to one third of patients are affected, including cases of progressive neurodegeneration without identifiable infectious causes (1,2). This study evaluated neurologic and neuropsychiatric symptoms in a Slovenian XLA cohort.

Methods: In this retrospective cohort study, patients with confirmed XLA were identified from the national immunodeficiency registry. Clinical and demographic data were collected from medical records. Structured telephone interviews with patients/caregivers, developed with input from neurology, immunology, and psychology specialists, were used to evaluate neurologic and neuropsychiatric symptoms. Only chronic symptoms were included, except seizures.

Results: Fourteen patients were identified, and 13 participated (age 6–61 years). Median age at diagnosis was 4.8 years (range 0.2–10.9). Self-reported neurologic symptoms of individual patients are presented in Table 1. Three patients had died (ages 18, 36, and 61). Four patients (30%) reported no neurologic symptoms. Patient 5 developed epilepsy at 18 years with MRI evidence of generalized cerebral atrophy. Patient 9 had enteroviral meningitis at 21 years. Patient 11 presented at age 8 with learning difficulties that progressed to motor decline and cognitive impairment, leading to death at 36; no definitive cause was established. Aside from this case, no significant motor, gait, or continence issues were reported.

Table 1. Self-reported neurologic and neuropsychiatric symptoms in patients with X-linked agammaglobulinemia

Patient	Age [years]	Age at dg [years]	None	Headache	Vertigo	Memory/ attention problems	Speech problems	Impaired motor coordination_falls	Epileptic seizures	Neuropsychiatric/ psychological	Sleep disturbances
1	6	0.5				X	X				
2	6	4.8					X	X		X	
3	9	2.0				X					
4*	18	early childhood	X								
5	19	0.8							X		
6	20	5.1	X								
7	21	6.0	X								
8	24	8.6								X	
9	24	0.2		X	X		X		X	X	X
10	26	10.9				X					
11*	36	1.1					X		X	X	X
12	40	6.0		X	X	X					
13*	61	early childhood	X								

*Deceased

Conclusions: Neurologic and neuropsychiatric symptoms were frequent in this Slovenian XLA cohort. Two notable cases—one with progressive neurologic decline and another with cerebral atrophy—underscore the need for more detailed diagnostics and a better understanding of neurologic involvement in this condition.

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S7.05.I**INBORN ERRORS OF IMMUNITY SCREENING IN SLOVAKIA - FIRST EXPERIENCES**Peter Čížnár¹, Miloš Jeseňák², Róbert Ostró³, Zuzana Mydlová⁴

¹Comenius University Medical Faculty, Bratislava, Paediatric Department; ²Comenius University Jesenius Medical Faculty, Martin, Paediatric Department; ³Paediatric University Hospital Košice; ⁴National Screening Centre, Banská Bystrica
Presenting author e-mail: ciznarpeto@gmail.com

Introduction: Newborn screening using quantitative determination of T-lymphocyte excision-related circular DNA (TREC) in dried blood spots was primarily introduced to enable the early identification of severe combined immunodeficiency (SCID), a group of inborn errors of immunity severely affecting T cell development. Clinical experience with this screening has demonstrated a broader application of the screening than initially anticipated.

Methods: As of January 1, 2024, the National Screening Centre of the Slovak Republic has included the TREC and KREC (B-lymphocyte DNA excision circles) parameters in the nationwide newborn screening program, along with analysis of the SMN1 gene (spinal muscular atrophy). Over the course of more than two years, 113,064 children were screened. The presentation compares screening results in the U.S., Europe and Slovakia.

Results: TREC screening demonstrated high sensitivity for identifying classic and leaky SCID cases. The incidence was comparable to that in other European countries. During the study period, two cases of SCID with confirmed mutations in the RAG1 gene were diagnosed. In 6 cases, severe lymphopenia was caused by 22q11del (3), FOXP1 haploinsufficiency (1), PRDM12 deficiency (1), and other (1) syndrome. In 5 patients, screening was positive due to secondary causes. All severe B-lymphopenias had a secondary aetiology. In both SCID cases, successful, uncomplicated HSCT from unrelated donors was performed.

Conclusions: Newborn screening reliably identifies all severe T- and B-lymphocyte disorders, of both primary and secondary origin. The identification of syndromic and secondary T-lymphocytopenias enables early genetic diagnosis and intervention, which improves patient prognosis. International collaboration continues to focus on harmonizing various laboratory platforms and expanding the screening panel to include a broader spectrum of inborn errors of immunity.

S7.06.I**COMPLEXITY OF IEI IN ADULTS – ONE CENTRE EXPERIENCE**

Anna Ružinák Bobčáková^{1,2,3}, Adam Markocsy^{2,3}, Martina Schniederová², Otilia Petrovičová^{2,3}, Lenka Kapustová^{2,3}, Branislav Šlenker³, Eva Jurková Malicherová³, Andrej Čereš², Miloš Jeseňák^{1,2,3}

¹Outpatient Department of Allergy and Clinical immunology, Department of Pneumology and Phthisiology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin University Hospital, Martin, Slovakia; ²Institute of Clinical Immunology and Medical Genetics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Teaching Hospital in Martin, Martin, Slovakia; ³Outpatient Department of Allergy and Clinical immunology, Department of Paediatrics and Adolescent Medicine, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin University Hospital, Martin, Slovakia

Presenting author e-mail: abobcakova@gmail.com

Background: Inborn errors of immunity (IEI) have a very heterogenous clinical presentation, far beyond the increased susceptibility to infections. One third of IEIs does not have an infectious symptoms in the clinical presentation.

Methods: We retrospectively analysed the clinical presentation of patients with IEI followed in our centre focusing on CVID patients. The CVID cohort included 34 male and 30 female patients with the mean age 41.4 years.

Results: Infections were observed in 88% of our CVID patients. The infectious-only phenotype (CVID_{inf}) was present in 26 patients (40.6%) while 38 patients (59.4 %) had the dysregulatory phenotype (CVID_{dys}). Women were more likely to develop CVID_{dys} compared to men. Lymphoproliferation was confirmed in 5 patients (8 %), lymphadenopathy in 22 patients (34 %), splenomegaly in 13 patients (20 %), granulomatous lesions in 11 patients (17 %, including 9 patients with GLILD and 2 patients with sarcoidosis/sarcoid-like reaction). Bronchiectasis developed in 2 patients, autoimmunity was detected in 27 patients (42 %, including 11 cases of enteropathy, 20 cases of cytopenias, 6 cases of rheumatic disease and 5 cases of autoimmune thyroiditis), allergic complication in 27 patients (42 %). Molecular-genetic testing was performed in 17 patients (28 %) and a causal pathogenic variant was detected in 4 patients. The different phenotypes also reflected in immunophenotyping differences.

Conclusions: CVID is a complex disease with heterogenous manifestations. In addition to severe, persistent, unusual, and recurrent infections, unusually severe, difficult to treat, or multiple immunodysregulatory manifestations should also raise suspicion of an inborn error of immunity.

S8.02**MECHANISTIC INSIGHTS INTO THE PROTECTIVE ROLE OF LACTOFERRICIN IN SARS-COV-2 INFECTION**

Vladimir Leksa

Institute of Molecular Biology SAS, Laboratory of Molecular Biology, Bratislava, Slovakia

Presenting author e-mail: vladoleksa@gmail.com

Since Coronavirus disease 2019 (COVID-19) remains a significant threat, it is beneficial to develop therapeutic supplements against it. In this respect, glycoprotein lactoferrin (LF) and lactoferricin (LFC), a natural bioactive peptide yielded upon digestion from the N-terminus of LF, are of utmost interest, since both have been shown by us to reduce infections of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus responsible for COVID-19, in particular via blockade of the virus priming and binding. Specifically, we have revealed two mechanisms whereby LF and LFC block SARS-CoV-2 infection: Firstly, LF directly binds to the Spike (S) protein of SARS-CoV-2. We determined thermodynamic and kinetic characteristics of the complex formation and mapped the mutual binding sites involved in this interaction, namely the N-terminal region of LF and the receptor-binding domain of the S-protein (RBD). Secondly, LFC inhibits the proteolytic activity of TMPRSS2, a host protease implicated in virus priming. These results may not only explain many of the observed protective effects of LF and LFC in SARS-CoV-2 infection but also be instrumental in proposing potent, cost-effective supplemental tools for the management of COVID-19.

S8.03.P**INFLAMMATION AND BONE REMODELING**

Maša Filipović^{1,2}, Sara Aničić^{1,2}, Marta Radošević^{1,2}, Ozana Jakšić^{1,2}, Pavao Planinić³, Marina Ikić Matijašević⁴, Tomislav Kelava^{1,2}, Sanja Novak⁵, Danka Grčević^{1,2*}

¹Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; ²Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; ³Department of Physiology, University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina; ⁴Department of Clinical Immunology, Rheumatology and Pulmonology, University Hospital "Sveti Duh", Zagreb, Croatia, ⁵Department of Physiology and Cell Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Presenting author e-mail: danka.grcevic@mef.hr

Background: Dysregulated immune responses observed in immune-mediated inflammatory diseases (IMIDs) may cause systemic adverse effects in different organs, including bone. Inflammatory and autoimmune reactions can be localized in the vicinity of the bone (i.e. in arthritis) or affect tissues involved in the systemic regulation of bone mass (i.e. in diabetes). Inflammation-induced bone loss may be ascribed to enhanced activity of osteoclasts, exclusive bone resorbing cells of hematopoietic origin. Majority of osteoclast progenitors (OCPs) physiologically reside within the marrow cavity. However, 1-2% of hematopoietic cells in the peripheral lymphoid tissues and blood are susceptible to chemoattraction to bone surfaces, where they may eventually fuse to form active osteoclasts. IMID-associated pathogenetic processes may be especially supportive for peripheral OCP attraction and enhanced osteoclast maturation.

Methods: We combined several monocyte markers and chemokine receptors to identify population of human (CD45⁺CD15⁻CD3⁻CD19⁻CD56⁻CD11b⁺CD14⁺CD16⁻CXCR3⁺CCR2⁺) and mouse (CD45⁺CD3⁻B220⁻NK1.1⁻Ly6G⁻CD11b^{low/+}CD115⁺CX3CR1⁺CCR2⁺) OCPs in arthritis and diabetes. These OCPs were functionally tested for their ability to migrate toward the chemokine gradient and to mature into functional osteoclasts.

Results: Mice with collagen-induced arthritis had increased systemic and local bone resorption, due to induced peripheral and intramedullar OCP population. In both, collagen-induced arthritis and streptozotocin-induced diabetes, OCPs exhibited enhanced chemotactic activity toward CCL2 gradient. In addition, CCR2 expression by OCPs was associated with enhanced osteoclast functions, and blockade of CCL2/CCR2 axis suppressed osteoclast activity in arthritis. Human OCP population was increased 20-30% among peripheral blood cells in patients with rheumatoid arthritis and type 1 diabetes compared to controls. Transcriptome profiling showed high osteoclastogenic potential of CCR2^{hi} OCPs, and, in addition, increased inflammation-associated pathways.

Conclusions: We showed increased OCP frequencies in arthritis and diabetes. Also, we identified several genes and their corresponding proteins involved in inflammatory response and cell adhesion as OCP markers. Although they were already associated with autoimmune pathology, our investigation revealed their novel role in inflammatory OCP biology.

Funding: This work was supported by the European Union through National Recovery and Resilience Plan under grant agreement NPOO.C3.2.R3-I1.04.0093 (CHIPART); project No. 10106-25-3013 (MEDIS); project No. 10106-25-2987 (LIOS); Croatian Science Foundation HRZZ-IP-2022-10-2285 (OPTIMIDAL).

S8.04.I**HOW SIGNALING PATHWAYS SHAPE T-CELL DIVERSITY IN PHENOTYPE AND FUNCTION**

Valeria Uleri, Ondrej Stepanek

Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czechia

Presenting author e-mail: ondrej.stepanek@img.cas.cz

LCK is a central SRC-family kinase that initiates T-cell receptor signaling and is essential for positive and negative selection during thymocyte development. However, its role in mature peripheral T cells has remained less well defined, in part because conventional LCK deficiency profoundly disrupts T-cell development. To overcome this, we used a whole-body and a tamoxifen-inducible conditional LCK knockout mouse model, allowing acute deletion of LCK in mature TCR-transgenic OT-I T cells. Using this system, we compared wild-type and LCK-deficient T cells in vivo in two infection models and an autoimmune diabetes model.

Unexpectedly, acute loss of LCK uncoupled two major components of the T-cell response. Although LCK-deficient T cells showed reduced antigen-driven proliferation, they underwent enhanced effector differentiation in vivo. This phenotype was associated with selective disruption of TCR downstream signaling, as LCK was more important for ERK and NFAT activation than for AKT/mTOR signaling. T cells lacking the related SRC-family kinase FYN showed a similar, albeit weaker, bias toward effector differentiation, suggesting that LCK and FYN jointly regulate this process rather than acting through strictly non-overlapping functions.

Together, these findings identify LCK as a dual intrinsic regulator of peripheral T-cell responses: it promotes clonal expansion while restraining effector differentiation. Our study refines the current view of proximal TCR signaling in vivo and has implications for understanding immune dysregulation in human LCK deficiency as well as for the design of adoptive T-cell therapies.

Funding: ERC CoG ActSwiftly, ID: 101125695 (Horizon Europe).

S9.01.P**INNATE IMMUNE RESPONSES AGAINST CYTOMEGALOVIRUS INFECTION IN THE OVARY**

Marija Mazor¹, Jelena Železnjak¹, Colin Sparano², Tina Ružić¹, Sonia Tugues Solsona², Berislav Lisnić¹, Stipan Jonjić¹, Vanda Juranić Lisnić^{1,*}

¹Center for Proteomics, University of Medicine Faculty of Rijeka, Croatia; ²Institute of Experimental Immunology, University of Zürich, Switzerland

Presenting author e-mail: vanda.juranic@uniri.hr

Background: Cytomegaloviruses (CMVs) are widespread herpesviruses infecting majority of vertebrates, including humans. CMVs are also widespread within the body of the infected host. We have previously demonstrated that mouse CMV (MCMV), the most commonly used model virus for studying the pathogenesis of CMVs, strongly infects the ovaries, especially corpora lutea and stroma. The strong infection of the corpora lutea resulted in early pregnancy loss due to the diminished serum progesterone levels. Interestingly, MCMV was completely excluded from the ovarian follicles. Since viral infection of ovarian follicles can result in infertility, we investigated which immune system components are responsible for the protection of ovarian follicles from CMV.

Methods: Systemic, intravenous infection of adult female mice of reproductive age with MCMV, utilizing various wild-type, transgenic and knock-out mouse strains lacking components of the innate immune system. High-parameter flow cytometry for characterization of immune cell subsets.

Results: The strongest protection to the follicles was mediated by interferon type I signaling due to the strong expression of NFκB within the follicles. In addition to type I IFN signaling, NK and ILC cells played significant protective roles, in collaboration with macrophages. Considering that a lot is known about uterine NK cells but very little about ovarian, we performed thorough characterization of ovarian NK and ILC cells.

Conclusions: Multiple layers of innate immunity protect the ovarian follicles from an ubiquitous virus like the CMV. Studying mechanisms of how ovaries are protected against common viral threats can yield better understanding of infertility.

Funding: Croatian Science Foundation grants HRZZ IP-2016-06-5980 and IP-2022-10-2324 to Vanda Juranić Lisnić and University of Rijeka grant PU-140, uniri-mzi-25-26 to Berislav Lisnić.

S8.02.I**MICROGLIAL PRIMING BALANCES LATENT VIRUS CONTROL AND SYNAPTIC LOSS**

Ilija Brizić

Center For Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Presenting author e-mail: ilija.brizic@uniri.hr

Microglia are resident myeloid cells of the central nervous system (CNS) that play key roles in maintaining tissue homeostasis. They also mount diverse immune responses to pathogenic challenges, including CNS infections. Herpesviruses establish lifelong latency and periodically reactivate when immune surveillance diminishes, yet how microglia contribute to the monitoring of latent herpesvirus infections remains poorly understood. Using cytomegalovirus (CMV) as a model, we examined how a persistent viral presence in the brain influences microglial function. We discovered that mouse CMV (MCMV) latency in the CNS leads to sustained microglial priming. Persistent infection drives continuous interferon γ -dependent activation and triggers profound single-cell transcriptional reprogramming, resulting in the expansion of a distinct microglial subset associated with latent infection. Importantly, this primed state strengthens microglial control over latent virus and enhances recall responses. However, it also causes substantial loss of dendritic spine synapses, a process mediated by the activated microglia. Together, these findings show that latent CMV infection disrupts microglial homeostasis, generating chronic neuroinflammation that both restricts viral reactivation and negatively affects neuronal synaptic integrity.

S8.03.I**IFN γ IS NECESSARY FOR *LACTIPLANTIBACILLUS PLANTARUM*-MEDIATED GROWTH PROMOTION IN UNDERNOURISHED MICE**

Martin Schwarzer

Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Nový Hrádek, Czech Republic

Presenting author e-mail: schwarzer@biomed.cas.cz

The intestinal microbiota is known to influence postnatal growth. We have shown that the intestinal microbiota and a specific *L. plantarum* WJL (LpWJL) strain can sustain juvenile mouse growth under chronic undernutrition conditions [1]. More recently, we described that daily administration of LpWJL improves the growth of conventional (microbiota-bearing) undernourished juvenile mice. We identified cell walls isolated from LpWJL as sufficient cues to stimulate animal growth despite undernutrition. Furthermore, we found that NOD2 is necessary in intestinal epithelial cells (IEC) for LpWJL-mediated postnatal growth promotion in malnourished conventional animals [2]. The growth-promoting effects of LpWJL feeding were accompanied by an increased number of proliferating crypt cells in the small intestine and elevated levels of IFN-response genes, both dependent on intact NOD2 signaling in IEC. The increased proliferation of IEC after LpWJL treatment was linked to increased IFN γ production by lamina propria CD4⁺ T cells. Next, we tested whether other bacteria have strain- and species-specific growth-promoting properties. Mice monocolonized with *Bifidobacterium longum* (Bl) strains showed improved systemic growth compared to mice monocolonized with *Bifidobacterium adolescentis* (Bad) strains or the control germ-free (GF) group. Monocolonization with the B1372 strain resulted in significantly enhanced weight and length gains. Similarly to LpWJL feeding, bulk RNA sequencing of jejunal tissue revealed differences in gene expression, and interferon gamma-response genes were significantly upregulated upon Bl colonization. Interestingly, when we blocked IFN γ production by injecting an anti-IFN γ antibody, the beneficial effect of LpWJL feeding on linear growth was lost, and the growth rate did not differ from that of anti-IFN γ antibody-injected placebo-treated animals. This prompted us to test the growth-promoting properties of LpWJL in RAG-1-deficient mice, which lack mature B and T lymphocytes. When we used littermate RAG^{+/-} (wild-type) and RAG^{-/-} cohorts and fed them LpWJL, only the RAG^{+/-} animals showed growth improvement. Thus, IFN γ appears to be necessary for bacteria-induced juvenile growth promotion during chronic undernutrition.

Funding: This work has been funded by a grant from the Programme Johannes Amos Comenius under the Ministry of Education, Youth and Sports of the Czech Republic (project Nr CZ.02.01.01/00/22_008/0004597).

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2. Schwarzer, M., et al., *Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice*. *Science*, 2023. **379**(6634): p. 826-833.

Poster presentations

Poster presentations list

MESIA 2026: Poster session (P1.01 - P1.20)		
DAY 2 THURSDAY, 16.4.2026, 16:40 - 17:50		
P1.01	Sara Simonič	Performance evaluation of indirect immunofluorescence kits for endomysial antibodies (EMA IgA) detection in the diagnostic algorithm of coeliac disease
P1.02	Manca Svetina	Baseline serum tryptase level is defined by genetic variation at the tryptase locus
P1.03	Urša Žibert	The role of fibulin-3 in plasma and pleural effusion as a potential biomarker of mesothelioma
P1.04	Maja Černilec	Prevalence of Severe Immunoglobulin A Deficiency among Slovene Blood Donors
P1.05	Katarina Kouter	Altered gene expression of the complement system genes and suicidality
P1.06	Valeriya Musina	Protease-dependent activation of a superantigen for local T-cell stimulation
P1.07	Maša Omerzel	Combination of Electrochemotherapy and Anti-PD-1 Immunotherapy in Murine Tumors
P1.08	Urša Lampreht Tratar	Evaluation of antitumor immune response induced by intratumoral or peritumoral interleukin-12 gene electrotransfer combined with electrochemotherapy in canine mast cell tumors
P1.09	Tanja Jesenko	Frequency and morphological characteristics of circulating tumour cell clusters isolated from central venous blood of patients with early breast cancer
P1.10	Dóra Adamecz	Metal nanoparticles as potential modulators of tumor cell-macrophage crosstalk
P1.11	Špela Konjar	Metabolic reprogramming of intestinal CD8 T cells during activation
P1.12	Anikó Kapitány	LL37 As A Potential Biomarker To Distinguish Between Atopic Eczema And Psoriasis
P1.13	Viktória Nagy	Expression pattern of IL-1 family cytokines in the epidermis of atopic dermatitis patients
P1.14	Renata Toth	microRNA-mediated regulation of Oral Squamous Cell Carcinoma responses during Candida infection
P1.15	Maja Cokarič Brdovčak	Development of novel monoclonal antibodies targeting viral proteins
P1.16	Gbenga Folurunsho Oginni	Quantitative detection of microcystis aeruginosa in fresh water using single domain antibodies (VHHS)
P1.17	Katja Leben Zupet	Precise immunophenotyping of mouse regulatory T cells
P1.18	Urška Rupnik	Development of approaches for isolation, activation and expansion of gamma delta T cells
P1.19	Andrea Šarac	Optimization of CRISPR/Cas9-Mediated TCR Knock-Out in primary mouse T cells
P1.20	Marija Rakić	Vitamin B complex suppresses LPS-induced neuroinflammation in activated microglia: in vitro and in silico insights

MESIA 2026: Poster session (P2.01 - P2.20)		
DAY 3 FRIDAY, 17.4.2026, 10:00 – 11:00		
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P2.02	Monika Gregorič Tasič	De novo designed minibinder proteins targeting NIN1
P2.03	Nina Naprudnik	Persistent Eosinophilia in Severe Atopic Dermatitis: Differential Diagnostic Evaluation and Identification of Subtherapeutic Cyclosporine
P2.04	Judit Danis	Nucleic acid-driven expression and alternative splicing of Z-DNA binding Protein 1 in human keratinocytes
P2.05	Csikós Máté Lajos	Priming the Oral Epithelium: Candida parapsilosis Pre-Exposure Enhances Cytokine Responses and Alters Candida albicans Interactions
P2.06	Tímea Berki	Autoantibody mediated neurological diseases: laboratory diagnostics
P2.07	Péter Németh	Are immune dysregulation-associated alterations in pathological and natural autoantibody profiles associated with vaccine-induced anti-SARS-CoV-2 humoral responses?
P2.08	Tina Vesel Tanjšek	Case Report: Antiparasitic treatment can cure an ill child with eosinophilia despite unconfirmed parasitosis
P2.09	Böröcz Katalin	Altered natural autoantibody signatures associated with pathological autoantibody co-positivity in SAD-suggestive profiles
P2.10	Larisa Janžič	Dynamic macrophage immune responses distinguish inflammatory from immune-evasive strains of Group B Streptococcus
P2.11	Le Roux Alain	Serological quantification of lost immune tolerance: pathological autoantibody accumulation coincides with depletion of homeostatic natural autoantibody in the anti-TG/TPO-positive patients
P2.12	Jasna Omersel	HLA-B allele frequencies and HLA-B*27 subtypes in Slovenian patients with ankylosing spondylitis treated with targeted therapies
P2.13	Nataša Kopitar Jerala	Cystatins as Endogenous Regulators of Innate Immune Responses in LPS-Induced Sepsis
P2.14	Ivana Bertović	Pathogenesis of MCMV infection in the adrenal gland
P2.15	Tomislav Kelava	Osteoclast Activity and Bone Mass Changes in a 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine-Induced Cholestatic Liver Disease Model
P2.16	Iva Vlačić	ILC1s mediate control of perinatal murine cytomegalovirus infection via NKG2D
P2.17	Simona Ivančan	Early etoposide is critical in Epstein-Barr virus-associated haemophagocytic lymphohistiocytosis: a paediatric case report
P2.18	Tanja Lunič	Immunomodulatory potential of moss Hypnum cupressiforme Hedw. extracts: in vitro and in silico study
P2.19	Lucie Zahradníčková	Impact of Early Postnatal Administration of Escherichia coli O83:K24:H31 on Immune System Maturation and Gut Barrier Function Authors
P2.20	Martin Petřek	Risk HLA variants for severe COVID-19 – a study from Olomouc, Czechia

Poster presentation abstracts

P1.01**PERFORMANCE EVALUATION OF INDIRECT IMMUNOFLUORESCENCE KITS FOR ENDOMYSIAL ANTIBODIES (EMA IGA) DETECTION IN THE DIAGNOSTIC ALGORITHM OF COELIAC DISEASE**

Sara Simonič¹, Renata Berdajs¹, Neja Šamec¹, Mojca Zrimšek¹, Alenka Trampuš Bakija¹, Tinka Hovnik^{1,2}

¹Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, UMC, Ljubljana, Slovenia; ²Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: sara.simonic@kclj.si

Background: EMA IgA is a serological marker used in the diagnostic algorithm for coeliac disease, its analysis using primate oesophagus tissue. In light of the global decrease in the availability of primate oesophagus tissue, largely related to COVID-19 related export restrictions, we conducted an evaluation of alternative reagent kits for the detection of EMA IgA.

Methods: We compared two different indirect immunofluorescence kits; NOVA Lite Monkey Oesophagus Kit (Inova Diagnostics, Inc.) and ImmuGlo anti-Endomysial IgA Kit (Immco Diagnostics) to Antiendomysium IgA (Eurospital Diagnostics). All samples were selected based on previously obtained tTG IgA and EMA IgA results, analysed with the Antiendomysium IgA kit. For the comparison between Antiendomysium IgA and NOVA lite, 38 patient serum samples were analysed. For the ImmuGlo comparison, 36 diluted sample preparations were analysed according to two protocols with different dilution schemes (Protocol A and Protocol B) as shown in Table 1. All analyses were performed using indirect immunofluorescence on primate oesophageal tissue sections, and results were evaluated by 3 laboratory technicians under fluorescence microscope. Results were interpreted as positive or negative based on the presence of characteristic fluorescence patterns. Statistical analysis was conducted using the Chi-square test and Fisher's exact test.

Protocol A					
Dilution step	Patient volume (µL)	Volume of previous dilution (µL)	Diluent volume (µL)	Total volume (µL)	Dilution factor
1	200	-	300	500	1:2,5
2	-	200	200	400	1:5
3	-	200	200	400	1:10
4	-	200	200	400	1:20
Protocol B					
Dilution step	Patient volume (µL)	Volume of previous dilution (µL)	Diluent volume (µL)	Total volume (µL)	Dilution factor
1	100	-	400	500	1:5
2	-	200	200	400	1:10
3	-	200	200	400	1:20
4	-	200	200	400	1:40

Table 1: Dilution scheme

Results: The comparison between Antiendomysium IgA and NOVA Lite demonstrated complete agreement across all 38 samples, with no statistically significant difference ($X^2(df=1, N=38)=0, p=1,000$). Similarly, comparison between the Antiendomysium IgA and ImmuGlo showed 100% concordance in both Protocol A(20 samples) and Protocol B(16 samples) ($p=1.000$). Overall, no statistically significant differences were observed between any of the reagent kits.

Conclusions: The newly evaluated reagent kits demonstrate complete diagnostic comparability with the previously used Antiendomysium IgA, and both are suitable for diagnostic use. The NOVA Lite slides provided clearer and more consistent fluorescent patterns, and the accompanying protocol offered more structured instructions. Based on these advantages, the NOVA Lite kit was selected for routine implementation in our laboratory. Limitations of this evaluation include the relatively small number of samples tested with the ImmuGlo and the challenging classification of borderline positive fluorescence pattern.

P1.02**BASELINE SERUM TRYPTASE LEVEL IS DEFINED BY GENETIC VARIATION AT THE TRYPTASE LOCUS**

Manca Svetina^{1,2}, Peter Kopač^{1,3}, Julij Šelb^{1,3}, Peter Korošec^{1,4}, Matija Rijavec^{1,2,5}

¹University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; ²Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ⁴Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia; ⁵Faculty of Medicine, University of Maribor, Maribor, Slovenia
Presenting author e-mail: manca.svetina@klinika-golnik.si

Background: Mast cell derived tryptase is a key mediator in allergy. Even modestly higher baseline serum tryptase (BST) levels are associated with anaphylaxis. Markedly elevated BST levels (>11.4 ng/mL) are most commonly due to hereditary α -tryptasemia (H α T) or clonal mast cell disorders (cMCD). However, the contribution of genetic variation at the tryptase locus to BST levels in individuals without these conditions remains incompletely understood.

Methods: We evaluated the diagnostic accuracy of BST for predicting cMCD and/or H α T and investigated the relationship between genetic variation at the tryptase locus and BST levels. BST levels were measured using ImmunoCAP Tryptase fluoroenzyme immunoassay. KIT p.D816V was determined using a highly sensitive qPCR test. In addition, multiplex ddPCR assays were developed and optimized for comprehensive tryptase genotyping, enabling quantification of α - and β -tryptase sequences and detection of the β III frameshifted allele (β IIIFS). These assays were applied to assess the contribution of tryptase locus variation to BST levels in 536 individuals referred to the University Clinic Golnik.

Results: All 25 individuals with elevated BST levels (>11.4 ng/mL) had cMCD and/or H α T (sensitivity: 35%, specificity: 100%): 16 (64%) had H α T, 8 (32%) cMCD, and 1 (4%) had both. The optimal BST cutoff for predicting H α T was 7.2 ng/mL (sensitivity 97%, specificity 92%, AUC 0.96). In contrast, BST was a weak predictor of cMCD, with an optimal cutoff of 5.5 ng/mL (sensitivity 70%, specificity 74%, AUC 0.77). The ImmunoCAP Tryptase immunoassay quantifies total serum tryptase, including all mature and pro α - and β -tryptase. Based on this, we hypothesized that BST levels primarily reflect the total number of functional tryptase alleles, excluding the β IIIFS. The c.980_981insC insertion in β IIIFS is predicted to trigger nonsense-mediated mRNA decay. Consistent with this hypothesis, increasing numbers of tryptase alleles were associated with progressively higher BST levels ($r = 0.34$, $p < 0.0001$).

Conclusion: BST with a cutoff of 7.2 ng/mL is a strong predictor of H α T. In contrast, BST has limited utility for predicting cMCD, as 30% (12/40) of individuals with the KIT p.D816V had BST levels below the optimal cutoff of 5.5 ng/mL. Genetic variation at the tryptase locus, particularly the presence of the nonfunctional β IIIFS allele, significantly influences BST levels and should be considered in future studies investigating tryptase.

P1.03**THE ROLE OF FIBULIN-3 IN PLASMA AND PLEURAL EFFUSION AS A POTENTIAL BIOMARKER OF MESOTHELIOMA**

Urša Žibert¹, Katja Adamič¹, Urška Bidovec-Stojković¹, Peter Korošec¹

¹University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia

Presenting author e-mail: ursa.zibert@klinika-golnik.si

Background: Malignant pleural mesothelioma (MPM) is a global health concern linked to asbestos exposure. In Slovenia, regions with high asbestos exposure rates make MPM a significant public health issue. Although thoracoscopy is the gold standard for MPM diagnosis, its invasiveness highlights the need for reliable, non-invasive diagnostic biomarkers. The study aimed to examine the potential role of fibulin-3 in plasma and pleural effusion as an early marker of MPM.

Methods: This study compares plasma and pleural fibulin-3 levels in subjects with MPM and those with benign asbestos-related pleural disease or carcinomatosis. The cohort comprises subjects evaluated at the Clinic Golnik between 2013 and 2025 with histologically confirmed MPM, as well as individuals who underwent thoracoscopy for pleural effusion and have a confirmed histopathological diagnosis (pleural carcinomatosis, asbestos pleuritis, chronic nonspecific pleuritis). We also include healthy volunteers. Fibulin-3 levels were measured using ELISA, and clinical data were analysed using descriptive statistical methods, with p-values <0.05 considered statistically significant. The Mann–Whitney test for independent samples was applied.

Results: Plasma samples were obtained from 48 patients with MPM, 50 with pleural carcinomatosis, 28 with asbestos pleuritis, 48 with chronic pleuritis, and 50 healthy volunteers. Plasma fibulin-3 levels tend to be higher in MPM patients compared to those with asbestos pleuritis (p=0.0724) and chronic nonspecific pleuritis (p=0.0577); however, there was no difference with healthy volunteers (p=0.3434). We found elevated plasma fibulin-3 levels in a group with pleural carcinomatosis compared to those with asbestos pleuritis (p=0.0112), chronic pleuritis (p=0.0061), and healthy volunteers (p=0.0436). Pleural effusion was obtained from 50 patients with MPM, 47 with pleural carcinomatosis, 20 with asbestos pleuritis, and 49 with chronic pleuritis. We found a statistically significant difference in pleural effusion fibulin-3 levels between patients with asbestos pleuritis compared to those with MPM (p=0.0016), carcinomatosis (p=0.0106), and chronic pleuritis (p=0.0330), with higher levels observed in the asbestos group.

Conclusion: Our findings demonstrate that plasma and pleural fibulin-3 levels tend to be higher in MPM compared to both group of pleuritis and carcinomatosis. In the future, we want to expand those groups of patients and to investigate the prognostic value of fibulin-3 in plasma and pleural effusion in patient follow-up, across different histological subtypes and clinical stages of MPM, in relation to biochemical and haematological markers of inflammation.

P1.04**PREVALENCE OF SEVERE IMMUNOGLOBULIN A DEFICIENCY AMONG SLOVENE BLOOD DONORS**

Maja Černilec, Marjeta Maček Kvanka, Petra Jovanovič, Melita Gracar, Danijela Fabjan, Aleš Ladiha, Suzana Đorđević, Valerija Kovač, Vladka Čurin Šerbec, Slavica Stanišić

Slovene Institute for Transfusion medicine, Ljubljana, Slovenia

Presenting author e-mail: maja.cernilec@ztm.si

Background: Selective IgA deficiency is the most common primary immunodeficiency. Severe IgA deficiency is a form of selective IgA deficiency, where the IgA antibody concentration is below 0.5 mg/L. In individuals with IgA deficiency, antibodies against IgA (anti-IgA) can be observed. Individuals with IgA deficiency may develop anti-IgA antibodies, which can cause anaphylactic reactions upon exposure to standard blood products containing IgA. IgA deficiency is rare, therefore maintaining a registry of IgA-deficient donors without anti-IgA antibodies is essential to ensure the availability of safe blood products for patients with severe IgA deficiency when needed. As the prevalence of IgA deficiency in Slovenia is unknown, we evaluated IgA deficiency and the presence of anti-IgA antibodies in IgA-deficient donors among regular blood donors of Slovenia.

Methods: We screened a random sample of 4,560 regular male blood donors aged 18–55 years who had no prior history of blood transfusion and had previously donated blood successfully at least once. Severe IgA deficiency and the presence of IgG class anti-IgA antibodies were assessed using validated in-house ELISA assays.

Results: We found 9 individuals with severe IgA deficiency. This resulted in the prevalence of 1:507. Among them, six had blood group O, two had blood group A, one had blood group B, and none had blood group AB. Two individuals were RhD negative, both belonging to blood group O. Anti-IgA antibodies were detected in three of the nine IgA-deficient donors (titer 1:316 or higher): one with blood group O, RhD positive; one with blood group O, RhD negative; and one with blood group A, RhD positive.

Conclusions: Data on the prevalence of severe IgA deficiency among blood donors are limited. A study conducted among Canadian blood donors reported a prevalence of 1:546, which is comparable to our findings (prevalence 1:507).

Optimal donors for IgA-deficient patients are severely IgA-deficient donors who have not developed anti-IgA antibodies. In our cohort, only six donors met these criteria, of whom four had blood group O. While blood group O accounts for approximately 38% of blood donors in the Slovenian population, it was more frequently observed among IgA-deficient individuals in our study (6/9; 67%). However, to determine whether there is a true association between certain ABO blood group and IgA deficiency, a larger study with balanced representation across all blood groups would be required.

P1.05**ALTERED GENE EXPRESSION OF THE COMPLEMENT SYSTEM GENES AND SUICIDALITY**

Žana Radivo¹, Alja Videtič Paska², Tomaž Zupanc³, Katarina Kouter⁴

¹Faculty of Medicine, Ljubljana, Slovenia; ²Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, Ljubljana, Slovenia; ³Institute of Forensic Medicine, Faculty of Medicine, Ljubljana, Slovenia; ⁴Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia

Presenting author e-mail: katarina.kouter@mf.uni-lj.si

Background: Suicide and the mental disorders involved in the development of the suicidal process represent a major public health problem. The relationship between immune and inflammatory processes and mental disorders has been intensively researched. Most studies have focused on inflammatory markers such as cytokines, while studies looking at the complement system remain scarce. The physiological and pathological roles of complement factors in the central nervous system have mainly been demonstrated in animal models, but increasingly strong links are being made between the expression of complement factors and schizophrenia and major depressive disorder. Complement in the central nervous system of those who have died by suicide and its possible role in the development of the suicidal process is a largely unexplored area. Our aim was to investigate whether there is a statistically significant difference in the expression of genes encoding elements of the complement system between a group of men who died by suicide and healthy controls.

Methods: The study is retrospective. 23 subjects who died by suicide (specifically by hanging) and 28 controls were included. All subjects were men aged between 18 and 65 years. mRNA was isolated from the brain (BA46 and hippocampus) and converted into cDNA. The qPCR method was used to measure the amount of cDNA in the tissue of each sample. The genes of interest were *CFB*, *CFH*, *C3*, *CIQA*, *CIQB*, *CIQC*, *C4A* and *C4B*; with *GAPDH* and *DCTN2* used as reference genes for normalisation of expression values. Statistical analysis was performed using the unpaired Student t-test and the Mann-Whitney test. The p-value indicating statistical significance was set at ≤ 0.05 .

Results: We observed altered gene expression in both brain regions. The expression of *C3* (BA46 $p=0.0155$, hippocampus $p=0.0311$) and *CIQC* (BA46 $p=0.0296$, hippocampus $p=0.0123$) was higher in the brain tissue of suicide completers. In BA46 the expression of *C4A* ($p=0.0025$) was higher in the control group, while in the hippocampus the expression of *CIQA* was increased in suicide completers ($p=0.0174$).

Conclusions: There is a difference in the expression of complement system genes between the two groups. Based on the findings of other research in this field, we can conclude that the complement system plays a role in the development of the suicidal process.

P1.06**PROTEASE-DEPENDENT ACTIVATION OF A SUPERANTIGEN FOR LOCAL T-CELL STIMULATION**

Valeriya Musina^{1,2}, Tadej Satler¹, Roman Jerala^{1,3,4}, Mateja Manček Keber^{1,3}

¹Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia. ²Interdisciplinary Doctoral Study of Biomedicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. ³EN-FIST Centre of Excellence, Ljubljana, Slovenia. ⁴CTGCT, Center for Technologies of the Gene and Cell Therapy, National Institute of Chemistry, Ljubljana, Slovenia.

Presenting author e-mail: valeriya.musina@ki.si

Superantigens, such as Staphylococcal enterotoxin A (SEA), are potent T-cell activators, capable of stimulating up to 20% of T cells. They act by directly cross-linking MHC class II molecules on antigen-presenting cells with T-cell receptors, completely bypassing classical antigen processing. Although their strong immunogenicity makes them promising candidates for cancer immunotherapy, systemic use is limited by toxicity from uncontrolled cytokine release. We aim to develop a tumour-specific system that enhances local T-cell activity in the immunosuppressive tumour microenvironment while minimizing systemic effects.

SEA was fused via short linkers to inhibitory molecules, including de novo designed minibinders, nanobodies, and a modified T-cell receptor β -chain. The inhibitory domains were designed to block the T-cell receptor binding site, while the linkers incorporated cleavage sites for tumour-associated proteases such as metalloproteinases and urokinase-type plasminogen activator. Constructs were produced in both mammalian cells and *E. coli*, then screened in vitro for inhibition of SEA activity prior to cleavage and reactivation following protease treatment. Protease-specific cleavage was confirmed by either SDS-PAGE or western blot, and functional T-cell activation was evaluated using peripheral blood mononuclear cells and cytokine release quantification.

Several constructs containing de novo designed minibinders effectively inhibited SEA activity before cleavage and were reactivated upon protease treatment. Functional assays demonstrated selective cytokine release upon protease-mediated activation, confirming tumour-specific potential.

Our approach offers a modular, protease-activated system that enables local T-cell stimulation and may be adapted to different tumour environments. This approach provides a foundation for safer, tumour-specific immunomodulatory therapies.

Funding: Supported by the Slovenian Research Agency (research core no. P4-0176).

P1.07**COMBINATION OF ELECTROCHEMOTHERAPY AND ANTI-PD-1 IMMUNOTHERAPY IN MURINE TUMORS**

Maša Omerzel^{1,2}, Simona Kranjc Brezar^{1,3}, Urša Lampreht Tratar^{1,4}, Tanja Jesenko^{1,3}, Barbara Liseč¹, Gregor Sersa^{1,2}, Maja Čemažar^{1,5}

¹Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ⁴Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia; ⁵Faculty of Health Sciences, University of Primorska, Izola, Slovenia
Presenting author e-mail: momerzel@onko-i.si

Background: Electrochemotherapy (ECT) is a clinically validated local ablative treatment that induces immunogenic cell death and stimulates antitumor immune responses. Its combination with immune checkpoint inhibitors, such as anti-PD-1 antibodies, may enhance systemic immunity and improve therapeutic efficacy, particularly in poorly immunogenic tumors.

Methods: The antitumor efficacy of ECT combined with a murine analogue of an anti-PD-1 antibody was evaluated in four syngeneic murine tumor models with distinct histology and immune characteristics: WEHI fibrosarcoma, CT26 and MC38 colorectal carcinoma, and 4T1 mammary carcinoma. Tumor cell sensitivity to ECT was assessed using in vitro cytotoxicity assays. In vivo experiments (permission no. U34401-3/2022/17) evaluated complete response (CR) rates, immune cell infiltration, and long-term immune memory through secondary tumor challenge. Immunohistochemical analysis was performed to assess infiltration of CD4⁺, CD8⁺, and granzyme B⁺ effector cells.

Results: In vitro, WEHI cells exhibited the highest sensitivity to ECT. In vivo, ECT monotherapy induced CR in 100% of WEHI, 60% of CT26, 17% of 4T1, and 15% of MC38 tumors. The addition of anti-PD-1 significantly improved therapeutic outcomes in less responsive models, increasing CR rates to 90% in CT26, 91% in MC38, and 53% in 4T1 tumors. Combined therapy promoted marked infiltration of CD4⁺, CD8⁺, and granzyme B⁺ T cells and induced the formation of tertiary lymphoid structures, particularly in MC38 tumors. Secondary challenge experiments confirmed long-term immune memory in CT26 and MC38 models and induced immune memory in the 4T1 model, which was absent following ECT monotherapy.

Conclusions: Electrochemotherapy combined with PD-1 blockade enhance both local and systemic antitumor immunity, overcoming immune resistance in poorly immunogenic tumors. These findings support further clinical development of ECT combined with immune checkpoint inhibitors as part of personalized cancer immunotherapy strategies.

P1.08**EVALUATION OF ANTITUMOR IMMUNE RESPONSE INDUCED BY INTRATUMORAL OR PERITUMORAL INTERLEUKIN-12 GENE ELECTROTRANSFER COMBINED WITH ELECTROCHEMOTHERAPY IN CANINE MAST CELL TUMORS**

Urša Lampreht Tratar^{1,2,3}, Nina Milevoj², Maja Čemažar^{1,3,4}, Katarina Žnidar^{1,3}, Katja Uršič Valentinuzzi^{1,3,5}, Andreja Brožič¹, Katerina Tomšič², Gregor Serša^{1,3,6}, Nataša Tozon²

¹Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of medicine, University of Ljubljana, Ljubljana, Slovenia; ⁴Faculty of Health Sciences, University of Primorska, Izola, Slovenia; ⁵Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; ⁶Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: ulampreht@onko-i.si

Electrochemotherapy (ECT) is an established local treatment for solid tumors, while interleukin-12 (IL-12) is a key immunostimulatory cytokine capable of inducing potent antitumor immune responses. Combining ECT with IL-12 gene electrotransfer (GET) represents a strategy to convert local tumor control into a systemic, immune-mediated therapeutic effect. This study aimed to evaluate the immunological and clinical impact of different IL-12 GET administration routes when combined with ECT in canine mast cell tumors (MCTs). Seventy-seven dogs with spontaneous MCTs were enrolled and treated with ECT combined with intratumoral IL-12 GET, ECT combined with peritumoral IL-12 GET, or ECT alone. Tumor immune status was assessed in pretreatment biopsies, and systemic immune effects were monitored by flow cytometric analysis of peripheral blood mononuclear cell (PBMC) subpopulations before and after therapy. Clinical outcomes included local tumor control, disease-free interval, and progression-free survival. Intratumoral IL-12 GET combined with ECT resulted in significantly improved local tumor control, longer disease-free intervals, and superior progression-free survival compared with peritumoral IL-12 GET or ECT alone, accompanied by an increased proportion of circulating antitumor immune cell subsets after treatment. Additionally, in the intratumoral IL-12 GET group, higher expression of PD-L1 and PD-1 in the tumors was associated with a poorer early treatment response. Intratumoral delivery of IL-12 via gene electrotransfer synergizes with ECT to enhance systemic antitumor immunity and improve clinical outcomes in canine MCTs. This combined therapeutic approach is safe, immunologically active, and supports intratumoral IL-12 GET as the preferred administration route in veterinary oncology.

P1.09**FREQUENCY AND MORPHOLOGICAL CHARACTERISTICS OF CIRCULATING TUMOUR CELL CLUSTERS ISOLATED FROM CENTRAL VENOUS BLOOD OF PATIENTS WITH EARLY BREAST CANCER**

Tanja Jesenko^{1,2}, Veronika Skrjanc^{1,2}, Ziva Pisljar¹, Simona Miceska¹, Maja Cemazar^{1,3}, Veronika Kloboves-Prevodnik^{1,4}, Cvetka Grasic Kuhar^{1,2}

¹Institute of Oncology Ljubljana, Ljubljana, Slovenia; ²Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of Health Sciences, University of Primorska, Izola, Slovenia; ⁴Faculty of Medicine, University of Maribor, Maribor, Slovenia

Presenting author e-mail: tjesenko@onko-i.si

Background: Circulating tumor cell (CTC) clusters are precursors of cancer distant metastases. Homotypic clusters are composed of tumor cells only, whereas heterotypic CTC clusters contain tumor cells together with non-tumor cells such as immune cells, platelets, or stromal cells. In the past trials with different isolation methods and routes of collecting blood, CTC clusters were considered a very rare event in early breast cancer.

Methods: A prospective, non-interventional clinical study GALIA is being conducted at the Institute of Oncology Ljubljana to investigate the presence and characteristics of CTC clusters in patients with early breast cancer. Prior to the initiation of neoadjuvant systemic therapy, 10 mL of central venous blood was collected in K2EDTA tubes. CTCs were isolated based on their physical properties using the Parsortix™ system (CellBxHealth plc.). Detection and characterization were performed using routine cytopathological assessment and immunofluorescence staining using the Portrait+ CTC Staining Kit (CellBxHealth plc.) including a nuclear dye, epithelial and mesenchymal markers, and a panel of hematopoietic cell markers.

Results: CTC clusters were detected in 69% of patients. Based on morphological and immunophenotypic analysis, CTCs forming clusters were classified into two major populations: non-degenerated and degenerated CTCs. Clusters of non-degenerated CTCs consisted of cells with preserved malignant morphology, a clearly detectable nuclear signal, and positivity for epithelial markers, vimentin, or both, while being negative for hematopoietic cell markers. In contrast, clusters of degenerated CTCs were composed of cells lacking a nuclear signal and exhibiting pronounced features of cellular degeneration, including membrane blebbing and villous protrusions. In addition, rare multicellular structures consistent with highly metastatic microemboli were identified in some of the patients. Overall, patients exhibited a higher number of degenerated CTC clusters compared with non-degenerated clusters. Degenerated CTC clusters were on average smaller than clusters composed of non-degenerated CTCs. Notably, CTCs within heterotypic clusters were most frequently associated with neutrophils and platelets.

Conclusions: The high frequency of CTC clusters identified in this study suggests that CTC clustering may be more prevalent in early breast cancer than previously assumed. Further analysis in a larger patient cohort, together with long-term follow-up of treatment outcomes, will be required to determine the clinical relevance of the observed CTC cluster populations.

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P1.10**METAL NANOPARTICLES AS POTENTIAL MODULATORS OF TUMOR CELL-MACROPHAGE CROSSTALK**

Dóra Izabella Adamecz^{1,2}, Éva Veres², Hédi Árva¹, Csaba Papp², Annamária Marton⁶, Andrea Rónavári⁷, Csaba Vizler⁶, Zoltán Kónya⁷, Attila Gácser^{3,4,5}, Mónika Kiricsi¹, Nóra Igaz¹

¹SZTE TTIK Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary; ²SZTE TTIK Department of Biotechnology and Microbiology, University of Szeged, Szeged, Hungary; ³HCEMM-SZTE Pathogen Fungi Research Group, University of Szeged, Szeged, Hungary; ⁴HUN-REN-SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Szeged, Hungary; ⁵IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, University of Szeged, Szeged, Hungary; ⁶HUN-REN Biological Research Centre, Centre of Excellence of the European Union, Laboratory of Tumor Immunology and Pharmacology, Szeged, Hungary; ⁷SZTE TTIK Department of Applied and Environmental Chemistry, University of Szeged, Szeged, Hungary

Presenting author e-mail: doraadamecz@gmail.com

Background: Tumor-associated macrophages (TAMs) contribute to tumor progression by establishing an immunosuppressive microenvironment through tumor-driven reprogramming. Macrophages can polarize toward classically activated (M1) or alternatively activated (M2) phenotypes, which differ in cytokine profiles, surface markers, and biological functions. This study aimed to investigate whether the presence of gold (AuNP) or silver nanoparticles (AgNP) could disrupt biological interactions between cells, interfere with polarization, and modulate the TAM phenotype.

Methods: Murine 4T1 breast cancer cells were exposed to AuNPs or AgNPs and either co-cultured with murine J774 or bone marrow-derived macrophages (BMDMs), or used to generate nanoparticle-tumor-conditioned media (CM) applied to both macrophage types. We examined the expression of selected M1/M2 polarization markers at both RNA (qPCR) and protein levels (Proteome Profiler assay), as well as measuring matrix metalloproteinase (MMP) activity and cell migration.

Results: Co-culture altered M1 and M2 marker expression, increased MMP activity, and enhanced macrophage migration, reflecting TAM-like traits. Nanoparticle treatment of 4T1 cells reduced M2 markers, and while co-culture increased MMP activity and migration, AuNPs and AgNPs attenuated these effects. The CM induced results and trends comparable to those observed in the co-culture system.

Conclusions: The CM and the co-culture significantly altered the RNA and protein expression of both M1 and M2 markers and were associated with increased MMP-activity and elevated macrophage migratory capacity, features commonly associated with TAM-like behavior. Our data highlight the importance of CM media and co-culture systems in enhancing research on tumor-immune cell communication.

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P1.11**METABOLIC REPROGRAMMING OF INTESTINAL CD8 T CELLS DURING ACTIVATION**

Š. Konjar^{1,2,6,7,*}, C. Ferreira¹, F. Carvalho¹, U. Frising², P. Figueiredo-Campos¹, V. Morais¹, Q. Zhang², N. Haberman⁴, D. Strathdee⁵, P. Rožman⁷, B. Jabri³, M. Veldhoen¹

¹Instituto de Medicina Molecular, Av. Professor Egas Moniz, Lisbon, Portugal; ²The Babraham Institute, Babraham Research Campus, Cambridge, UK; ³Department of Medicine, University of Chicago, Chicago, USA; ⁴Department of Molecular Neuroscience, UCL, Queen Square, London, UK; ⁵Beatson Institute for Cancer Research, Garscube Estate, Glasgow, Scotland; ⁶Centre of Proteomics, Medical faculty Rijeka, Rijeka, Croatia; ⁷Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Presenting author e-mail: spelakonjar@yahoo.com

The metabolic functions of immune cells are tightly regulated and adapt to the demands of their local environment and pathogenic challenges. The intestinal epithelial barrier contains one of the largest T cell populations in the body, known as intestinal intraepithelial CD8 T lymphocytes (IELs). Located within an environment rich in gut microbiota and continually exposed to pathogens, dietary antigens, and toxins, IELs require specialized adaptations to mount effective immune responses. One such adaptation is their high expression of cytotoxic molecules, including granzymes, as well as activation markers such as CD69 and CD44, reflecting a heightened but controlled activation state.

We demonstrate that this regulated activation state is driven, at least in part, by alterations in mitochondrial membranes that constrain metabolic activity, proliferation, and effector functions in IELs. During inflammation, mitochondrial activity is dynamically remodeled to support IEL effector responses. These findings reveal a specialized mechanism of metabolic control unique to IELs and identify a novel role for mitochondria in maintaining these cells in a metabolically poised state while allowing rapid transition to full effector functionality.

Additionally, we show that IELs exhibit a distinct metabolic response to immune challenge, activating metabolically more rapidly than circulating CD8 T cells. In IELs, glycolysis and oxidative phosphorylation (OXPHOS) are tightly and mutually regulated, a metabolic feature not observed in circulating CD8 T cells. We further identify nutritional metabolites that support this metabolic adaptation and enhance IEL-mediated clearance of intestinal pathogens through increased interferon- γ secretion. Collectively, these metabolic properties enable IELs to execute tightly regulated immune responses within the fragile and complex environment of the intestinal epithelial barrier.

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P1.12**LL37 AS A POTENTIAL BIOMARKER TO DISTINGUISH BETWEEN ATOPIC ECZEMA AND PSORIASIS**

László Hagymási¹, Eszter Anna Janka¹, Viktória Nagy¹, Tünde Toka-Farkas¹, Krisztián Gáspár¹, Zsolt Dajnoki¹, Andrea Szegedi¹, Anikó Kapitány¹

¹Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; ²HUN-REN-DE Allergology Research group, Debrecen, Hungary

Presenting author e-mail: anikokapitany@gmail.com

Background: Classic plaque-type psoriasis (PSO) is easily distinguishable from typical eczema (atopic dermatitis, AD) based on clinical features. However, cases with overlapping phenotypes are still often challenging for specialists. While several research efforts aimed to find reliable biomarkers to distinguish these two inflammatory skin diseases, no single marker has yet been proven effective in making this distinction independently.

Patients and Methods: Our objective was to identify a single biomarker, that can discriminate the two diseases. Based on literature review, we selected 7 potential biomarkers (IL-17A, IL-17C, IL-25, IL-36RA, CCL27, INOS, LL37) for investigation by immunohistochemistry (IHC) in skin samples from patients with classical AD, PSO and healthy controls. Sensitivity and specificity of the most promising candidate were calculated in an extended cohort. In addition, the diagnostic accuracy of the candidate molecule was also assessed in patients with clinically ambiguous diagnoses.

Results: Among the 7 selected molecules only LL37 (cathelicidin) and INOS showed significantly different expression in AD and PSO, while the other molecules exhibited no significant differences between the two diseases. We further investigated the promising LL37 in an extended cohort, its sensitivity was 85.7% and specificity 73.5% (AUC=0.87; p<0.001). This model was applied on patients with uncertain diagnosis, giving 90.9% diagnostic accuracy with the diagnoses made by the pathologist on histological examination.

Conclusions: The LL37 molecule may be a promising biomarker when AD and PSO are difficult to distinguish from each other based on clinical symptoms alone. Its investigation, especially on the stratum corneum by the non-invasive tape-stripping sample collection could be a potentially useful diagnostic method.

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P1.13**EXPRESSION PATTERN OF IL-1 FAMILY CYTOKINES IN THE EPIDERMIS OF ATOPIC DERMATITIS PATIENTS**

Viktória Nagy¹, Zsolt Dajnoki^{1,2}, László Sajtos¹, István Rebenku³, Eszter Anna Janka^{1,2}, Tünde Toka-Farkas¹, Andrea Szegedi^{1,2}, Anikó Kapitány^{1,2}

¹Department of Dermatology, MTA Centre of Excellence, Faculty of Medicine, University of Debrecen, Debrecen, Hungary;

²HUN-REN-DE Allergology Research Group, Hungary; ³Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Presenting author e-mail: nagyviktoria4.22@gmail.com

Background: The pathogenesis of atopic dermatitis (AD) involves barrier failure and keratinocyte cytokine networks, driving inflammation. Therapies mainly target late, T-cell-mediated steps or single early mediators, with limited efficacy. We assumed that mapping epidermal cytokines could reveal relevant targets. The aim of this study is to comprehensively map and quantify keratinocyte-derived members of the IL-1 cytokine family in chronic atopic dermatitis (AD), with the goal of identifying novel therapeutic targets and elucidating potential compensatory mechanisms underlying the limited efficacy of IL-33-targeted therapies. Furthermore, we seek to identify cytokines involved in the earliest stages of AD initiation by analyzing clinically unaffected skin from AD patients. Finally, we aim to determine whether the observed cytokine expression patterns are specific to AD or are shared with other inflammatory skin diseases by comparison with psoriatic skin samples.

Methods: For the study, we used skin biopsy samples from lesional atopic dermatitis (ADL), non-lesional atopic dermatitis (ADNL), and lesional psoriasis (PSL), as well as healthy skin samples obtained from gland-poor (GP) areas as controls. Protein-level expression of pro-inflammatory (IL-1 α , IL-1 β , IL-18, IL-33, IL-36 $\alpha/\beta/\gamma$) and anti-inflammatory (IL-1Ra, IL-36Ra, IL-37, IL-38) members of the IL-1 cytokine family was analyzed by immunofluorescence staining.

Results: Among pro-inflammatory cytokines, IL-33 is significantly elevated in AD and represents an AD-specific signal, while IL-18 and IL-36 β are increased in both AD and psoriasis, indicating a general inflammatory marker rather than an AD-specific one. The anti-inflammatory cytokines IL-1Ra and IL-37 are similarly upregulated in AD lesional and psoriatic lesional skin, suggesting a compensatory anti-inflammatory response that is not specific to AD.

Conclusions: As IL-33 was the only IL-1 family member showing significant AD-specific upregulation at the protein level, no evidence was found for compensatory upregulation of other IL-1 family cytokines following IL-33 inhibition. The limited efficacy of IL-33-targeted therapy in AD may be explained by restricted monoclonal antibody penetration into the epidermis compared with mucosal tissues, as well as by the predominant role of IL-33 in disease initiation rather than in maintaining established, self-sustaining chronic inflammation.

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P1.14**MICRORNA-MEDIATED REGULATION OF ORAL SQUAMOUS CELL CARCINOMA RESPONSES DURING *CANDIDA* INFECTION**Renata Toth¹, Marton Horvath¹, Attila Gacser^{1,2,3,4}¹Department of Biotechnology and Microbiology, Institute of Biology, University of Szeged, Szeged, Hungary; ²HCEMM-SZTE Pathogen Fungi Research Group, University of Szeged, Szeged, Hungary;³HUN-REN-SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Szeged, Hungary;⁴University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Szeged, Hungary

Background: The contribution of *Candida* species to the development of oral squamous cell carcinoma (OSCC) has recently been recognized as a distinct pathological phenomenon. However, the molecular mechanisms underlying early *Candida*-tumor cell interactions remain poorly understood. In this study, we investigated post-transcriptional regulatory mechanisms mediated by microRNAs during the initial stages of fungal-tumor cell interactions.

Methods: Using a tumorous oral epithelial cell line, we compared host cellular responses (qPCR, ELISA) to *C. albicans*, a pathogenic species capable of forming invasive hyphae, and *C. parapsilosis*, a less virulent commensal species. Gene expression profiles, miR expression profiles and miR-target interactions were analyzed *in silico* to identify affected signaling pathways.

Results: Exposure to *C. albicans* induced pronounced inflammatory and oncogenic responses in tumor cells. This was characterized by the upregulation of miRs associated with tumor progression and the downregulation of tumor-suppressive miRs. miR-target analyses indicated that the dysregulated genes were mainly associated with inflammatory signaling pathways, particularly TNF α / NF κ B signaling, as well as tumor progression processes such as tumor growth and invasion, as defined by the PanCancer Progression Panel. In contrast, *C. parapsilosis* induced only modest tumor-associated responses, primarily involving hypoxia and metabolic regulation and did not markedly affect miRNA-mediated regulatory networks.

Conclusions: These results highlight a distinct role for *C. albicans* in OSCC progression through miRNA-mediated regulation of inflammatory and tumorigenic pathways, uncovering a previously unrecognized mechanism of fungal-driven oral carcinogenesis.

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P1.15**DEVELOPMENT OF NOVEL MONOCLONAL ANTIBODIES TARGETING VIRAL PROTEINS**

Maja Cokarić Brdovčak¹, Paola Kučan Brlić¹, Iva Vladić¹, Lucija Šakota¹, Karmela Miklič¹, Suzana Malić¹, Leonarda Mikša¹, Anne-Marie Patenaude², Anto Vrdoljak², Ilija Brizić¹, Tihana Lenac Roviš¹

¹Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; ²Vaxxinoa Zagreb d.o.o., Zagreb, Croatia

Presenting author e-mail: maja.cokaric@uniri.hr

Background: Viral diseases pose a major threat to global poultry production, causing significant economic losses and challenges in disease control. Among these, avian influenza virus (AIV), infectious bursal disease virus (IBDV), infectious laryngotracheitis virus (ILTV), and avian metapneumovirus (AMPV) are of particular concern due to their high pathogenicity and widespread prevalence. Viral surface glycoproteins play essential roles in host cell attachment, entry, and immune recognition, making them prime targets for virus-neutralizing antibodies and diagnostic assays. The development of monoclonal antibodies (mAbs) against these key glycoproteins can greatly enhance our ability to study viral pathogenesis, improve diagnostics, and support vaccine design. This study aimed to develop and characterize a novel panel of monoclonal antibodies targeting five major viral glycoproteins from important poultry pathogens: hemagglutinin (HA) from AIV, VP2 from IBDV, glycoproteins gI and gD from ILTV, and the fusion (F) protein from AMPV.

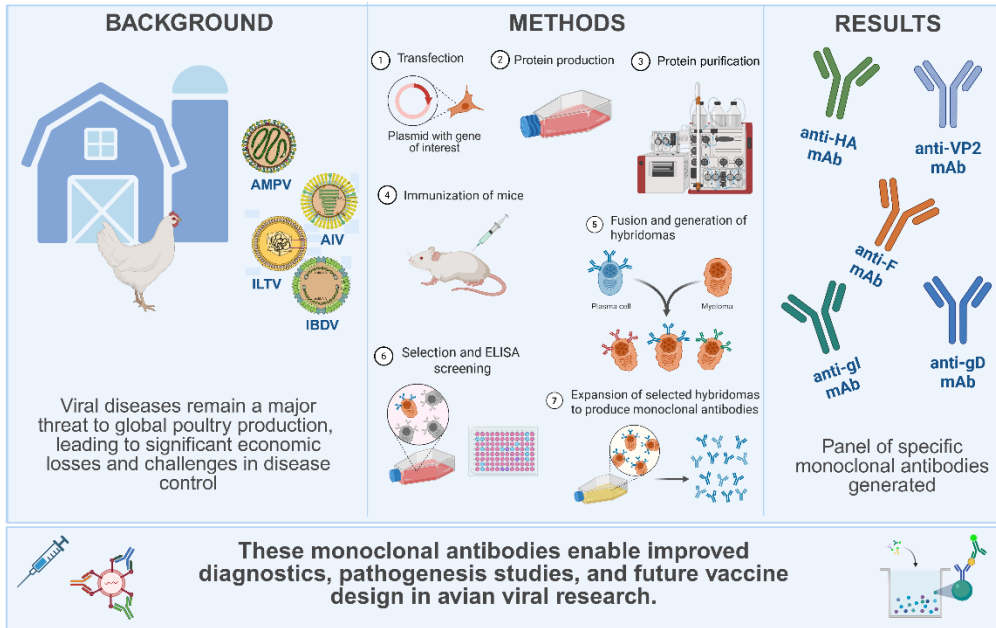
Methods: Recombinant viral glycoproteins were expressed in optimized expression systems and purified by affinity chromatography. Purified proteins were used to immunize BALB/c mice to induce antigen-specific immune responses. Splenocytes from immunized mice were then fused with myeloma cells using standard hybridoma technology to establish stable mAb-producing cell lines. Hybridoma supernatants were systematically screened by enzyme-linked immunosorbent assay (ELISA) and Western blotting to assess binding affinity and antigen specificity toward the corresponding recombinant glycoproteins.

Results: A diverse panel of monoclonal antibodies was successfully generated against all five viral glycoproteins. Screening assays confirmed strong and specific binding of individual mAbs to their respective target antigens.

Conclusions: This monoclonal antibody panel provides a valuable resource for poultry virology research. Owing to their high specificity and reliability they offer powerful tools for elucidating viral biology and may also contribute to the development of targeted antiviral therapeutics and diagnostic applications.

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Graphical abstract:



P1.16**QUANTITATIVE DETECTION OF *MICROCYSTIS AERUGINOSA* IN FRESH WATER USING SINGLE DOMAIN ANTIBODIES (VHH)**

Oginni Gbenga Folorunsho¹, Oloketuyi Sandra Folarin¹, Elisa Mazzega¹, Hanna Budasheva¹, Alfred Beran², Marina Cabrini², Dorota Korte¹, Mladen Franko¹, Ario de Marco¹

¹Laboratory for Environmental and Life Sciences, University of Nova Gorica, 5000 Nova Gorica, Slovenia; ²Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS), Trieste, Italy

Presenting author e-mail: Gbenga.oginni@yahoo.com

Background *Microcystis aeruginosa* accumulation in fresh water poses a significant threat to aquatic organisms and human health. The toxicity of Cyanobacterial metabolites has prompted the development of methods for their rapid and efficient detection, but what remains almost completely missing is the availability of reagents for quantifying *M. aeruginosa* cells in water to monitor fluctuations in its population (1).

Methods In this study, nanobodies against cell surface antigens of toxic Cyanobacteria *Microcystis aeruginosa* were recovered by whole-cell panning of a naïve phage display library. Six unique sequences were identified, and three were subcloned and purified as fusion immunoreagents, either with green fluorescent protein or Avi-Tag, for diagnostics. Their specificity and sensitivity were evaluated using immunofluorescence, fluorescent and colorimetric cell ELISA, and thermal lens spectrometry (TLS) (2).

Results: Binding specificity analysis by ELISA using purified nanobodies revealed no cross-reactivity with other unrelated algae cells tested. However, TLS was superior to other techniques, enabling a limit of detection of 1.2 cells/mL and a linear range of 0-10,000 cells.

Conclusions: The data obtained in this study show that *in vitro* selection of antibodies from a naïve phage library via biopanning enables the recovery of binders that selectively detect *M. aeruginosa*. Fluorescence ELISA showed linearity within a certain measurement range, while enzyme ELISA was better, showing linearity across the measured data range. Thermal lens spectrometry confirms the binding of *M. aeruginosa* to our nanobodies, with a low detection limit and a wide linear range.

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P1.17**PRECISE IMMUNOPHENOTYPING OF MOUSE REGULATORY T CELLS**

Katja Leben Zupet¹, Kristina Manzoni², Rebeka Prosen³, Anja Nagode⁴, Jon Žnidarčič⁴, Karen Butina Ogorelec⁵, Andrea Šarac⁶, Maša Čater⁶, Simon Horvat⁶, Uršula Prosenc Zmrzljak⁷, Ana Marentič⁷, Monika Savarin⁷, Anže Smole¹, Jelka Pohar¹

¹Immunology and Cellular Immunotherapy, Department of Gene Toxicology and Cancer Biology, National Institute of Biology, Slovenia; ²Faculty of Biotechnology and Drug Development, University of Rijeka, Rijeka, Croatia; ³Faculty of Pharmacy, University of Ljubljana, Slovenia; ⁴Biotechnical Faculty, University of Ljubljana, Slovenia; ⁵Present address: InnoRenew CoE, Andrej Marušič Institute, University of Primorska, Koper, Slovenia; ⁶Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; ⁷Molecular Biology Department, BIA Separations CRO, Labena d.o.o, Ljubljana, Slovenia

Presenting author e-mail: katja.leben.zupet@nib.si

Background: Regulatory T cells (Tregs) are a rare subset of CD4⁺ T cells characterized by a CD4⁺CD25⁺FOXP3⁺ phenotype. They are essential for maintaining homeostasis and self-tolerance, as they suppress immune responses to self-antigens and foreign antigens such as those from pathogens, commensal bacteria, foetal antigens, or food-derived antigens. Deregulation of self-tolerance leads to overactivation of the immune system and ultimately to the development of autoimmune diseases. Cellular immunotherapy with modified Tregs represents a step towards an alternative therapeutic option for patients with autoimmune diseases, with the potential to restore immune homeostasis and induce regeneration of damaged tissue. In 2025, Mary E. Brunkow, Fred Ramsdell, and Shimon Sakaguchi, whose discoveries elucidated the biology of Treg cells and established the field, were recognised with the Nobel Prize in Physiology or Medicine.

Our work addresses the need for reliable immunophenotype identification of Tregs and determines the conditions required to prepare highly enriched, viable Tregs with a consistent phenotype *ex vivo*.

Methods: Tregs were isolated from C57BL/6JOLA^{Hsd} mice using a CD4⁺CD25⁺ enrichment kit, then expanded and profiled at five time points. Single-cell RNA sequencing was used to identify surface markers associated with *ex vivo* expanded FOXP3⁺ Treg cells. Multiparametric spectral flow cytometry was used to monitor the proportion of FOXP3⁺ Tregs and to validate new marker candidates.

Results: We established isolation and expansion protocols for mouse Tregs and determined the conditions yielding the highest fraction of FOXP3⁺ cells and identified candidate surface markers associated with *ex vivo* expanded FOXP3⁺ Treg cells through differential expression analysis of *Foxp3*⁺ versus *Foxp3*⁻ clusters. Expression of these markers was validated by spectral flow cytometry and incorporated into the development of a spectral flow panel, expanding a classical T-cell/Treg panel from 12 to 17 markers. In parallel, we developed a multiparametric Treg–Teff co-culture suppression assay that captures Teff proliferation and phenotype, including the expression of activation markers on Treg and Teff cells within the co-culture.

Conclusions: The development of improved tools for Treg cell analysis confirmed that several of the newly included markers are preferentially expressed on Treg cells, although not consistently at all stages of the expansion process. We identified the specific time points when individual newly introduced markers show preferential expression on FOXP3⁺ Treg cells, with markedly lower expression on FOXP3⁻ cells. This temporal resolution enhances the utility of the expanded panel for distinguishing Treg-associated phenotypes during *ex vivo* manipulation and suppression assays. Our pipeline forms the foundation for generating and analysing potent engineered Tregs for cellular immunotherapy.

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P1.18**DEVELOPMENT OF APPROACHES FOR ISOLATION, ACTIVATION AND EXPANSION OF GAMMA DELTA T CELLS**

Urška Rupnik¹, Pia Bertoncej¹, Katja L. Zupet¹, Valerija Kovač², Vladka Čurin Šerbec², Jelka Pohar¹, Anže Smole¹

¹Immunology and Cellular Immunotherapy (ICI) Group, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia; ²Centre for Immunology and Development, Slovenian Institute for Transfusion Medicine, Ljubljana, Slovenia

Presenting author e-mail: urska.rupnik@nib.si

Background: Gamma delta ($\gamma\delta$) T cells make up 5–10% of human blood lymphocytes and are important in inflammation, defence against viruses and bacteria, and antitumour immunity. Because of their unique properties they present an alternative for CAR-T immunotherapy to currently prevailing $\alpha\beta$ T cells. $\gamma\delta$ T cells recognize diverse antigens via TCR and other receptors, often independently of MHC, potentially offering broader accessibility and improved safety. However, *ex vivo* isolation, activation, expansion and modification of $\gamma\delta$ T cells remain poorly studied, limiting their therapeutic use.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy adult donors. Part of the PBMCs underwent further isolation using magnetic-activated cell sorting. Cells were then *ex vivo* expanded using various activation approaches and culturing conditions. Cultures pre- and post-expansion were analyzed with flow cytometry and ELISA assays were conducted on expanded cultures.

Results: $\gamma\delta$ T cells accounted for $3,5 \pm 1,7$ % of PBMCs with the dominant subtype being V γ 9V δ 2. Further isolation increased the $\gamma\delta$ T cell proportion to $75,4 \pm 7,9$ %. Adding various activators to the relevant cell cultures induced activation and robust expansion of $\gamma\delta$ T cells. Flow cytometry confirmed activation-associated phenotypic changes, while ELISA assays demonstrated the ability of the expanded cells to secrete effector molecules.

Conclusions: The developed approaches led to the successful expansion of $\gamma\delta$ T cells. These efforts provide a solid foundation for the production of $\gamma\delta$ T cell-based products with potential clinical applications.

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P1.19**OPTIMIZATION OF CRISPR/CAS9-MEDIATED TCR KNOCK-OUT IN PRIMARY MOUSE T CELLS**

Andrea Šarac^{1,2}, Klemen Kunej^{1,3,†}, Kristina Manzoni^{1,†}, Karen Butina Ogorelec^{1,4}, Katja Leben Zupet¹, Simon Horvat^{2,a}, Jelka Pohar^{1,b,*}, Anže Smole^{1,b}

¹Immunology and Cellular Immunotherapy (ICI) Group, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia; ²Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; ³Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia; ⁴Present address: InnoRenew CoE, Andrej Marušič Institute, University of Primorska, Koper, Slovenia; [†]These authors contributed equally to this work; ^aSenior author; ^bThese authors contributed equally to this work and share last authorship
Presenting author e-mail: Andrea.Sarac@bf.uni-lj.si

Background: CRISPR-based genome editing has revolutionized biomedical research and accelerated therapeutic development by enabling precise and efficient genetic modifications. Ribonucleoprotein (RNP)-based gene knockout provides a non-viral strategy for generating loss-of-function mutations and represents a powerful tool for further immunotherapy and adoptive cell therapy development. Here, we present an optimized CRISPR/Cas9 RNP-based genome editing pipeline for primary mouse CD4⁺, CD8⁺, and regulatory T cells (Tregs). As a benchmarking target, we disrupted the T cell receptor alpha constant (Trac) locus, a key genomic site for engineering universal T cells and synthetic immune circuits. Since the TCR α constant region is encoded by a single TRAC gene, its disruption provides an efficient strategy to abolish TCR surface expression.

Methods: CD4⁺, CD8⁺ T cells and Treg cells were isolated from single-cell suspensions prepared from the spleens of C57BL/6JOLA^{Hsd} mice, followed by activation, RNP precomplexing and nucleofection. To assess the functionality of the edited T cells, they were re-stimulated on the fourth day post-electroporation. Their activation potential was evaluated by measuring the upregulation of activation markers CD69 and CTLA-4 following polyclonal stimulation with either PMA and ionomycin or anti-CD3/CD28 beads. Flow cytometry was used to analyze both the KO efficiency and activation potential of edited cells.

Results: Editing efficiency was strongly influenced by sgRNA design, Cas9:sgRNA molar ratio, RNP dose, and cell number. Optimization of editing parameters yielded up to 95% TCR knockout in CD8⁺ T cells and 80% in CD4⁺ T cells. Editing posed minimal impact on viability, and edited cells maintained their activation capacity. Furthermore, we achieved efficient Trac editing in primary mouse Tregs, reaching up to 85% knockout. While Tregs showed slightly reduced viability after nucleofection, editing efficiency remained high.

Conclusions Together, these findings establish an optimized non-viral genome-editing platform for primary mouse T cells with potential to advance mechanistic studies in syngeneic models and support the development of next-generation cellular immunotherapies.

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P1.20**VITAMIN B COMPLEX SUPPRESSES LPS-INDUCED NEUROINFLAMMATION IN ACTIVATED MICROGLIA: *IN VITRO* AND *IN SILICO* INSIGHTS**

Marija Rakić¹, Tanja Lunić¹, Marina Bekić², Sergej Tomić², Katarina Mitić¹, Bojan Božić^{1*}, Biljana Božić Nedeljković^{1,*}

¹University of Belgrade, Faculty of Biology, 11000 Belgrade, Serbia; ²University of Belgrade, Institute for the Application of Nuclear Energy, INEP, 11080 Belgrade, Serbia

Presenting author e-mail: marija.mandic@bio.bg.ac.rs

Background: Vitamin B deficiency has been associated with cognitive impairment and neurodegenerative disorders, where neuroinflammation represents a key pathogenic component. Microglia, as resident immune cells of the central nervous system, are major drivers of inflammatory responses following immune stimulation. Although individual B vitamins have been reported to exert neuroprotective effects, the combined anti-inflammatory potential and underlying molecular mechanisms of the vitamin B complex in microglial activation remain poorly understood.

Methods: The anti-inflammatory effects of a vitamin B complex (VBC; B1, B2, B3, B5, B6, and B12) were investigated in LPS-stimulated BV2 microglial cells. Microglial inflammatory output was assessed through secreted mediator profiling and phenotypic evaluation. To explore microglia–neuron crosstalk, SH-SY5Y neuronal metabolic activity was measured following exposure to conditioned media from VBC-treated microglia. In parallel, molecular docking was performed to evaluate binding of individual B vitamins to key proteins in the TLR4 signaling cascade and inducible nitric oxide synthase (iNOS).

Results: VBC treatment reduced the secretion of inflammatory mediators in LPS-activated BV2 microglia and attenuated the neurotoxic effects of microglia-derived factors on SH-SY5Y cells. In addition, VBC promoted a shift toward an anti-inflammatory (M2-like) microglial phenotype. Docking analysis indicated that several B vitamins may interact with multiple targets within the LPS/TLR4 axis, including LBP, CD14, TLR4/MD2, as well as iNOS, suggesting a multi-target mechanism of action.

Conclusion: Our findings support a synergistic anti-inflammatory potential of the vitamin B complex in suppressing microglial activation and limiting neuron-damaging effects. These results highlight vitamin B supplementation as a promising adjunct strategy for reducing neuroinflammation and potentially slowing neurodegenerative processes.

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P2.01**THE ROLE OF NINJURINS IN PLASMA MEMBRANE RUPTURE**

Borna Bacinger, Sara Orehek, Taja Železnik Ramuta, Roman Jerala, Iva Hafner Bratkovič

Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia

Presenting author e-mail: Borna.Bacinger@ki.si

The regulation of cell death plays a vital role in ensuring tissue homeostasis, influencing the progression of tumours and modulating inflammatory responses. Cells can undergo different cell death pathways, yet the process of plasma membrane rupture has long been considered a passive event. Recently, ninjurin-1 (NINJ1) was identified as a key molecule mediating the plasma membrane rupture after lytic types of cell death (1). The ninjurin family consists of two members, NINJ1 and NINJ2, which were initially characterized as adhesion molecules (2). NINJ1 and NINJ2 share high sequence similarity and were both shown to make fibrils in the plasma membrane, yet only NINJ1 potently breaks the membrane apart, leading to the release of large damage-associated molecular patterns and contributing to excessive inflammation (3).

The main aim of this study is to elucidate the role of NINJ2 in the plasma membrane rupture. To achieve this, we made a panel of NINJ1/NINJ2 chimeras identifying segments crucial for mediating PMR and tested the variants in HEK293 overexpression systems as well as in biologically relevant cell death induction systems. In agreement with recently published studies, we identified the crucial role of NINJ1 N-terminus. Furthermore, we revealed an unexpected regulatory role of NINJ2 that can suppress NINJ1 mediated release of large cellular cargo. These results highlight NINJ1 as a key mediator of immunogenic cell death and suggest that modulation of the NINJ1–NINJ2 axis may represent a novel therapeutic strategy in inflammatory diseases.

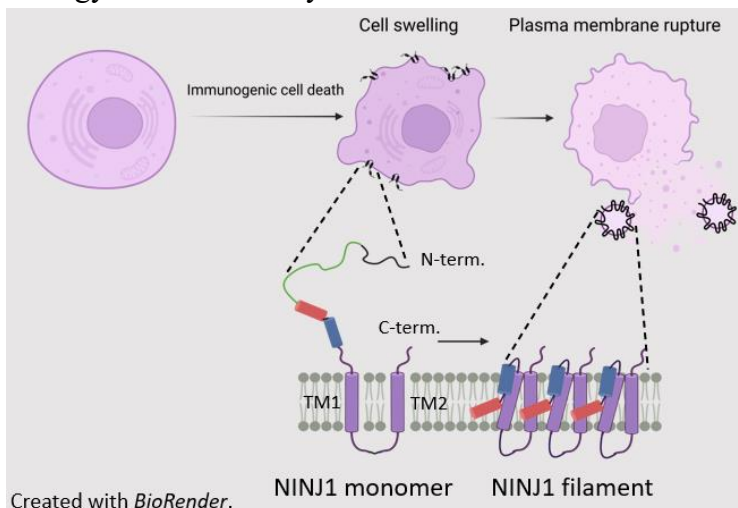


Figure 1. Mechanism of plasma membrane rupture

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P2.02**DE NOVO DESIGNED MINIBINDER PROTEINS TARGETING NINJ1**

Monika Gregorič Tasić^{1,2}, Teja Koblar³, Tadej Satler¹, Roman Jerala^{1,4}, Iva Hafner Bratkovič^{1,5,6}

¹Department of Synthetic Biology and Immunology, National Institute of Chemistry, 1000 Ljubljana, Slovenia;

²Interdisciplinary Doctoral Study of Biomedicine, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia;

³Interdisciplinary Master's Study of Biotechnology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia;

⁴Centre for the Technologies of Gene and Cell Therapy, National Institute of Chemistry, 1000 Ljubljana, Slovenia; ⁵EN-FIST Centre of Excellence, 1000 Ljubljana, Slovenia; ⁶Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia

Presenting author e-mail: monika.gregoric.tasic@ki.si

Ninjurin 1 (NINJ1) is a transmembrane protein that mediates plasma membrane rupture [1] during secondary necrosis after apoptosis, pyroptosis, and ferroptosis, and thus promotes inflammation in a variety of inflammatory diseases, making it a promising therapeutic target. However, the precise mechanism of NINJ1-mediated plasma membrane rupture is not understood and consequently, few inhibitors of NINJ1 have been identified to date. The aim of our work was to develop *de novo* designed small minibinder proteins against NINJ1, to modify NINJ1-mediated plasma membrane rupture. Previous studies demonstrated the potential of *de novo* designed binders that bind to their targets with similar affinity as antibodies; however, they are smaller, more stable, less immunogenic, and can be easily produced in large quantities in bacteria [2,3,4].

Minibinders were engineered to selectively bind to different regions of NINJ1 using *in silico* design tools [5,6,7,8]. The designed minibinder proteins and their protein targets (human and murine Ninjurin 1 and/or Ninjurin 2) were produced in a bacterial expression system, purified, and analyzed using a binding assay to identify the most promising candidates for further investigation. We identified several candidates that bind specifically to NINJ1, have appropriate secondary protein structure and stability. Those minibinders will be further evaluated in cell culture models of plasma membrane rupture during different forms of cell death by measuring the release of lactate dehydrogenase and propidium iodide uptake.

We expect that our study will not only provide new insights into the mechanism of NINJ1-mediated plasma membrane rupture, but also evaluate the potential of *de novo* designed minibinders targeting NINJ1 for suppressing inflammation.

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P2.03**PERSISTENT EOSINOPHILIA IN SEVERE ATOPIC DERMATITIS: DIFFERENTIAL DIAGNOSTIC EVALUATION AND IDENTIFICATION OF SUBTHERAPEUTIC CYCLOSPORINE**

Nina Naprudnik¹, Tina Vesel Tajnšek², Tadej Avčín², Mateja Starbek Zorko^{1,3}

¹Faculty of Medicine, University of Ljubljana, Slovenia; ²Clinical Department of Pediatric Allergology, Clinical Immunology, and Rheumatology, University Children's hospital, Ljubljana, Slovenia; ³Dermatovenerology Clinic, Ljubljana University Medical Centre

Presenting author e-mail: nina.naprudnik@gmail.com

Background: Blood eosinophilia is defined as an absolute eosinophil count of more than 0.5×10^9 /L and may accompany a wide range of clinical conditions, making differential diagnosis complex. In pediatric clinics, elevated eosinophils are most often associated with parasitic infections. However, a similar laboratory profile is often observed in patients with allergic asthma, atopic dermatitis (AD), and chronic rhinosinusitis with nasal polyposis. Neoplastic conditions such as myeloid neoplasms, clonal myeloid leukemia, and rare forms of myelodysplastic syndromes are associated with eosinophils. Recurrent hypereosinophilia can cause tissue eosinophilic infiltration, leading to clinically significant organ damage, which is called hypereosinophilic syndrome [1].

Methods: The abstract presents a case report of an 8-year-old girl with severe AD on dual therapy with dupilumab (300 mg every 4 weeks) and cyclosporine A (0.6 ml every 12 hours). Despite the therapy, she still has frequent exacerbations. She has allergic asthma, allergy to eggs, peanuts and inhalant allergens. Her blood tests consistently show moderate eosinophilia (absolute eosinophil count between 2.2×10^9 /L and 2.7×10^9 /L, reference value $0.02 - 0.70 \times 10^9$ /L). Due to prolonged elevated eosinophil levels, it was necessary to investigate differential diagnostic causes.

Results: In line with current recommendations, a screening test for HIV was performed and was non-reactive. Serological tests for Toxocara spp. IgG, Strongyloides IgG and Trichinella spiralis IgG were negative. Three consecutive stool examinations showed no protozoan cysts, helminth eggs, Cryptosporidium spp., Giardia duodenalis, or Entamoeba histolytica. Abdominal ultrasound did not show focal changes or changes in the organ parenchyma. X-ray of the lungs and heart was unremarkable. We measured the level of cyclosporine A (47.7 µg/L), which was lower than the desired value (80–150 µg/L).

Conclusions: With the above examinations, we excluded the most common reasons for eosinophilia. The dose of cyclosporine A was increased to 0.9 ml every 12 hours. Laboratory evaluation 13 days later showed a rise in cyclosporine A level (94.3 µg/L) and decrease in eosinophils (1.7×10^9 /L). AD was in remission. At future check-ups, we will check clinical response, the eosinophil values and the cyclosporine A level. We hypothesize that persistent eosinophilia was related to subtherapeutic cyclosporine A levels.

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P2.04**NUCLEIC ACID-DRIVEN EXPRESSION AND ALTERNATIVE SPLICING OF Z-DNA BINDING PROTEIN 1 IN HUMAN KERATINOCYTES**

Judit Danis^{1,2}, Sára Gógl, Evelyn Kelemen³, Kornélia Szabó^{2,3,4}, Éva Ádám^{5,6}, Márta Széll^{5,6}

¹Department of Immunology, University of Szeged, Szeged, Hungary; ²HUN-REN-SZTE Dermatological Research Group, University of Szeged, Szeged, Hungary; ³Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary; ⁴HCEMM Skin Research Group, University of Szeged, Szeged, Hungary; ⁵HUN-REN-SZTE Functional Clinical Genetics Research Group, University of Szeged, Szeged, Hungary; ⁶Department of Medical Genetics, University of Szeged, Szeged, Hungary

Presenting author e-mail: danis.judit@med.u-szeged.hu

Background: Keratinocytes actively contribute to cutaneous immunity by recognizing danger signals such as nucleic acids. Z-DNA binding protein 1 (ZBP1/DAI) is a cytosolic sensor of Z-DNA structures that triggers inflammatory responses. While its role in immune cells is well established, its regulation in human keratinocytes is less understood. Therefore we aimed to study the induction and splicing of ZBP1 in normal human epidermal keratinocytes (NHEK) following exposure to nucleic acids.

Methods: Synthetic nucleic acid analogues polyinosinic:polycytidylic acid [poly(I:C)] or poly(deoxyadenylic-deoxythymidylic) acid [poly(dA:dT)] and inflammatory cytokines and bacterial ligands were used to induce inflammation in NHEKs. Expression of ZBP1 and its splice variants was analysed by qPCR, conventional PCR and Western blot. Pathway inhibitors and luciferase-reporter systems were used to study the signalling cascades up- and downstream of ZBP1.

Results: While ZBP1 expression is negligible in resting NHEKs, transfection with nucleic acid mimics and Type I and II interferons robustly induces its transcription, in contrast to the inert response to other inflammatory cytokines or bacterial ligands. We also identified all four major NCBI transcript variants (NM_030776, NM_001160418, NM_001160419, NM_001323966), with corresponding protein isoforms. The different isoforms had distinct effects on downstream pathways. Blockade of cytokine secretion by Brefeldin A reduced ZBP1 induction, indicating that nucleic acid sensing triggers an autocrine/paracrine cytokine loop, which activates ZBP1 expression.

Conclusions: Although ZBP1 is not constitutively expressed in keratinocytes, it is induced via autocrine/paracrine cytokine loops after nucleic acid sensing, acting as an inducible sentinel that links environmental nucleic acids to skin inflammation. The appearance of distinct isoforms also alters ZBP1 functions, thus, splicing can serve as a regulatory mechanism in inflammation and skin diseases.

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P2.05**PRIMING THE ORAL EPITHELIUM: *CANDIDA PARAPSILOSIS* PRE-EXPOSURE ENHANCES CYTOKINE RESPONSES AND ALTERS *CANDIDA ALBICANS* INTERACTIONS**Máté Lajos Csikós¹, Zsolt Tasi¹, Kitti Vecsernyés-Nagy¹, Attila Gácser^{2,3,4}

¹University of Szeged, Department of Biotechnology and Microbiology, Szeged, Hungary; ²HCEMMSZTE Pathogen Fungi Research Group, University of Szeged, Szeged, Hungary; ³HUNRENSZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Szeged, Hungary; ⁴University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Szeged, Hungary
Presenting author e-mail: mate1260@gmail.com

Trained immunity refers to the ability of innate immune cells and certain non-immune cells, including epithelial cells, to develop memory-like responses through epigenetic modifications and metabolic reprogramming. While trained immunity has been extensively studied in myeloid cells, its role in oral epithelial biology and oral squamous cell carcinoma (OSCC) remains poorly understood. Given the strong association between *Candida* colonization, mucosal inflammation, and oral cancer progression, elucidating how fungal exposure modulates epithelial immune responses is of significant clinical relevance.

In this study, we investigated whether the presence of *Candida parapsilosis* can induce trained immunity-like effects in healthy oral epithelial cells and OSCC cell lines. Cells were pre-treated for 24 hours with *C. parapsilosis* strains GA1 or CLIB214. Following a five-day resting period, during which no additional stimuli were applied, the epithelial cells were re-challenged with *Candida albicans* strains SC5314 or WO-1. We assessed the impact of fungal pre-exposure on epithelial cell damage, cytokine and chemokine production, fungal adhesion dynamics, and transcriptional changes in the host cells.

Pre-treatment with *C. parapsilosis* markedly reduced *C. albicans*-induced cytotoxicity in both healthy and OSCC epithelial cells. Additionally, primed healthy epithelial cells produced significantly lower levels of the pro-inflammatory cytokines while primed OSCC cells produced higher levels of IL-6 and IL-8 upon secondary fungal challenge. Notably, prior exposure enhanced the adhesion of *C. albicans* to epithelial cells, suggesting alterations in surface receptor expression or epithelial barrier properties. Gene expression analyses revealed that the pre-treatments downregulated genes, which are involved in tumour progression and metastasis.

These findings indicate that *C. parapsilosis* can induce trained immunity-like responses in oral epithelial cells and that this phenomenon may influence host-pathogen interactions in the oral mucosa under healthy and tumour conditions. Understanding these mechanisms may provide new insights into fungal-epithelial crosstalk in health and disease, with potential implications for oral cancer biology and mucosal immunotherapy strategies.

P2.06**AUTOANTIBODY MEDIATED NEUROLOGICAL DISEASES: LABORATORY DIAGNOSTICS**

Timea Berki, Diána Simon, Zoltán Kellermayer, Peter Balogh

Department of Immunology and Biotechnology, University of Pécs, Medical School, Pécs, Hungary

Presenting author e-mail: berki.timea@pte.hu

Background: Except myasthenia gravis caused by autoantibodies against the acetylcholine receptor (AChR and Musk) the autoimmune background of many neurological diseases were not known. Over the past 10 years, thanks to advances in molecular immunology, it has been confirmed that a number of previously unclassified neurological or psychiatric syndromes are caused by an autoimmune process. In the case of paraneoplastic limbic encephalitis, an immune reaction is triggered against intracellular antigens (anti-Hu/ANNA1, anti-Ri/ANNA2, anti-CV2/CRMP5, and anti-Ma2/Ta) caused by lung, ovarian, or hereditary tumors and characterized by a poor prognosis. Autoimmune encephalitis with a varied clinical picture, is often associated with the presence of autoantibodies against neuronal cell surface receptors (NMDAR, GABABR, AMPAR) or synaptic proteins (LGI1, CASPR2), which respond well to immunosuppressive treatment.

Method: After describing the diseases, our laboratory began laboratory diagnostics using indirect immunofluorescence tests performed on HEK293 cell lines transfected with individual receptor genes. Serum or liquor samples were tested on a BIOCHIP consisting of 6 cell lines transfected with different receptor genes. For detection of onconeural autoantibodies we used an immunoblot containing 12 purified brain proteins.

Results. Our laboratory is center for autoantibody detection against neuronal antigens in Hungary. The evaluation of the results can highlight the incidence of the diseases in Hungarian population. From the serum/liquor samples of 2290 patients, only 80 proved to be positive for IgG autoantibodies against one of the receptor proteins (=3.4%). The distribution of receptor-specific autoantibodies was the following: NMDAR(29;36%) >LGI1(20;25%) >CASPR(16;20%) >GABA_BR(12;15%) >AMPA(2;3%) >DPPX(1;1%), which correlates with the literature. The average age was 56 years as expected.

Conclusion: A clinical laboratory study on autoantibody testing of AIE in the Hungarian population was presented. Some results show coherence with the literature, but some content deviated significantly from selected observations. Some of the anti-NMDAR encephalitis-specific properties were also outside the well-established range and CASPR2 occurred at a significantly greater level than international average. Detection of autoantibodies helps in the early recognition of the disease and in establishing a diagnosis and therapy.

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P2.07**ARE IMMUNE DYSREGULATION–ASSOCIATED ALTERATIONS IN PATHOLOGICAL AND NATURAL AUTOANTIBODY PROFILES ASSOCIATED WITH VACCINE-INDUCED ANTI–SARS-COV-2 HUMORAL RESPONSES?**Péter Németh¹, Katalin Böröcz¹, Szinger Dávid¹, Berki Timea¹¹Department of Immunology and Biotechnology, Clinical Centre, Medical School, University of Pécs, Pécs, Hungary

Presenting author e-mail: nemeth.peter@pte.hu

Background: Recent studies indicate that anti–SARS-CoV-2 antibody dynamics might be altered in autoimmune settings [3-5]. In rheumatoid arthritis (RA), systemic immune dysregulation and high rheumatoid factor (RF) burden may promote immune-complex formation with vaccine-induced IgG, leading to Fcγ receptor–mediated Mφ activation, altered cytokine signaling, and reduced protective IgG availability. Disrupted immune homeostasis may further affect natural autoantibody (nAAb) production [4,5].

Aim: To serologically quantify nAAb associated immune regulation and IgG responses post–SARS-CoV-2 vaccination, using a surrogate model of an anti-CCP IgG–seropositive, RF-rich cohort.

Methods: We performed a retrospective, anonymized cohort analysis within the anti-CCP–positive cluster. Index samples for anti–citrate synthase (a-CS) nAAb (IgG, IgM) assessment included strongly positive a-CCP IgG non-overlap samples (n= 20), overlap samples positive >1 pathological autoantibody (n=8), and negative controls (n_{NC} =37). Post-vaccination anti–SARS-CoV-2 IgG responses to Wuhan-Hu-1 and Omicron BA.1 were measured by commercial ELISAs (n_{a-CCP+} =49, n_{NC} =70).

Results: Anti-CCP IgG+ samples exhibited significantly reduced anti-SARS-CoV-2 IgG titers compared against NCs ($p_{\text{holm}} < 0.004$). This difference became even more pronounced at higher seropositivity thresholds. In the ungrouped dataset, RF IgM, IgG, and IgA levels showed weak to moderate negative correlations with anti-SARS-CoV-2 IgG titers. Anti-CCP IgG + RF IgG/A/M + samples showed lower antiviral IgG levels relative to NCs. a-CCP IgG+ nAAb patterns showed significantly lower a-CS IgM ($p_{\text{holm}} = 0.005$, $p_{\text{holm}} < 0.001$) and higher a-CS IgG ($p_{\text{holm}} < 0.001$) titers.

Conclusion: We link diminished anti–SARS-CoV-2 IgG responses to RF-enriched autoimmune serological profiles in anti-CCP IgG–positive sera. In parallel, we demonstrate reduced protective IgM natural autoantibody responses and compensatory or pathology-associated increases in IgG isotype nAAbs in RA suggestive samples. Although RFs may contribute to altered antiviral IgG homeostasis, our findings point to broader B-cell dysfunction and immune-regulatory imbalance.

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P2.08**CASE REPORT: ANTIPARASITIC TREATMENT CAN CURE AN ILL CHILD WITH EOSINOPHILIA DESPITE UNCONFIRMED PARASITOSIS**

Tina Vesel Tajnšek¹, Simona Ivančan², Saša Šetina Šmid³, Nuša Cesar⁴, Katarina Vincek⁵, Tadej Avčin¹

¹Clinical Department of Pediatric Allergology, Clinical Immunology, and Rheumatology, University Children's hospital, Ljubljana, Slovenia; ²Clinical Department of Pediatric Hematology and Oncology, University Children's hospital, Ljubljana, Slovenia; ³Department of Pulmonary Diseases, University Children's hospital, Ljubljana, Slovenia; ⁴Clinical Department of Pediatric Gastroenterology, Hepatology and Nutrition, University Children's hospital, Ljubljana, Slovenia; ⁵Clinic for Infectious Diseases and Febrile Conditions, University Clinical Center, Ljubljana, Slovenia

Presenting author e-mail: tina.vesel@kclj.si

A two-year-old boy became ill with abdominal pain, diarrhea, vomiting, a transient rash, irritability, and reduced appetite. Laboratory tests showed leukocytosis, thrombocytosis, severe eosinophilia, elevated LDH, and increased total IgE. Bilateral peribronchovascular infiltrates were observed. The spleen was slightly enlarged, and the small-bowel loops had thickened mucosa. Microbiological tests, including those for parasitic infections, were negative. Low-positive atypical cANCA was present. A possible genetic cause of eosinophilia was not confirmed. After treatment with mebendazole, he gradually recovered over the following two months.

Conclusions: Antiparasitic treatment may be the solution for a sick child with eosinophilia despite previously negative microbiological tests for parasitic infection.

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P2.09**ALTERED NATURAL AUTOANTIBODY SIGNATURES ASSOCIATED WITH PATHOLOGICAL AUTOANTIBODY CO-POSITIVITY IN SAD-SUGGESTIVE PROFILES**

Katalin Böröcz¹, Szinger Dávid¹, Alain le Roux, Szabina Erdő-Bonyár¹, Diána Simon¹, Péter Németh¹, Berki Tímea¹

¹Department of Immunology and Biotechnology, Clinical Centre, Medical School, University of Pécs, Pécs, Hungary
Presenting author e-mail: borocz.katalin@pte.hu

Background: In systemic autoimmune diseases (SADs), impaired immune homeostasis reshapes both natural (nAAb) and pathological (pAAb) autoantibody titers. Inflammatory cytokine -driven modulation of B1b and marginal zone B cells, the primary producers of nAAbs, may lead to altered nAAb secretion, differentiation, and isotype switching [1-5].

Aim: Leveraging our Autoimmune Diagnostics Laboratory (Southern Transdanubia Regional Centre, accredited MSZEN ISO 15189:2023) database, we examined how perturbed homeostatic regulation, particularly in overlap SADs, may influence nAAb signatures.

Study design: We conducted an 11-year retrospective study (January 1, 2014–December 31, 2024) to analyse patterns of coexisting disease-associated pAAb sero-positivities and to characterize accompanying nAAb networks. Longitudinal evaluation of pAAb was based on anonymized, SAD-suggestive test results (anti-dsDNA IgG n=27,118; ANA screen n=41,764; anti-SSA IgG n=3,279; anti-CCP IgG n=26,437; aCL/anti-β2GPI screen n=30,362; aCL IgG/IgM n=3,475; anti-β2GPI IgG/IgM n=4,475; parallel requests: anti-dsDNA IgG + aCL/anti-β2GPI IgG n=3,772; anti-dsDNA + anti-SSA n=2,980; anti-dsDNA + anti-CCP IgG n=16,626). Samples for nAAb profiling (ELISA, IgM/IgG anti-citrate synthase, HSP60/70, n= 126 SAD samples + 37 negative controls) were selected from our Diagnostic Serum Bank.

Results: Across parallel pAAb test panels, anti-dsDNA IgG + aCL/anti-β2GPI IgG co-positivity was observed in 220/3,772 patients (overall 5.83%; annual incidence 7.5-5.8%), anti-dsDNA + anti-SSA in 281/2,980 patients (overall 9.42%; annual incidence 11.1-5.9%), and anti-dsDNA + anti-CCP IgG in 149/16,626 patients (overall 0.89%; annual incidence 1.38-0.63%). Protective IgM nAAb levels (a-HSP60/70, a-CS) were consistently reduced across pAAb positive groups (p<0.05), whereas anti-CS IgG nAAbs were highest in pAAb overlap clusters (p<0.01).

Conclusion: Our analysis indicates that pAAb co-positivity is a relatively frequent diagnostic finding, reflecting multilevel impairment of immune tolerance. Pronounced reductions in protective nAAbs in overlap-positive clusters suggest inadequate compensatory capacity, while increased IgG nAAb patterns may reflect the severity of immune dysregulation.

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P2.10**DYNAMIC MACROPHAGE IMMUNE RESPONSES DISTINGUISH INFLAMMATORY FROM IMMUNE-EVASIVE STRAINS OF GROUP B *STREPTOCOCCUS***

Larisa Janžič¹, Sara Petrin², Lucija Sršen¹, Alojz Ihan¹, Andreja Nataša Kopitar¹

¹Laboratory for Cellular Immunology, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia; ²Institute of Pathology, Faculty of Medicine, University of Ljubljana, Slovenia

Presenting author e-mail: larisa.janzic@mf.uni-lj.si

Background: Group B *Streptococcus* (GBS) remains a leading cause of neonatal sepsis and meningitis despite preventive strategies. Clinical outcomes vary significantly and are influenced by strain-specific virulence traits. Macrophages are central innate immune sentinels during GBS infection; however, how genetically distinct clinical GBS isolates dynamically shape macrophage inflammatory, immunoregulatory, metabolic, and cell death responses over time remains poorly understood.

Methods: Human THP-1–derived macrophages were infected with a panel of 12 fully characterised clinical GBS isolates representing multiple serotypes, sequence types, clinical presentations, specimen origins, and neonatal gestational ages. Cytokine and chemokine production was quantified at 3 and 24 hours post-infection using bead-based multiplex immunoassays. Caspase-1 activity was assessed by bioluminescence, and expression of inflammatory, immunoregulatory, metabolic, and cell death–associated genes was analysed by RT-qPCR at 4 and 24 hours post-infection.

Results: Macrophage responses to GBS were highly isolate- and time-dependent. Serotype Ia and Ib isolates induced rapid inflammasome-associated responses characterised by early IL-1 β and IL-18 release, elevated caspase-1 activity, and inflammatory cell death consistent with pyroptosis. In contrast, serotype II and hypervirulent serotype III isolates showed minimal early inflammasome activation but promoted delayed immunoregulatory programmes marked by increased IL-10 and *ACOD1* expression, accompanied by distinct metabolic reprogramming at later time points. Reciprocal analyses revealed an inverse relationship between *ACOD1* and IL-1 β and a positive association between *ACOD1* and IL-10, indicating coordinated immunometabolic regulation. Stratification by clinical metadata demonstrated that isolates derived from preterm infants elicited stronger early inflammatory responses.

Conclusions: This study demonstrates that GBS pathogenicity is not a uniform species-level trait but reflects isolate-specific capacities to reprogramme macrophage immunity over time, ranging from early hyperinflammatory responses to delayed immune evasion. By integrating temporal resolution with strain diversity, these findings provide mechanistic insight into preterm birth–associated inflammation and heterogeneous outcomes in neonatal sepsis, supporting a precision-oriented approach to risk stratification and targeted intervention in GBS infection.

P2.11**SEROLOGICAL QUANTIFICATION OF LOST IMMUNE TOLERANCE: PATHOLOGICAL AUTOANTIBODY ACCUMULATION COINCIDES WITH DEPLETION OF HOMEOSTATIC NATURAL AUTOANTIBODY IN THE ANTI-TG/TPO-POSITIVE PATIENTS**

Alain le Roux^{*}, Katalin Böröcz¹, Szinger Dávid¹, Szabina Erdő-Bonyár¹, Diána Simon¹, Péter Németh¹, Berki Timea¹

¹Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs, Pécs, Hungary
Presenting author e-mail: leroux.alain@pte.hu

Background: Hashimoto thyroiditis (HT) is characterised by anti-thyroglobulin (a-TG) and anti-thyroperoxidase (a-TPO) pathological autoantibodies (pAAb), which mediate glandular injury and serve as diagnostic markers. A pro-inflammatory cytokine milieu sustains systemic inflammation, contributing to the high comorbidity burden (14–81%), and may activate B1b and marginal zone (MZ) B cells, reshaping natural autoantibody (nAAb) secretion, differentiation, and isotype switching.

Aim: Investigate bidirectional pAAb and nAAb dysregulation in a HT-suggestive serological cluster.

Methods: We retrospectively (2014–2024) analysed a-TG/TPO IgG+ (N = 2,519) anonymized cohort for disease-specific pAAb co-positivities: index patient samples were selected for longitudinal pAAb evaluation stratified by age and gender. Groups for overlap Systemic Autoimmune Disease (SAD)-suggestive pAAb assessments included: ANA/a-dsDNA IgG; aCL/a-β2GPI IgG; a-CCP IgG, a-MPO/PR3 IgG, ASCA IgG/IgA; a-Ro/SSA IgG. For cross-sectional nAAb profiling, we selected a-Tg/TPO IgG–positive samples (n=73), a-Tg/TPO IgG positive - pAAb overlap samples (n=32), and pAAb negative controls (n=85). nAAb levels were measured using in-house ELISA assays for IgM and IgG against citrate synthase (CS).

Results: Overall, 23% of anti-Tg/TPO IgG–positive patients exhibited ≥1 additional SAD-related pAAb, most frequently ANA IgG (17.1%), followed by ASCA IgG/IgA (8.2%), anti-dsDNA IgG (5.6%), anti-CL/β2GPI IgM/G (4.4%), anti-CCP IgG (4.1%), anti-Ro/SSA IgG (3.9%), and ANCA (0.8%). Patients with overlapping pAAb profiles had a higher median age (p < 0.001). Females predominated among a-Tg/TPO IgG–positive patients (76%), with subgroup-specific prevalence ranging from 76.9% to 100%. Homeostatic a-CS IgM nAAb levels were consistently reduced across all pAAb-positive groups (p < 0.01), while a-CS IgG titers peaked in the a-dsDNA IgG+ overlap cluster (p < 0.001) and increased with age (p = 0.001).

Conclusion: Bidirectional (pAAb/nAAb) profiling indicates that pAAb accumulation coincides with depletion of homeostatic nAAb, with older age further associating with heightened a-CS IgG titres and broader SAD pAAb overlap. Together, these findings suggest that SAD-associated immune dysregulation disrupts nAAb homeostasis, with IgG isotype shifts potentially reflecting compensatory class switching or maladaptive immune priming.

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P2.12**HLA-B ALLELE FREQUENCIES AND HLA-B27 SUBTYPES IN SLOVENIAN PATIENTS WITH ANKYLOSING SPONDYLITIS TREATED WITH TARGETED THERAPIES**

Jasna Omersel¹, Joanna Wielińska², Saša Čučnik³, Borut Božič^{1,3}, Žiga Rotar³, Katja Lakota^{3,4} on behalf of the Slovenian rheumatologist contributing to biorx.si.

¹Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia; ²Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Poland; ³Department of Rheumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia; ⁴FAMNIT, University of Primorska, Koper, Slovenia

Presenting author e-mail: jasna.omersel@ffa.uni-lj.si

Background: Ankylosing spondylitis (AS) is an immune-mediated inflammatory disorder with a well-recognized genetic component. Human leukocyte antigen B27 (HLA-B27) is the major genetic risk factor, with HLA-B27:05 and 27:02 most strongly associated with AS risk in Caucasians. Other HLA-B alleles are also associated with susceptibility to AS but also with disease phenotype, severity, and possibly treatment response. Due to the high polymorphism of HLA-B, which varies significantly across countries, races, and ethnic groups, population-based studies and allele frequency data are essential for further gene association research in AS. The Slovenian national registry of AS patients treated with biologics was established in 2009. The aim of this study was to determine HLA-B allele frequencies and HLA-B27 subtypes among Slovenian AS patients receiving either biologic or targeted synthetic therapy.

Methods: A cohort of 61 AS patients (46 females, 15 males) was typed for HLA-B alleles using next-generation sequencing. Library preparation was performed using the NGSgo® Library Full Kit (GenDx), including NGSgo®-LibrX, NGSgo®-IndX Plate I, and GenDx-AMPure XP beads. Sequencing was carried out on the Illumina MiSeq platform, data were analyzed with NGSengine®, statistical analyses included Fisher's exact test and non-parametric Mann-Whitney U or t-tests to investigate sex- and age-related differences in HLA-B27 status. The presence of uveitis and its association with HLA-B27 positivity were also evaluated.

Results: We identified 20 different HLA-B alleles. The most frequent were HLA-B*27:05:02 (29.5%) and HLA-B*27:02:01 (8.2%), followed by B*08:01:01 (7.4%), B*15:01:01 (6.6%), B*18:01:01 (5.7%), B*35:01/03 (5.7%), and B*44:02/03/05 (7.4%). Overall, 75.4% of patients tested positive for HLA-B27. Differences in onset age between B*27:05 and B*27:02 subtypes were not statistically significant. Uveitis occurrence and age of biologic therapy initiation showed no significant association with any specific allele.

Conclusion: This is the first study reporting HLA-B allele frequencies among AS patients in the Slovenian population. High frequency of B*27:05 and B*27:02:01 in our AS patients as compared to frequency reported for Caucasian population is in consistent with other studies and expected, given that our AS cohort has high disease activity requiring biologics. Further studies with larger sample sizes are needed to confirm allele distribution and explore associations with disease susceptibility and treatment outcomes.

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P2.13**CYSTATINS AS ENDOGENOUS REGULATORS OF INNATE IMMUNE RESPONSES IN LPS-INDUCED SEPSIS**

Monika Biasizzo^{1,2}, Mojca Trstenjak-Prebanda¹, Klemen Dolinar³, Sergej Pirkmajer³, Boris Turk^{1,4}, Nataša Kopitar-Jerala¹

¹Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia; ²International Postgraduate School Jožef Stefan, Ljubljana, Slovenia; ³Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ⁴Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia
Presenting author e-mail: natasa.kopitar@ijs.si

Cystatins are endogenous cysteine protease inhibitors that play important roles in inflammation and cancer. Stefin B (cystatin B) inhibits nuclear and lysosomal cysteine cathepsins, and loss-of-function mutations in the *CSTB* gene cause Unverricht–Lundborg disease (EPM1), a neurodegenerative disorder characterized by progressive myoclonus epilepsy and ataxia. We have previously shown that steffin B-deficient mice are highly susceptible to lethal lipopolysaccharide (LPS)-induced sepsis, associated with enhanced activation of the NLRP3 inflammasome.

In the present study, we demonstrate that steffin B trisomic mice, carrying an additional copy of the *CSTB* gene, exhibit reduced caspase-11 expression and decreased interleukin-1 β (IL-1 β) processing in bone marrow-derived macrophages (BMDMs). Steffin B trisomy prevented mitochondrial reactive oxygen species (ROS) formation and impaired NLRP3 inflammasome activation. These effects were accompanied by increased AMP-activated protein kinase (AMPK) activation and suppressed mTOR signaling in both steffin B trisomic macrophages and cells overexpressing steffin B.

In contrast, mice deficient in cystatin C (CstC), a type 2 cystatin, were significantly more sensitive to lethal LPS-induced sepsis. This increased susceptibility correlated with elevated caspase-11 expression, enhanced NLRP3 inflammasome activation, and increased secretion of IL-1 β and IL-18 in CstC-deficient BMDMs following LPS and ATP stimulation. Cystatin C deficiency did not affect MAPK signaling or anti-inflammatory IL-10 secretion but resulted in impaired autophagy, mediated through dysregulated mTOR and AMPK signaling, similar to steffin B-deficient macrophages.

Collectively, our findings indicate that excessive inflammatory responses in both steffin B- and cystatin C-deficient mice are driven by increased caspase-11 expression and defective autophagy. These results highlight the anti-inflammatory properties of cystatins and suggest their therapeutic potential in preventing pathological NLRP3 inflammasome overactivation.

P2.14**PATHOGENESIS OF MCMV INFECTION IN THE ADRENAL GLAND**

Ivana Bertović¹, Marija Mazor¹, Jelena Železnjak¹, Tina Ružić¹, Magdalena Medved¹, Maja Cokarić Brdovčak¹, Martina Brnjčić¹, Jelena Tomac², Stipan Jonjić¹, Vanda Juranić Lisnić¹, Berislav Lisnić¹

¹Center for Proteomics, Faculty of Medicine, University of Rijeka; ²Department of Histology and Embryology, Faculty of Medicine, University of Rijeka

Presenting author e-mail: ibertovic@uniri.hr

Background: Murine cytomegalovirus (MCMV) is an established model for studying beta-herpesvirus infections and host-pathogen interactions. The adrenal gland, a central organ in stress adaptation and hormone synthesis, is a known target of MCMV infection; however, the mechanisms governing viral pathogenesis and immune-endocrine interactions in this tissue remain poorly defined.

Methods: Mice were intravenously infected with MCMV, and viral replication in the adrenal glands was assessed by viral titration and immunohistological detection of IE1-positive cells. Local immune responses were characterized by cytokine profiling and immune cell depletion studies. Endocrine function was evaluated by measuring adrenal hormone production. The role of type I interferon signaling was investigated using mice deficient in type I interferon receptor.

Results: Viral titers in the adrenal glands peaked at day 5 post-infection, with IE1-positive cells predominantly localized in the adrenal cortex. Despite robust viral replication, adrenal hormone production remained preserved. Cytokine levels in the adrenal glands generally reflected viral loads in infected organs; notably, IL-6 was consistently absent, which correlated with the lack of corticosterone induction during infection. In the absence of type I interferon signaling, viral replication was markedly enhanced and accompanied by increased adrenal hormone secretion and strong induction of proinflammatory cytokines, including IFN γ , TNF, CCL2, and IL-6. Immune cell depletion studies identified NK cells and CD8⁺ T cells as key mediators of viral control, whereas CD4⁺ T cells were required for the establishment of viral latency in the adrenal glands.

Conclusions: These findings reveal a complex interplay between MCMV infection, immune responses, and endocrine regulation in the adrenal gland. Type I interferon signaling is crucial for limiting viral replication and maintaining hormonal homeostasis, while distinct immune cell populations exert tissue-specific roles in viral control and latency. Together, this study provides novel insights into the mechanisms shaping MCMV pathogenesis in an immune-endocrine organ.

P2.15**OSTEOCLAST ACTIVITY AND BONE MASS CHANGES IN A 3,5-DIETHOXYCARBONYL-1,4-DIHYDROCOLLIDINE-INDUCED CHOLESTATIC LIVER DISEASE MODEL**

Pavao Planinić¹, Marta Radošević^{2,3}, Ivo Krešić¹, Sara Aničić^{2,3}, Darja Flegar^{2,3}, Alan Šućur^{2,3}, Nataša Kovačić^{2,4}, Danka Grčević^{2,3}, Antonio Markotić¹, Ivan Čavar¹, Tomislav Kelava^{2,3}

¹Department of Physiology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina; ²Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia;

³Department of Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia; ⁴Department of Anatomy, School of Medicine, University of Zagreb, Zagreb, Croatia

Presenting author e-mail: tkelava@mef.hr

Background: Chronic liver diseases are often associated with osteoporosis, creating significant clinical challenges. The liver-bone axis is modulated by mediators released from the liver, with bone loss severity and underlying mechanisms varying across different liver diseases. Cholestatic liver fibrosis linked to pronounced bone loss. In this study, we aimed to investigate the mechanisms of bone loss and the potential activation of myeloid osteoclast progenitors (OCPs) in this model.

Methods: Female C57BL/6 mice were fed either a standard diet (control) or a DDC-supplemented diet for 4–8 weeks. Bone changes were assessed using micro-computed tomography (μ CT), focusing on femoral and vertebral trabecular bone parameters (BV/TV, Tb.Th, Tb.N, Tb.Sp) and femoral cortical thickness (C.Th). Osteoclast number and surface area were quantified on TRAP-stained femoral sections. OCPs were characterized by flow cytometry (CD45+Ly6G-CD3-B220-NK1.1-CD11b^{lo}CD115⁺). To differentiate between direct and secondary effects of DDC, OCPs were cultured in osteoclastogenic media (RANKL/M-CSF) with varying DDC concentrations, and osteoclast differentiation was assessed by TRAP staining.

Results: DDC treatment led to liver fibrosis, confirmed by Sirius Red staining and increased expression of hepatic Colla1, ACTA2, Krt19, and proinflammatory cytokines. Fibrosis was associated with reduced femoral C.Th. In contrast, an increase in trabecular bone volume was observed in both femoral and spinal regions, indicated by higher femoral BV/TV and Tb.N, along with decreased Tb.Sp. Additionally, DDC-fed mice exhibited lower number of TRAP positive cells in trabecular bone after four weeks, however, after eight weeks these findings reversed and increase in number of TRAP positive cells was associated with higher number of OCPs, possibly reflecting a reactive response. In culture, DDC inhibited osteoclast differentiation, as evidenced by a dose-dependent reduction in TRAP-positive osteoclasts.

Conclusion Our findings highlight model-specific mechanisms linking liver disease to specific changes in bone morphology. Due to a possible direct inhibitory effect of DDC on osteoclasts, other models of cholestatic liver disease might be better suited for investigation of bone loss in these diseases.

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P2.16**ILC1S MEDIATE CONTROL OF PERINATAL MURINE CYTOMEGALOVIRUS INFECTION VIA NKG2D**

Iva Vladić¹, Carmen Rožmanić¹, Berislav Lisnić¹, Vanda Juranić Lisnić¹, Lucija Šakota¹, Stipan Jonjić¹, Ilija Brizić¹

¹Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Presenting author e-mail: iva.vladic@uniri.hr

Background: NK cells are key mediators of defense against cytomegalovirus (CMV) infection. However, the contribution of NK cells to the control of early-life infection is limited, as shown by the use of a model of perinatal mouse CMV (MCMV) infection [1]. CMVs are well-known manipulators of the immune system, with numerous strategies to evade NK cell surveillance. Here, we aimed to assess whether, during its coevolution with its host, activating NK cell receptors may have contributed to viral control during early life.

Methods: NK cells were characterised during ontogeny by performing RNA-seq analysis at different postnatal time-points and compared to adult mice NK cells. To investigate the capacity of NK cells to control MCMV using NKG2D, neonatal mice were infected with an MCMV mutant lacking the immune evasion gene m145 (Δ m145), which promotes intracellular retention of NKG2D ligand MULT-1. NK1.1+ cells (NK cells and ILC1s) were depleted to assess their role in viral control. To discriminate the role of ILC1s and NK cells in control of Δ m145 MCMV infection, NCR1^{iCre}Eomes^{fl/fl} mice were used, in which Eomes is conditionally deleted in NKp46+ cells, resulting in a significantly reduced NK cell number.

Results: Transcriptional analysis demonstrated that NK cells in early neonatal life display enhanced cytokine and diminished cytotoxic capacity. Expression of a set of NK cell receptors, including NKG2D, was similarly expressed by NK cells in early life and adulthood. Depletion of NK1.1+ cells (NK cells and ILC1s) resulted in increased titres of MCMV following infection of neonatal mice. Experiments using NCR1^{iCre}Eomes^{fl/fl} demonstrated that ILC1s confer viral control via NKG2D.

Conclusions: This study identifies a novel mechanism of neonatal antiviral immune defence, revealed upon disruption of viral immune escape mechanisms. The findings demonstrate that ILC1s mediate control of perinatal MCMV infection through the NKG2D pathway, indicating that this pathway could have played a role during host-virus coevolution.

Reference

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P2.17**EARLY ETOPOSIDE IS CRITICAL IN EPSTEIN–BARR VIRUS-ASSOCIATED HAEMOPHAGOCYTTIC LYMPHOHISTIOCYTOSIS: A PAEDIATRIC CASE REPORT**

Simona Ivančan¹, Tina Plankar Srovin², Tina Vesel Tanšek¹, Katarina Vinček²

¹University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; ²Clinic for Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

Presenting author e-mail: simona.ivancan@kclj.si

Background: Haemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome caused by uncontrolled immune activation. Epstein–Barr virus (EBV) is the most common infectious trigger in children and is associated with high viral load and severe clinical course [1,2]. Early recognition and timely initiation of etoposide-based therapy are crucial for survival [1,3].

Methods: We report a case of EBV-associated HLH in a previously healthy 3-year-old boy treated at a tertiary paediatric centre. Clinical features, laboratory parameters, virological findings and treatment response were analysed retrospectively.

Results: The patient presented with 11 days of persistent fever, splenomegaly and rash. Laboratory investigations revealed cytopenia (WBC $4.2 \times 10^9/L$, haemoglobin 96 g/L), hyperferritinaemia (2,855 $\mu g/L$), elevated transaminases and lactate dehydrogenase. EBV DNAemia was markedly elevated. Initial bone marrow examination showed no haemophagocytosis. Treatment with intravenous immunoglobulin, high-dose methylprednisolone and cyclosporine resulted in transient improvement. However, progressive elevation of ferritin, soluble IL-2 receptor and EBV DNAemia (1,200,000 IU/mL) required addition of rituximab. Clinical deterioration followed, with pancytopenia, coagulopathy and worsening hyperinflammation; repeat bone marrow confirmed haemophagocytosis and EBV DNAemia increased to nearly 10,000,000 IU/mL. Escalation to HLH-2004 protocol therapy including dexamethasone and etoposide led to rapid clinical and laboratory improvement within three days. EBV DNAemia became undetectable after four weeks. Genetic testing excluded primary HLH.

Conclusions: This case highlights the pivotal role of early etoposide initiation in paediatric EBV-associated HLH. Antiviral therapy alone is ineffective due to viral latency and immune dysregulation. Prompt escalation to etoposide-based therapy is essential to prevent multiorgan failure and improve survival [1,3].

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P2.18**IMMUNOMODULATORY POTENTIAL OF MOSS *HYPNUM CUPRESSIFORME* HEDW. EXTRACTS: *IN VITRO* AND *IN SILICO* STUDY**

Tanja Lunić¹, Marija Rakić¹, Aneta Sabovljević¹, Marko Sabovljević¹, Bojan Božić¹, Biljana Božić Nedeljković¹

¹University of Belgrade, Faculty of Biology, Belgrade, Serbia

Presenting author e-mail: tanja.lunic@bio.bg.ac.rs

Background: Mosses are a rich but underexplored source of specialised metabolites with potential immunomodulatory effects. This study aimed to characterise phenolic constituents of *Hypnum cupressiforme* Hedw. extracts and evaluate their immunomodulatory and neuroprotective potential using *in vitro* and *in silico* approaches.

Methods: Extracts were chemically profiled for phenolic acids and flavonoids. Antioxidant activity and biocompatibility were assessed. Anti-neuroinflammatory effects were evaluated in lipopolysaccharide (LPS)-stimulated BV2 microglia by measuring nitric oxide (NO), reactive oxygen species (ROS) and pro-inflammatory cytokines (IL-6, TNF- α). Neuroprotection was assessed in SH-SY5Y neurons exposed to soluble mediators from LPS-activated BV2 cells. Molecular docking was performed to estimate binding affinities of selected compounds towards acetylcholinesterase and tyrosinase.

Results: Extracts contained diverse phenolics; *p*-hydroxybenzoic acid was the most abundant phenolic acid and kaempferol the predominant flavonoid. The extracts were biocompatible and exhibited antioxidant and antineurodegenerative properties. In BV2 cells, treatment with moss extracts reduced NO and ROS production and lowered IL-6 and TNF- α levels compared with LPS stimulation alone, indicating anti-neuroinflammatory activity. Conditioned media from extract-treated BV2 cultures attenuated microglia-mediated neurocytotoxicity in SH-SY5Y neurons, supporting a neuroprotective effect. Docking suggested eriodictyol had the highest predicted affinity for acetylcholinesterase, whereas quercetin-3-O-rutinoside and caffeic acid showed the strongest predicted affinity for tyrosinase.

Conclusions: *Hypnum cupressiforme* extracts can be a promising source of bioactive phenolics that modulate inflammatory signalling in microglia and protect neurons from microglia-derived soluble mediators. These findings support further evaluation of *H. cupressiforme*-derived compounds as adjunct candidates for conditions where neuroinflammation contributes to pathology.

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P2.19**IMPACT OF EARLY POSTNATAL ADMINISTRATION OF *ESCHERICHIA COLI* O83:K24:H31 ON IMMUNE SYSTEM MATURATION AND GUT BARRIER FUNCTION**

J. Hrdý¹, L. Súkeníková¹, V. Černý¹, J. Věcek¹, E. Krčmářová¹, P. Petrásková¹, O. Novotná¹, I. Kocourková², J. Procházka³, L. Prokešová¹, L. Zahradníčková¹

¹Department of Clinical Immunology and Allergology, First Faculty of Medicine, Charles University, Prague, Czech Republic; ²Institute for the Care of Mother and Child, Prague, Czech Republic; ³Czech Centre for Phenogenomics, Institute of Molecular Genetics, Czech Academy of Sciences, Czech Republic

Presenting author e-mail: lucie.zahradnickova@lf1.cuni.cz

Background: The early postnatal period is critical for immune system maturation and the establishment of host–microbiota interactions. Early microbial colonization influences immune tolerance and intestinal barrier development. Probiotic supplementation during this period may modulate immune responses and provide long-term protection against inflammatory diseases. This study evaluated the effects of early postnatal administration of *Escherichia coli* O83:K24:H31 on immune maturation, gut barrier function, and susceptibility to intestinal inflammation.

Methods: A neonatal mouse model was used to investigate the impact of early-life supplementation with *E. coli* O83:K24:H31. Colonization capacity in the gastrointestinal tract was assessed during the early postnatal period. The maturation and functional properties of dendritic cells were analyzed using bone marrow-derived and cord blood-derived dendritic cells. Cytokine production and T-cell polarization were evaluated by *in vitro* and *in vivo* assays. Gut barrier function was assessed by measuring intestinal permeability. In adulthood, susceptibility to inflammation was examined using a TNBS-induced colitis model, with evaluation of weight loss and macroscopic and histological disease scores.

Results: Early postnatal administration of *E. coli* O83 resulted in efficient intestinal colonization and promoted dendritic cell maturation. Treated animals exhibited increased production of the immunoregulatory cytokine interleukin-10 and a shift toward a tolerogenic immune profile. Probiotic supplementation improved gut barrier integrity and reduced inflammatory responses. In the TNBS-induced colitis model, supplemented animals showed significantly reduced weight loss and lower macroscopic and histological scores compared with controls, indicating attenuated disease severity.

Conclusion: Early-life supplementation with *E. coli* O83:K24:H31 supports immune system maturation, enhances intestinal barrier function, and reduces susceptibility to inflammatory bowel disease in adulthood. These findings suggest that early probiotic intervention may represent an effective strategy for long-term modulation of immune homeostasis and prevention of immune-mediated disorders.

P2.20**RISK HLA VARIANTS FOR SEVERE COVID-19 – A STUDY FROM OLOMOUC, CZECHIA**Martin Petřek¹, Kateřina Sikorová¹, Adriana Gavroňová², Jana Petřková¹

¹Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czechia; ²Department of Forensic Medicine and Medical Law, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czechia

Presenting author e-mail: martin.petrek@upol.cz

Background: The major histocompatibility complex in humans (HLA) is a central component of the human immune response, mediating self/non-self-recognition and antigen presentation. HLA polymorphisms have an important role in immune response against viral infection. Based on emerging association studies, HLA variation may be linked with infectious diseases, including COVID-19. We have used next-generation sequencing (NGS) to investigate the role of HLA variation in Czech patients with COVID-19, focusing on severe disease.

Methods: HLA polymorphisms were determined in 67 patients (44 males, 23 females) who died due to severe SARS-CoV-2 infection at University Hospital Olomouc during 2021; the cause of death was viral interstitial pneumonia and respiratory failure. Seven HLA loci (HLA-A,-B,-C,-DRB1,-DQA1,-DQB1,-DPB1) were genotyped by Omixon Holotype kit and HLATwin software. HLA allele frequencies in patients were compared with frequencies in 290 unrelated Czech healthy subjects; 4-digit characteristics of typing results were used for the analysis.

Results: Six HLA variants associated with the risk of severe COVID-19 with subsequent death were identified, two at HLA class I loci (HLA-A, -B) and four at class II loci (HLA-DRB1, -DQB1, -DPB1). The most prominent associations were the following, HLA-A*01:01 ($p=0.048$; Odds Ratio, OR:1.62), HLA-B*38:01 ($p=0.029$; OR:2.52), HLA-DRB1*04:08 ($p=0.026$; OR:5.89), HLA-DQB1*03:04 ($p=0.043$; OR:4.47) and HLA-DPB1*06:01 ($p=0.033$; OR:3.87).

Conclusion: The study in this particular Czech population implicated six HLA variants as the susceptible risk variants for severe COVID-19 resulting in patients' death. Some of the reported alleles were reported for associations with severe COVID-19 in other populations (e.g., HLA-A*01:01). Still, as the study power is limited due to a lower number of patients, similarly to the majority of COVID-HLA studies performed, validation of findings in larger, possibly multicentre cohort(s) would be necessary. A current alternative could be the combination with / addition to HLA genotyping data in existing global consortia, where populations other than Czech have been contained.

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