THE ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY 2025

22ND TO 23TH SEPTEMBER, 2025
HOTEL MAGAL, NJIVICE

Welcome letter

Dear colleagues and friends,

On behalf of the Organizing Committee, it is our great pleasure to welcome you to the Annual Meeting of the Croatian Immunological Society 2025, which will take place in the beautiful coastal town of Njivice, on the Island of Krk, from September 22nd to 23rd.

Njivice and the Island of Krk, rich in history and natural beauty, provide an inspiring backdrop for our shared mission — a commitment to advancing immunology with a clear vision focused on supporting young scientists, strengthening international collaborations, and fostering inclusivity across diverse research communities.

Continuing our tradition, this year's meeting will bring together outstanding speakers from abroad alongside renowned researchers from Croatia, reflecting the growing strength and visibility of our scientific community both locally and internationally. We are especially proud to support and encourage early-career researchers, offering them a valuable platform to present their work, engage with established scientists, and become an integral part of our immunological society.

The past year has been particularly fruitful for our Society, marked by several important scientific events that underscored both the collaborative spirit and scientific excellence of our members. Among these, the 1st Adriatic Immunology Symposium with EFIS on Tour, co-organized by the Faculty of Medicine, University of Rijeka and the Croatian Immunological Society, stood out as a milestone event. It brought together leading immunologists from across Europe under the auspices of the European Federation of Immunological Societies (EFIS), highlighting cutting-edge research and fostering high-level scientific exchange.

We are also proud that our distinguished members played key roles in organizing the 2025 European Congress of Virology in the historic city of Dubrovnik — one of the first places in the world to implement a mandatory quarantine, dating back to 1377. With the active participation of our members, this event further highlighted our Society's growing contribution to both immunological and virological research on a global stage.

We look forward to a stimulating and inspiring meeting in Njivice, filled with scientific exchange, new collaborations, and the opportunity to reconnect with colleagues in a relaxed and picturesque setting. Thank you for being part of this vibrant and growing community. We warmly welcome you to join us for what promises to be another memorable and impactful event.

With kind regards,

Mariastefania Antica

THE ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY 2025

Hotel Magal, Njivice, otok Krk 22. - 23.09.2025.

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY

Bijenička cesta 54, Ruđer Bošković Institute, Zagreb

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PROGRAM

Monday, September 22nd, 2025.

10:30-11:15 REGISTRATION

11:15-11:30 OPENING CEREMONY

Chairs: Vanda Juranić Lisnić & Bojan Polić

11:30-12:00 ***Invited Lecture*** **- CHIARA ROMAGNANI**, Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Germany

NK Cell Clonality and Memory

12:00-12:45 *Selected Oral Presentations – **SESSION 1***

12:00 Ivana Bertović, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Pathogenesis of MCMV Infection in the Adrenal Gland

12:15 Tomislav Glavan, Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

Interferon-Gamma Mediates Anapyrexia During Viral Infection

12:30 Marija Mazor, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Tissue-Resident NK Cells Orchestrate Innate Immune Defense Against Cytomegalovirus in the Ovary

12:45-13:15 ***Invited Lecture*** **- JELENA ŽELEZNJAK**, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

The Art of Immunoevasion: Cytomegalovirus vs. NK Cells

13:15 – 14:15 LUNCH BREAK

Chairs: Asja Stipić Marković & Tomislav Kelava

14:15-14:45 ***Invited Lecture*** **- GORAN ŠIMIĆ**, Department of Neuroscience, Croatian Institute for Brain Research, Medical School, University of Zagreb, Croatia

Activation of Neuronal NLRP1 Inflammasome in Alzheimer's Disease and Schizophrenia

14:45-15:30	*Selected Oral Presentations – **SESSION 2 - Bright Spark***
	14:45 Tomislav Balen, Dept. of Anatomy & Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia ***Low Grade Systemic Inflammation Induces Osteoarthritis in Mice Accompanied by Accumulation of Osteochondroprogenitors in Early Time Points***
	15:00 Iva Vladić, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia ***ILC1s Mediate Control of Perinatal Murine Cytomegalovirus Infection via NKG2D***
	15:15 Luca Grisetti, National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte, Italy ***Targeting PD-L1 to Boost T Cell–Mediated Killing in HCC: An In Vitro Analysis***
15:30-16:00	*Invited Lecture* - **MARINA BABIĆ ČAČ**, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia ***Regulation of Lymphocyte Responses During Neuroinflammation***
16:00-16:30	**COFFEE BREAK**
16:30-17:00	*Chair*: Mariastefania Antica *Invited Lecture* - **GRAHAM ANDERSON**, Department of Immunology and Immunotherapy, University of Birmingham, UK ***Control of Thymus Regeneration by Type 2 Immune Networks***
17:00-19:00	*Chairs: Alan Šućur & Ilija Brizić* **POSTER SESSION – SHORT PRESENTATIONS**
20:30	**GALA DINNER**

Tuesday, September 23rd 2025

8:45 – 9:15	**GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY**
	Chairs: Danka Grčević & Stipan Jonjić
9:15-9:45	*Invited Lecture* - **CRISTINA SOBACCHI**, IRCCS Humanitas Research Hospital & National Research Council-Institute for Genetic and Biomedical Research (CNR-IRGB), Rozzano, Italy ***The Long Pentraxin PTX3 at the Host-Pathogen Interface in Osteomyelitis***
9:45-10:30	*Selected Oral Presentations – **SESSION 3***
	9:45 Ivo Krešić, Dept. of Physiology and Immunology & Croatian Institute for Brain Research, School of Medicine, Univ. of Zagreb, Croatia ***Notch Signaling Modulates Human Trilineage Monocyte Progenitor Differentiation Under Inflammatory Conditions***
	10:00 Ozren Majstorović, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia ***Adiponectin is an Endocrine Modulator of $CD8^+$ Memory T Cells***
	10:15 Marko Šestan, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia ***Immune System-Mediated Changes in Amino Acid Metabolism Enhance the Immune Response During Viral Infections***
10:30-11:00	*Invited Lecture* - **MAJA LENARTIĆ**, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia ***IFN-γ: A Common Denominator of Infection-Induced Metabolic and Thermoregulatory Adaptations***
11:00-11:30	**COFFEE BREAK SPONSORED BY GOREA PLUS**

Chairs: Ines Mrakovčić-Šutić & Felix M. Wensveen

11:30-12:00 ***Invited Lecture*** **- ERIC ELDERING**, Department of Experimental Immunology, Amsterdam UMC location University of Amsterdam & Cancer Immunology, Amsterdam Institute for Infection and Immunity & Cancer Immunology, Cancer Center Amsterdam, the Netherlands

Imunotherapeutic Killing by CTLs Occurs Partly by Necroptosis

12:00-12:45 *Selected Oral Presentations – **SESSION 4***

12:00 Sanja Mikašinović, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

IGF-1 Receptor Signaling Promotes CD8 Memory T Cell Formation and Enhances Tumor Control In Vivo

12:15 Paola Kučan Brlić, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Evaluating Nectin-2 and Nectin-4 as Immunotherapeutic Targets in Hepatocellular Carcinoma

12:30 Asja Stipić Marković, University Hospital for infectious diseases „Dr Fran Mihaljević“, Zagreb, Croatia

Unknown Side Effect of Janus Kinase 1 Inhibitor: IgE Hyperproduction, Case Report

12:45-13:15 **AWARD CEREMONY AND CLOSING REMARKS**

Invited Lecture

NK Cell Clonality and Memory

Chiara Romagnani

Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Germany

Clonal expansion of cells with somatically diversified receptors and their long-term maintenance as memory cells is a hallmark of adaptive immunity. In order to understand pathogen-specific adaptation within the innate immune system, we study natural killer (NK) cell memory to human cytomegalovirus (CMV) infection. Leveraging single-cell multiomic maps of ex vivo NK cells and somatic mitochondrial and nuclear DNA mutations as endogenous barcodes, we reveal extreme clonal dominance of memory NK cells in healthy young CMV-seropositive donors. NK cell clonotypes share an inflammatory memory signature enriched for AP1 motifs and are enriched in cancer driver mutations. Our ongoing investigations aim to elucidate how mutations in expressed genes influence clonal selection and NK cell functions. Together, these findings provide unprecedented insights into NK cell clonality and suggest that the interplay between phenotypic and genetic heterogeneity drives clonal evolution of CMV-specific NK cells.

Invited Lecture

The Art of Immunoevasion: Cytomegalovirus vs. NK Cells

Jelena Železnjak[1,*], Magdalena Medved[1,*], Ivana Bertović[1], Maja Cokarić Brdovčak[1], Branka Popović[2], Marina Babić Čač[2], Marija Mazor[1], Anne Halenius[3,4], Hartmut Hengel[3,4], Silvia Vidal[5], Astrid Krmpotić[2], Ilija Brizić[1], Lars Dölken[6,7], Berislav Lisnić[1], Stipan Jonjić[1], Vanda Juranić Lisnić[1]

*[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia; [2]Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia; [3]Institute of Virology, Medical Center University of Freiburg, Germany; [4]Faculty of Medicine, University of Freiburg, Germany; [5]Dept. of Human Genetics, McGill University, Montreal, Quebec, Canada; [6]Institute of Virology and Immunobiology, Julius Maximilian University of Würzburg, Germany; [7]Institute of Virology, Hannover Medical School, Germany. * These authors contributed equally*

Cytomegaloviruses' (CMVs) ability to manipulate immune-cell recognition is well established, especially fine-tuning of MHC-I expression to simultaneously avoid both CD8 T cell surveillance and NK cell recognition. While mouse CMV (MCMV) causes significant downregulation of all MHC-I molecules from the cell surface, NK missing-self-mediated killing is prevented by the action of two viral proteins: m04 and MATp1 which form tri-molecular complexes with certain MHC-I alleles and allow their expression on the cell surface. There, these MATp1/m04-altered-self MHC-I molecules engage inhibitory Ly49 NK cell receptors more strongly than MHC-I molecules alone. As a result, MCMV efficiently evades NK cell lysis even when MHC-I surface levels are strongly reduced. This provides an explanation for the long-standing paradox of why licensed NK cells, despite their sensitivity to MHC-I loss, fail to control infection in MCMV-susceptible mouse strains.

Unexpectedly, MATp1 also regulates the viral MHC-I-like molecule m157 – the ligand for the potent activating receptor Ly49H in MCMV-resistant C57BL/6 mice. By modulating m157 surface expression independently of m04 and MHC-I, MATp1 inhibits Ly49H+ NK cell activation, limiting their expansion and cytotoxicity. Loss of MATp1 in MCMV results in robust Ly49H+ NK cell activation and enhanced viral control in C57BL/6 mice, even at later stages of infection.

Together, these findings identify MATp1 as a dual immunoevasin that maintains inhibitory NK signaling via altered MHC-I, while simultaneously inhibits activating NK responses via m157 regulation, highlighting the importance of complex viral modulation of NK cell responses.

Invited Lecture

Activation of Neuronal NLRP1 Inflammasome in Alzheimer's Disease and Schizophrenia

Goran Šimić

Department of Neuroscience, Croatian Institute for Brain Research, Medical School, University of Zagreb, Croatia

The neuronal NLRP1 inflammasome is a component of innate immunity that senses cellular danger and stress signals. In neurons, its activation leads to caspase-1–mediated maturation of IL-1β and IL-18 and induction of pyroptotic cell death, positioning it as a central mediator of neuroinflammatory responses. Although primarily an innate immune mechanism, inflammasome-derived cytokines can also influence adaptive immunity by modulating T cell activity and inflammatory balance. In Alzheimer's disease (AD), neuroinflammation is a core feature, with amyloid β (Aβ) and tau proteins promoting sustained microglial activation and inflammasome overactivation. Postmortem hippocampal tissue from AD patients shows significantly higher expression of NLRP1, ASC, and caspase-6 in neurons compared to controls. NLRP1 expression correlates with neurofibrillary tangle (NFT) burden and with microglial markers IBA1 and CD68, linking inflammasome activation to tau pathology and neuronal degeneration. Unlike Aβ deposits, NFTs and neuronal loss in hippocampal fields, particularly CA1, remain the strongest pathological correlates of cognitive decline. These findings implicate neuronal NLRP1 overactivation in synaptic dysfunction and progression of AD, supporting its potential as a diagnostic and therapeutic target. In schizophrenia, increasing evidence connects neuroinflammation with cortical dysfunction. Postmortem analyses revealed elevated NLRP1 mRNA in the medial orbitofrontal and dorsolateral prefrontal cortices, especially in the right medial orbitofrontal cortex. Immunohistochemistry confirmed more NLRP1-positive pyramidal neurons in layers III, V, and VI. Dysfunction in layer III is associated with working memory deficits, while abnormalities in deeper layers likely disrupt predictive processing. These results suggest that NLRP1 activation contributes to the pathophysiology of schizophrenia and may serve as a biomarker and therapeutic target.

Invited Lecture

Regulation of Lymphocyte Responses During Neuroinflammation

Marina Babić Čač

Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) associated with inflammation, demyelination, oligodendrocyte loss and axonal injury. T lymphocytes play a crucial role in the inflammatory processes that underlie MS and experimental autoimmune encephalomyelitis (EAE), a mouse model thereof. While autoreactive $CD4^+$ T cells have been extensively studied, the role of bystander $CD4^+$ T cells in modulating the disease remains unclear.

We identified a conserved population of circulating $NKG2D^+ID2^{hi}$ Th1 cells, both in mouse and human blood and enriched in patients with autoimmune conditions, particularly MS. By combining EAE and single-cell RNA sequencing we characterized $NKG2D^+CD4^+$ T cells accumulated in the central nervous system (CNS) and revealed two distinct transcriptional and activation states within this population. One comprises antigen-specific cells, while the other consists of bystander-like $ID2^{hi}$ cells that lack features of recent TCR engagement and transcriptionally resemble their blood-derived counterparts present at steady state. NKG2D augmented proinflammatory features in both antigen-specific and bystander cells, leading to increased myeloid cell recruitment into the CNS and demyelination at the peak of EAE. Pathogenic potential of $NKG2D^+CD4^+$ T cells was confirmed in the passive EAE model where transfer of NKG2D-sufficient polyclonal Th1 cells exacerbated the disease outcome.

Altogether, NKG2D seems to operate as a critical checkpoint that integrates cellular stress-induced ligand and cytokine cues to amplify both antigen-dependent and bystander $CD4^+$ T cell programs in neuroinflammation.

Invited Lecture

Control of Thymus Regeneration by Type 2 Immune Networks

Graham Anderson

Department of Immunology and Immunotherapy, University of Birmingham, UK

Although the thymus is the primary site of T-cell development, rates of thymus function are not constant. Instead, the thymus is sensitive to multiple forms of injury including environmental factors and therapeutic interventions, and thymus regeneration is needed to restore new T-cell production following tissue damage. Here, we examined cellular and molecular mechanisms controlling thymus regeneration. In particular, we build on our earlier observations demonstrating an essential role for eosinophils. In experiments involving either IL33 KO mice or recombinant IL33 administration to WT mice we show the alarmin IL33, which is expressed by thymic mesenchyme, is an essential regulator of regeneration. This requirement for alarmins is selective, as IL25, a product of thymic tuft cells, is neither necessary nor sufficient for thymus recovery. On the mode of action of IL33, we show it influences recovery through targeted expansion of intrathymic ILC2, known producers of the cytokine IL5, a key regulator of eosinophils. In turn, increased intrathymic availability of IL5+ ILC2 expands thymic eosinophils expressing IL4, enabling eosinophils to act as the major intrathymic source of this type 2 cytokine in the regenerating thymus. Finally, we show IL4 treatment rescues thymus regeneration in eosinophil deficient mice, and that IL4 is required for IL33+ thymic mesenchyme recovery following damage. These findings identify IL4 as an effector molecule of eosinophils in thymus regeneration. Collectively, our study describes multiple cellular and molecular components of a type 2 innate immune network that regulates the recovery of thymus function following tissue injury.

Invited Lecture

The Long Pentraxin PTX3 at the Host-Pathogen Interface in Osteomyelitis

Raffaella Parente[1,*], Valentina Possetti[1,2,*], Dario Strina[1,3], Valentina Granata[1], Maša Filipović[4,5], Nataša Kovačić[4,5], Erica Gianazza[6], Francesca R Liberati[1], Maria L Schiavone[1,3], Sonia Valentino[1], Ciro Menale[7], Eleonora Palagano[8], Paolo Kunderfranco[1], Susanna Skalicky[9], Matthias Hackl[9], Cristina Banfi[6], Barbara Bottazzi[1], Danka Grčević[4,5], Alberto Mantovani[1,2,10], **Cristina Sobacchi**[1,3], Antonio Inforzato[1,2]

[1]IRCCS Humanitas Research Hospital, Rozzano, Italy; [2]Dept of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy; [3]National Research Council-Institute for Genetic and Biomedical Research (CNR-IRGB), Rozzano, Italy; [4]Dept of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; [5]Lab for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; [6]Unit of Functional Proteomics, Metabolomics and Network Analysis, Centro Cardiologico Monzino IRCCS, Milan, Italy; [7]Dept of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy; [8]National Research Council- Institute of Biosciences and Bioresources (CNR-IBBR), Division of Florence, Sesto Fiorentino, Italy; [9]TAmiRNA, Vienna, Austria; [10]The William Harvey Research Institute, Queen Mary University of London, London, UK

Infections of the bone and joint tissue represent a serious clinical challenge due to high morbidity, increasing antibiotic resistance, and diagnostic complexity, resulting in huge health-related costs and impact on quality of life for patients. Understanding the molecular and cellular mechanisms involved is crucial to improve preventive and treatment strategies. The primary cause of infectious osteomyelitis (OM) is the Gram-positive bacterium Staphylococcus aureus (SA), and immune and non-immune components of the bone microenvironment cooperate in its recognition, containment, and disposal. We focused on the soluble pattern recognition molecule (PRM) long pentraxin-3, PTX3, exerting host-protective functions against opportunistic pathogens and recently involved in bone pathophysiology. We demonstrated that in patients, synovial fluid PTX3 is a specific and sensitive marker of periprosthetic joint infection and genetic variants in IL-1β are associated with the levels of PTX3 expression, possibly due to intermediate effects on IL-1β production. Moreover, in a mouse model of SA OM we found that Ptx3 expression increased in non-hematopoietic bone cells and the protein accumulated in the bone during the acute phase of infection. Interestingly, Ptx3-/- mice had lower bacterial burden in the infected limb and reduced systemic inflammation, but stronger local inflammatory response to SA, with high levels of antimicrobial chemokines and cytokines and enhanced expression of cell-borne and soluble PRMs in non-hematopoietic bone cells. This phenotype was species-specific, and both sex- and bacterial load-independent.
Overall, PTX3 plays a pathogenic role in the acute phase of SA OM and might be exploited as a novel target for prophylaxis and therapy of bone infections.

Invited Lecture

IFN-γ: A Common Denominator of Infection-Induced Metabolic and Thermoregulatory Adaptations

Maja Lenartić, Tomislav Glavan, Marko Šestan, Marko Ljesar, Sarah Furjan, Erik Etemaj, Felix M. Wensveen, Bojan Polić

Medical Faculty, University of Rijeka, Dept. of Histology and Embryology, Rijeka, Croatia

Infection triggers a complex metabolic and behavioral response known as sickness behavior, yet the immunological mediators remain incompletely understood. We and others have shown that systemic viral infections with murine cytomegalovirus (mCMV), lymphocytic choriomeningitis virus (LCMV), and influenza A virus induce transient hypoglycemia and adipose tissue remodeling, effects mediated primarily by IFN-γ. IFN-γ acts directly on pancreatic β cells to enhance insulin secretion and on adipocytes to promote lipolysis, thereby mobilizing nutrients that support antiviral immunity. Failure to restrict glucose availability, either by genetic or nutritional interventions, impairs viral control and increases mortality, underscoring the adaptive value of infection-induced metabolic reprogramming. Extending these observations, we investigated the role of IFN-γ in thermoregulation during viral infection. We identified a previously unrecognized phase of thermorestriction at the peak of T cell activity, which requires IFN-γ signaling but not TNF, as IFN-γ–deficient mice failed to exhibit thermorestriction upon infection. We further explored the role of brown adipose tissue (BAT) and found that BATectomy abolished thermorestriction, highlighting its central role in infection-induced temperature regulation, although genetic model showed BAT is not directly influenced by IFN-γ. Other conditional IFN-γ receptor knockout experiments also indicated that IFN-γ does not act directly on the CNS. Using immunocompromised mice highly susceptible to MCMV, we evaluated the physiological importance of thermoregulation and its potential clinical relevance. These findings suggested that transient thermorestriction is a protective mechanism that helps the body avoid excessive immunopathology during viral infection.

Invited Lecture

Imunotherapeutic Killing by CTLs Occurs Partly by Necroptosis

Eric Eldering

Department of Experimental Immunology, Amsterdam UMC location University of Amsterdam & Cancer Immunology, Amsterdam Institute for Infection and Immunity & Cancer Immunology, Cancer Center Amsterdam, the Netherlands

Autologous cellular immunotherapies, including T-cell-redirecting bispecific antibodies (BsAbs) and chimeric antigen receptor (CAR) T cells, are increasingly used in B-cell malignancies. Although cytotoxic lymphocytes (CTLs) are generally presumed to kill via granzyme-induced apoptosis, the exact mechanism triggered by BsAbs or CARs remains unclear. Using B cell lines and primary CLL samples as targets, we show that killing in fact occurs by a mix of apoptosis and necroptosis. Necroptotic killing was associated with the release of pro-, as well as anti-inflammatory cytokines, with their levels differentially altered by apoptosis versus necroptosis inhibition. These findings suggest that modulating these pathways could shape immune responses in immunotherapy.

*Selected oral presentations – **SESSION 1***

S1-O1 ***Pathogenesis of MCMV Infection in the Adrenal Gland***

Ivana Bertović[1], Marija Mazor[1], Jelena Železnjak[1], Tina Ružić[1], Magdalena Medved[1], Maja Cokarić Brdovčak[1], Martina Brnjić[1], Jelena Tomac[2], Stipan Jonjić[1], Vanda Juranić Lisnić[1], Berislav Lisnić[1]

[1]*Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia*
[2]*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

Murine cytomegalovirus (MCMV) serves as an established model for investigating beta-herpesvirus infections and host-pathogen interactions. The adrenal gland, a key organ in stress adaptation and hormone synthesis, has been recognized as a target of MCMV infection, yet the mechanisms governing its pathogenesis in this tissue remain largely undefined. In this study, we examined the course of MCMV infection in the adrenal glands, focusing on viral replication, immune responses, and endocrine regulation.

Following intravenous infection, viral titers reached a maximum at day 5 post-infection, coinciding with immunohistological detection of IE1-positive cells predominantly located in the adrenal cortex. Despite robust viral replication, adrenal hormone production was preserved, suggesting that endocrine function is not directly impaired. Cytokine profiling revealed that local levels reflected viral loads in infected organs; however, IL-6 was consistently absent in the adrenal glands, which may account for the lack of corticosterone induction during infection. In mice deficient in type I interferon signaling, viral replication was markedly enhanced, accompanied by increased adrenal hormone secretion and strong induction of proinflammatory cytokines, including IFNγ, TNF, CCL2, and IL-6. Depletion studies identified NK cells and $CD8^+$ T cells as critical for early viral control, while $CD4^+$ T cells were essential for the establishment of latency in the adrenal glands.

Collectively, these results uncover the complex interplay between MCMV and host immune responses in the adrenal gland. They emphasize the pivotal role of type I interferon signaling in restraining viral replication and preserving hormonal balance, while also providing novel insights into tissue-specific immune mechanisms shaping MCMV pathogenesis.

*Selected oral presentations – **SESSION 1***

S1-O2 Interferon-Gamma Mediates Anapyrexia During Viral Infection

Tomislav Glavan[1], Erik Etemaj[2], Maja Lenartić[1], Felix Wensveen[1], Bojan Polić[1]

[1]*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*
[2]*Department of Biotechnology, University of Rijeka, Croatia*

Changes in body temperature during infection are commonly seen across many different species. Typically, a fever response is observed, but in certain cases, the opposite occurs and anapyrexia is triggered. While the mechanism of fever has been well-characterized, how infection-induced thermorestriction is induced remains to be elucidated. After inoculation of mice with a virulent MCMV variant, a sharp and short-term hypothermic response was seen, typically 5-6 days post-infection. Mice with total-body deficiency of IFNg showed a marked reduction in infection-induced thermorestriction. We showed that mice depleted of macrophages had an attenuated hypothermic response, while in mice that were genetic knockouts for CD8 T cells the response was delayed. We hypothesized that anapyrexia mediated by IFN-γ is a strategy of disease tolerance, a mechanism important in limiting immunopathology during infection. We found that in vitro differentiation of CD8 T cells at hypothermic temperatures suppresses IFN-γ and TNF-α production. To confirm these findings in vivo, we used an IFNγ-dependent model of severe MCMV-induced hepatitis and prevented anapyrexia using heated cages. We observed significantly higher levels of tissue damage-associated markers in sera. Therefore, we propose that IFN-γ-mediated anapyrexia suppresses immune cell function in order to limit immune-mediated tissue damage during an overwhelming immune response to viral infection.

*Selected oral presentations – **SESSION 1***

S1-O3 Tissue-Resident NK Cells Orchestrate Innate Immune Defense Against Cytomegalovirus in the Ovary

Marija Mazor[1], Jelena Železnjak[1], Tina Ružić[1], Jelena Tomac[2], Berislav Lisnić[1], Stipan Jonjić[1], Vanda Juranić Lisnić[1]

[1]*Center for Proteomics, University of Rijeka, Faculty of Medicine, Rijeka, Croatia*
[2]*Department of Histology and Embryology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia*

Viral infections during pregnancy are a major cause of adverse outcomes, yet their mechanisms remain incompletely understood. Cytomegalovirus (CMV), the most common intrauterine infection in humans, is implicated in early pregnancy loss. Here, we demonstrate a striking tissue specificity of CMV in the ovary, with robust infection of corpora lutea (CL) but no detectable infection in follicles. High viral loads in CL cells indicated a failure of local immune control, leading to progesterone insufficiency and pregnancy loss, while overall fertility remained unaffected. The survival of the species relies on the capacity of the ovarian tissue to rapidly respond to perturbations including pathogens and return to homeostasis. Mechanisms that initiate, maintain, and regulate immune responses in tissues are therefore essential with tissue residency being cardinal features. We identified a unique population of ovarian tissue-resident natural killer (trNK) cells with cytotoxic potential, capable of rapidly responding to and controlling CMV infection. This response preserves ovarian tissue integrity and function, providing a species survival advantage. These findings reveal a fundamental aspect of ovarian immune defense, with potential implications for understanding fertility disorders and individual susceptibility to reproductive tract infections.

*Selected oral presentations – **SESSION 2 – BRIGHT SPARK***

*S2-BS1 **Low Grade Systemic Inflammation Induces Osteoarthritis in Mice Accompanied by Accumulation of Osteochondroprogenitors in Early Time Points***

Tomislav Balen[1,2], Sara Priselac[1,2], Pavao Planinić[2,4], Marta Radošević[2,3], Ivo Krešić[2,4], Sara Aničić[2,3], Katerina Zrinski Petrović[1, 2], Darja Flegar[2,3], Tomislav Kelava[2,3], Alan Šućur[2,3], Danka Grčević[2,3], Nataša Kovačić[1,2]

[1]Dept. of Anatomy, School of Medicine, University of Zagreb, Croatia; [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia; [3]Dept. of Physiology and Immunology, School of Medicine, University of Zagreb, Croatia; [4]Dept. of Physiology, School of Medicine, University of Mostar, Bosnia and Herzegovina

Introduction: Osteoarthritis (OA) is a complex joint disease driven by multiple factors, including mechanical trauma, inflammation and impaired regeneration. This study aims to characterize inflammatory and osteochondroprogenitor populations during OA induced by collagenase (CIOA), and investigate whether systemic inflammation alone is sufficient to initiate knee OA.

Methods: CIOA was induced in 12-16–week-old C57BL/6 male mice by two intraarticular injections of 1U collagenase type VII in 10µl of 0.1M PBS (right OA knee) or 10µl of 0.1M PBS (left control knee) separated by 48h. Mice were sacrificed on day 3, 7 and 14. Systemic inflammation was induced by subcutaneous injection of 100uL complete Freund's adjuvant. Mice were sacrificed on day 10, 20, and 30 post-injection. Knees were analyzed by uCT or flow cytometry, after labelling with antibodies to main hematopoietic (CD3, CD8, CD11b, Ly6G, F4/80) and osteochondroprogenitor (CD51, CD200, CD90, CD105) markers.

Results: Early CIOA (days 3-7) is characterized by subchondral osteopenia and accumulation of myeloid-lineage (CD11b+, Ly6G+, F480+) and osteochondroprogenitor populations (CD200+, CD90.2+) in injected knees. At day 14, all populations except CD11b+Ly6G+ cells were similar to control. Initial systemic inflammation (day 10) was characterized by increased frequency of CD200+ osteochondroprogenitors and thinning of subchondral epiphyseal bone. Accumulation of myeloid cells (CD11b+, Ly6G+, F4/80+) appeared at day 30 post-injection, accompanied by sclerotic changes in tibial subchondral bone.

Conclusion: Synovial inflammation and accumulation of osteochondroprogenitor populations are hallmarks of early CIOA. Alterations of subchondral bone observed in systemic inflammation point to its potential role in initiation or progression of OA.

*Selected oral presentations – **SESSION 2 – BRIGHT SPARK***

*S2-BS2 **ILC1s Mediate Control of Perinatal Murine Cytomegalovirus Infection via NKG2D***

Iva Vladić, Carmen Rožmanić, Berislav Lisnić, Vanda Juranić Lisnić, Lucija Šakota, Stipan Jonjić, Ilija Brizić

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

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 (Rožmanić et al, 2023. Nat Commun). 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. First, we characterized NK cells during ontogeny by performing RNA-seq analysis of NK cells at different postnatal time-points and compared them to NK cells of adult mice. Transcriptional analysis demonstrated that NK cells in early neonatal life display enhanced cytokine and diminished cytotoxic capacity. At the same time, expression of a set of NK cell receptors, including NKG2D, was similarly expressed by NK cells in early life and adulthood. In addition to NK cells, ILC1s express NKG2D. To investigate the capacity of NK cells to control MCMV using NKG2D, we infected neonatal mice with an MCMV mutant lacking the immune evasion gene m145 (Δm145), which promotes intracellular retention of NKG2D ligand MULT-1. Depletion of NK1.1+ cells (NK cells and ILC1s), resulted in increased titers of MCMV following infection of neonatal mice. To discriminate the role of ILC1s and NK cells in control of Δm145 MCMV infection, we used NCR1iCreEomesfl/fl mice, in which Eomes is conditionally deleted in NKp46+ cells, resulting in a significantly reduced NK cell number. Interestingly, these experiments demonstrated that ILC1s confer viral control via NKG2D, indicating that this pathway could have played a role during host–virus coevolution. Overall, our study identifies a novel mechanism of neonatal antiviral immune defense, revealed upon disruption of viral immune escape mechanisms.

Rožmanić, Carmen et al. "Perinatal murine cytomegalovirus infection reshapes the transcriptional profile and functionality of NK cells." Nature communications vol. 14,1 6412. 12 Oct. 2023, doi:10.1038/s41467-023-42182-w

*Selected oral presentations – **SESSION 2 – BRIGHT SPARK***

S2-BS3 Targeting PD-L1 to Boost T Cell–Mediated Killing in HCC: An In Vitro Analysis

Luca Grisetti[1], Paola Tarchi[2], Paola Kučan Brlić[3,4], Claudio Tiribelli[3], Devis Pascut[3]

[1]National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte, Italy
[2]Surgical Clinic, Azienda Sanitaria Universitaria Giuliano Isontina (ASUGI), Trieste, Italy
[3]Liver Cancer Unit, Fondazione Italiana Fegato ONLUS, AREA Science Park Campus Basovizza, Trieste, Italy
[4]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

PD-L1/CD274 is an immune checkpoint that enables tumor immune evasion by binding PD-1 on T cells. Immune checkpoint inhibitors (ICIs) targeting this axis, including atezolizumab and durvalumab, have improved outcomes in advanced hepatocellular carcinoma (HCC), particularly in combination regimens. However, in vitro data on PD-L1 blockade and $CD8^+$ T cell–mediated cytotoxicity in HCC remain limited.

We observed higher PD-L1 protein expression in tumor tissue compared to matched adjacent non-tumoral liver in 59% of patients (n=56, $p<0.05$, Western blot). Among HCC cell lines, PD-L1 surface expression (flow cytometry) was elevated in S1/TGFβ-Wnt–activated lines (JHH6, HLE, HLF) and minimal in S2/progenitor lines (Huh7, HepG2), consistent with DepMap total protein data. In JHH6 cells, atezolizumab or durvalumab (10μg/ml) completely bound surface PD-L1 at all tested time points (1–48h) without altering PD-L1 mRNA expression.

PBMCs from healthy donors were activated with CD3/CD28 beads and co-cultured with HCC cells. $CD8^+$ T cells upregulated CD69 and PD-1 after 24h, confirming activation. Impedance-based cytotoxicity assays (Maestro Z) revealed E:T-dependent killing with KT50 values of 72h (1:2), 48h (1:1), and 20h (1:0.5). ICIs increased target cell apoptosis at 4h (1.3–1.6-fold, Annexin V/PI) and enhanced PBMC-mediated killing at 60–72h (8–12% reduction in viability). Pre-treatment with IFN-γ (10 ng/ml), which upregulates PD-L1 expression, further accelerated killing and boosted ICI efficacy to 11–19% at 24h.

In conclusion, PD-L1 is upregulated in HCC and enriched in S1-like HCC and its blockade enhances $CD8^+$ T cell–mediated cytotoxicity, supporting ICI use in immunologically active HCC subtypes.

*Selected oral presentations – **SESSION 3***

S3-O1 Notch Signaling Modulates Human Trilineage Monocyte Progenitor Differentiation Under Inflammatory Conditions

Sara Aničić[1,2], Marta Radošević[2], Maša Filipović[1,2], Darja Flegar[1,2], Pavao Planinić[4], **Ivo Krešić**[4], Sara Priselac[2,3], Tomislav Balen[2,3], Ozana Jakšić[1,2], Terezija Knežević[1,2], Tomislav Kelava[1,2], Nataša Kovačić[2,3], Alan Šućur[1,2], Danka Grčević[1,2]

[1]Dept. of Physiology and Immunology, School of Medicine, University of Zagreb, Croatia; [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia; [3]Dept. of Anatomy, School of Medicine, University of Zagreb, Croatia; [4]Dept. of Physiology, School of Medicine, University of Mostar, Bosnia and Herzegovina

Human monocyte progenitors are bone-marrow derived cells with potential to differentiate into effector cells that contribute to pathogenesis of various inflammatory diseases. We identified peripheral blood trilineage monocyte progenitor with the ability to differentiate into macrophages, osteoclasts and dendritic cells (DCs). Considering the established role of Notch signaling in hematopoietic lineage commitment, we examined how Notch activation influences differentiation of human monocyte trilineage progenitor under inflammatory conditions. Progenitors were FACS sorted from peripheral blood mononuclear cells and differentiated in vitro under lineage-specific conditions – M-CSF for macrophages, GM-CSF/IL-4 for DCs, M-CSF/RANKL for osteoclasts – under the influence of immobilized Notch ligands (DLL1, JAG1) and inflammatory stimulation (LPS). Differentiation was assessed through flow cytometric and qPCR analysis of lineage-specific markers, alongside functional assays – tartrate-resistant acid phosphatase (TRAP) activity for osteoclasts, phagocytosis for macrophages and antigen-presentation for DCs. Trilineage monocyte progenitors (CD45+CD15-CD3-CD19-CD56-CD11b+CD14++) express Notch-receptors, confirming their responsiveness to Notch-stimulation. Sorted progenitors efficiently differentiated into functional macrophages, DCs and osteoclasts in vitro. JAG1 and DLL1 suppressed osteoclastogenesis and reduced the expression of osteoclast markers (CD11b, CD206) and genes (RANK, CTSK, CSFR1), while upregulating negative regulator IRF8. DLL1 also impaired macrophage phagocytosis, but enhanced MHC-II and CD1c expression, indicating increased antigen-presenting capacity. DLL1-stimulated DCs showed higher co-stimulatory molecule CD40 expression and enhanced antigen-presentation to autologous T lymphocytes.

Collectively, results suggest that Notch signaling inhibits osteoclast activity and macrophage phagocytosis while promoting DC and macrophage antigen-presenting features – highlighting its role in regulating monocyte progenitor lineage commitment during inflammation and inflammation-induced bone loss.

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*Selected oral presentations – **SESSION 3***

S3-O2 Adiponectin is an Endocrine Modulator of CD8⁺ Memory T Cells

Ozren Majstorović, Inga Kavazović, Sanja Mikašinović, Karlo Mladenić, Felix M. Wensveen

Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

Adiponectin is an adipokine abundantly expressed in adipose tissue and its decreased levels are linked to metabolic disorders such as type 2 diabetes (T2D). While endocrine hormones exert immune-modulatory effects that vary depending on the cell type, the specific effects at different stages of the immune response remain largely unknown. Given that T2D is also associated with increased susceptibility to infections, this study aimed to investigate the endocrine control adiponectin might exert on CD8 T-cells function.

OT-1 T-cell receptor transgenic cells were used to confirm the mRNA expression of different hormone receptors via RNAseq. The cells were stimulated by N4/anti-CD28 and differentiated into effector and memory CD8+ cells using IL-2 or IL-15, respectively, without or in presence of adiponectin. The expression of activation and differentiation markers, proliferation, survival, and cytokine production were confirmed using flow cytometry, while cellular metabolic function was measured using Seahorse analysis (Agilent Technologies).

OT-1 cells showed to constitutively express both adiponectin receptors (AdipoR1 and AdipoR2) with AdipoR2 being upregulated in activated cells and kept upregulated during memory formation. Treatment with adiponectin resulted in increased IFN-γ production of memory cells, as well as a concentration dependent increase in extracellular acidification rate (ECAR). No effect on cell survival or proliferation was observed.

This study demonstrates that adiponectin exerts endocrine control on memory CD8 T cell function by enhancing IFN-γ production and favoring glycolytic over mitochondrial metabolism, suggesting that adiponectin deficiency in T2D might represent an important underlying cause of impaired immune cell functionality.

*Selected oral presentations – **SESSION 3***

S3-O3 Immune System-Mediated Changes in Amino Acid Metabolism Enhance the Immune Response During Viral Infections

Sanja Mikašinović[1], Martina Brnjić[2], Felix M Wensveen[1], Bojan Polić[1], **Marko Šestan[1]**

[1]*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*
[2]*Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia*

Everyone knows that an infection can make us feel sick. While we often view infection-induced metabolic changes as pathological, they are part of a carefully regulated process that relies on tissue-specific interactions between the immune system and organs responsible for maintaining systemic homeostasis. Immune-mediated changes in homeostatic parameters lead to altered production and uptake of nutrients in the bloodstream, which modifies the metabolic rate of key organs. This is what we experience as being sick. The purpose of sickness metabolism is to create a metabolic environment where the body can effectively combat infection while depriving pathogens of the essential nutrients needed for their replication.

The effects of infections on systemic metabolism and the physiological reasons for these changes are best understood in relation to blood glucose and lipid levels. However, the impact of viral infections on amino acids has been poorly investigated. Additionally, the purpose and underlying mechanisms of this process remain largely unknown. Therefore, by utilizing cutting-edge immunology and metabolic approaches we uncover how unconventional multi-organ, immuno-metabolic circuits regulate amino acid metabolism following viral infection. We also explored whether activating these circuits can be used to prevent viral replication, especially as there are still no highly effective antiviral drugs.

*Selected oral presentations – **SESSION 4***

S4-O1 IGF-1 Receptor Signaling Promotes CD8 Memory T Cell Formation and Enhances Tumor Control In Vivo

Sanja Mikašinović, Marko Šestan, Bojan Polić, Felix M. Wensveen

Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

The interplay between the immune and endocrine systems is essential in preserving metabolic homeostasis and protecting tissues during infection. When a viral infection occurs, the immune system induces metabolic changes that contribute to “sickness behavior”, enhancing the body’s ability to combat the pathogen. Moreover, endocrine hormones modulate immune cell function, exerting both stimulatory and inhibitory effects. Despite their importance, many of these immunomodulatory effects of hormones remain poorly understood.

Insulin and insulin-like growth factor 1 (IGF-1) are two hormones that are cross-reactive for each other's receptors. Previously, we demonstrated that insulin stimulation enhances cytokine production by CD8 T cells; however, we also found that this effect occurs in cells deficient in the insulin receptor (IR). This indicates that insulin itself does not impact the anti-viral CD8 T cell response through the insulin receptor. Instead, our data revealed that CD8 T cells in the memory phase express high levels of the IGF1R, which binds insulin with low affinity. Stimulation with IGF-1 enhanced IFNγ production and glycolytic activity in memory CD8 T cells. In vivo, IGF-1 signaling was critical for the generation of functional memory CD8 T cells. Mice lacking IR and IGF1R on CD8 T cells showed impaired memory responses and failed to control B16 melanoma.

Our results indicate that IGF-1R signaling is important for shaping memory T cell function and tumor control. Disruption of hormonal control of the antiviral response in the context of metabolic disease might explain increased susceptibility to pathogens in individuals with metabolic disorders, such as diabetes.

*Selected oral presentations – **SESSION 4***

S4-O2 Evaluating Nectin-2 and Nectin-4 as Immunotherapeutic Targets in Hepatocellular Carcinoma

Paola Kučan Brlić[1,4], Ema Bellulovich[1], Mijo Golemac[1], Stipan Jonjić[1], Tihana Lenac Roviš[1], Luca Grisetti[2], Paola Tarchi[3], Claudio Tiribelli[4], Devis Pascut[4]

[1]*Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia*
[2]*National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte, Italy*
[3]*Surgical Clinic, Azienda Sanitaria Universitaria Giuliano Isontina (ASUGI), Trieste, Italy*
[4]*Liver Cancer Unit, Fondazione Italiana Fegato-ONLUS, Trieste, Italy*

Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, highlighting the urgent need for novel immunotherapeutic targets. This study explores the potential of Nectin-2 and Nectin-4, two members of the nectin family of immunoregulatory proteins, as candidates for antibody-based therapies in HCC.

Nectin-4 expression was found to be low or absent in most HCC cell lines and early stage HCC tissue samples analyzed, with limited membranous staining observed by immunohistochemistry (IHC). While this suggests limited therapeutic potential in our cohort, it does not exclude a role for Nectin-4 in advanced or metastatic disease, where previous studies have reported increased expression, particularly in the cytoplasmic compartment, which may also hold clinical relevance.

Conversely, Nectin-2 showed consistently high expression across HCC cell lines and tumor tissues, with a clear membranous localization in tumor cells and lower expression in adjacent non-tumor tissues. IHC scoring demonstrated a significant correlation between high Nectin-2 expression and reduced disease-free survival, though no association with overall survival was observed.

Functional assays targeting the Nectin-2 axis by anti-CD112R antibodies using real-time cell analysis based on impedance measurements, revealed donor-dependent enhancement of cytotoxicity, with variability potentially influenced by immune cell composition and CD112R expression on the effector cells.

In conclusion, our findings suggest that while Nectin-4 may be of limited relevance in early-to-intermediate stage HCC, Nectin-2 shows promise as an immunotherapeutic target, supported by its expression profile and prognostic value. However, inter-donor variability in immune response underscores the need for a personalized medicine approach when targeting the Nectin-2/CD112R axis in HCC.

*Selected oral presentations – **SESSION 4***

*S4-O3 **Unknown Side Effect of Janus Kinase 1 Inhibitor: IgE Hyperproduction, Case Report***

Asja Stipić Marković, Alemka Markotić

University Hospital for infectious diseases "Dr Fran Mihaljević", Zagreb, Croatia

Introduction: Upadacitinib, a Janus kinase 1 inhibitor, is effective in treating immune-mediated inflammation in atopic dermatitis (AD). It does not significantly alter total IgE in patients in whom it is often elevated. The normal range of serum IgE is 2-214 IU/mL. When the levels exceed 2000 IU/mL, they are considered "hyper IgE". We report the case of the patient in whom IgE was extremely raised after upadacitinib therapy.
Case description: The patient is a 19-year-old male with a medical history of AD allergic rhinitis and IgA deficiency. At the age of 15, his flares worsened without response to steroids. In past laboratory findings, IgE was 911 kU/ml, specific IgE positive to grasses and dog dander in 5 class. At the age of 17 upadacitinib was introduced. After several months marked AD deterioration, onset of urticaria and several febrile episodes of unknown etiology developed. Our laboratory evaluation revealed a markedly elevated total IgE level of 17.211 IU/mL with unchanged profile of specific IgE. Eosinophil percent was 9,1% eosinophilic cationic protein 102 μg/L, no signs of helminth infection. We have withdrawn upadacitinib, prescribed steroid, cephalexin, antihistamine and topical therapy. Slowly, IgE was decreasing: 15.130 IU/mL, 10.098 IU/ml, 8933 IU/mL, ECP 41 μg/L, eosinophils 6,5%. Patient was switched to IL-4 inhibitor, dupilumab.
Conclusion: We showed extreme IgE elevation after upadacitinib therapy and resolution of symptoms and lowering IgE after treatment withdrawn.

POSTER SESSION

No.	Presenter	Title
P1	**Karlo Mladenić**	*The IL-17A and IFN-γ dichotomy in MASLD compromises the immune response to mCMV infection*
P2	**Pavao Planinić**	*Osteoimmune and Mesenchymal Shifts in a Murine Model of Cholestatic Liver–Bone Disease*
P3	**Marta Radošević**	*Immune dysregulation and enhanced osteoclastogenesis in a mouse model of streptozotocin induced type 1 diabetes*
P4	**Inga Kavazović**	*Optimal Diabetes Management is Associated with Decreased Cytokine Production by Cytotoxic Lymphocytes*
P5	**Vedrana Jelenčić**	*Role of immune cells in regulating ketogenesis*
P6	**Lucija Mušak**	*Peripheral Cues Shape CNS Autoimmunity: Dietary Modulation of Immunity and Disease Outcome in EAE*
P7	**Fran Krstanović**	*Immune surveillance of latent virus in the brain*
P8	**Ivo Krešić**	*Psoriatic Arthritis and Ankylosing Spondylitis are driven by functionally different monocyte phenotypes*
P9	**Alojzija Brčić**	*Ribonucleotide reductase knockdown enhances monocytic differentiation in response to metabolic perturbation but not genotoxic stress*
P10	**Ema Bellulovich**	*Novel Antibodies Reveal Expression Patterns of Soluble and Tissue PVR in Urogenital Cancers*
P11	**Maja Cokarić Brdovčak**	*Development of Novel Monoclonal Antibodies Targeting Viral Proteins*
P12	**Beata Halassy**	*Quality Attributes of Mumps Virus and Vesicular Stomatitis Virus Preparations Developed for the Oncolytic Virotherapy Application*
P13	**Beata Halassy**	*Spontaneous Canine Mammary Tumors as a Platform for Human Breast Cancer Virotherapy Development*
P14	**Tanja Košutić Gulija**	*Monitoring the Viral Samples' Composition During the Wet-Lab Procedure for RNA-Seq*
P15	**Stefano A. Garagnani**	*HCMV Induces Senescence and Paracrine Inflammation in Human Endothelial Cells*
P16	**Francesca Vaino**	*Unveiling the Role of PTX4 in Viral Infections: Insights from Murine Models*

P1 *The IL-17A and IFN-γ Dichotomy in MASLD Compromises the Immune Response to mCMV Infection*

Karlo Mladenić, Felix M. Wensveen, Bojan Polić

Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

MASLD (Metabolic dysfunction-associated steatotic liver disease), formerly known as NAFLD, is a liver condition linked to metabolic syndrome and ranges from fat accumulation to cirrhosis. Chronic inflammation plays a central role in its progression. While inflammation is known to impair immune responses in metabolic diseases like type 2 diabetes, its impact on viral infections in MASLD remains poorly understood. Emerging evidence shows MASLD patients are more susceptible to severe infections, though the mechanisms are unclear.

In our previous work, we found elevated levels of IL-17A-producing cells in the liver and blood of both MASLD patients and mouse models, suggesting a systemic skewing toward a type 3 immune response. We hypothesized that this shift impairs the type 1 immune polarization needed to fight viral infections.

To test this, we induced MASLD in mice using a steatosis and steatohepatitis diet (SSD). Following mCMV infection, SSD-fed mice showed increased IL-17A levels in the liver and spleen, reduced IFN-γ production by liver NK cells, and higher viral loads compared to controls on a normal chow diet (NCD). We found that NK cells, key players in early viral defense, expressed more IL-17 receptors in SSD-fed mice. In vitro, IL-17A stimulation directly suppressed IFN-γ production by NK cells.

These results suggest that MASLD promotes a type 3 immune bias that weakens the type 1 response required to control viral infections—potentially explaining the increased infection risk in MASLD patients.

P2 *Osteoimmune and Mesenchymal Shifts in a Murine Model of Cholestatic Liver–Bone Disease*

Pavao Planinić[1], Ivo Krešić[1], Sara Aničić[2,3], Darja Flegar[2,3], Alan Šućur[2,3], Nataša Kovačić[2,4], Danka Grčević[2,3], Marta Radošević[2,3], Tomislav Balen[2,4], Sara Priselac[2,4], Antonio Markotić[1], Ivan Ćavar[1], Tomislav Kelava[2,3]

[1]Department of Physiology, School of Medicine, University of Mostar, Bosnia and Herzegovina; [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia; [3]Department of Physiology, School of Medicine, University of Zagreb, Croatia; [4]Department of Anatomy, School of Medicine, University of Zagreb, Croatia

Chronic liver diseases are commonly associated with osteoporosis, with the extent and mechanisms of bone loss varying by disease type. Cholestatic liver fibrosis, modeled in mice using a 0.025% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet, induces particularly severe skeletal changes. This study investigated mechanisms of bone loss and the roles of myeloid osteoclast progenitors (OCPs) and non-hematopoietic osteochondroprogenitors.

Female C57BL/6 mice were fed either a control or DDC-supplemented diet for 4–8 weeks. Bone microarchitecture was assessed via micro-computed tomography, evaluating trabecular (BV/TV, Tb.Th, Tb.N, Tb.Sp) and cortical (C.Th) parameters. OCPs ($CD45^+Ly6G^-CD3^-B220^-NK1.1^-CD11b^{\wedge}loCD115^+$) and osteochondroprogenitors ($TER119^-CD31^-CD45^-CD51^+CD200^+CD105^-$) were quantified by flow cytometry. To assess direct DDC effects, OCPs were cultured in RANKL/M-CSF osteoclastogenic media with varying DDC concentrations; differentiated osteoclasts were identified by TRAP staining.

DDC-treated mice developed liver fibrosis, confirmed by Sirius Red staining and increased hepatic Col1a1, Acta2, Krt19, and proinflammatory cytokine expression. Bone alterations included reduced femoral cortical thickness alongside increased femoral and vertebral trabecular volume. Despite a dose-dependent reduction of $TRAP^+$ osteoclasts in vitro, in vivo osteoclast activity was elevated, as indicated by increased TRAP levels (ELISA) and OCP

frequencies. Additionally, higher osteochondroprogenitor frequencies in DDC-treated mice suggest enhanced osteoblast lineage commitment may contribute to the observed bone phenotype.

These findings highlight complex, model-specific liver–bone interactions in cholestatic liver disease and suggest a dual contribution of osteoclast and osteoblast lineage alterations. Further investigation is needed to elucidate the underlying molecular mechanisms driving these skeletal changes.

P3 Immune Dysregulation and Enhanced Osteoclastogenesis in a Mouse Model of Streptozotocin Induced Type 1 Diabetes

Marta Radošević[1,2], Sara Aničić[1,2], Ozana Jakšić[1], Maša Filipović[1,2], Darja Flegar[1,2], Ivo Krešić[4], Pavao Planinić[4], Tomislav Kelava[1,2], Sanja Novak[5], Nataša Kovačić[2,3], Alan Šućur[1,2], Ivo Kalajzic[6], Danka Grčević[1,2]

[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia; [2]Department of Physiology and Immunology, School of Medicine, University of Zagreb, Croatia; [3]Department of Anatomy, School of Medicine, University of Zagreb, Croatia; [4]University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina [5]Department of Physiology and Cell Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA; [6]Center for Regenerative Medicine and Skeletal Development, UConn Health, CT, USA

Type 1 diabetes mellitus (T1D) is an autoimmune disease that results from destruction of pancreatic beta-cells, causing chronic hyperglycemia, systemic organ damage, and alterations in both innate and adaptive immune compartments. Osteoclast progenitors (OCPs), as part of the myeloid lineage, may be activated under chronic inflammatory conditions such as T1D, potentially enhancing bone resorption. This study aimed to investigate immune dysregulation and associated changes in the OCP pool in a T1D model.

T1D was induced in male C57BL/6 mice (11 weeks) by streptozotocin (50 mg/kg for 5 days). Bone marrow (BM) and spleen (SPL) were analyzed by flow cytometry at 3 weeks and 2 months post-treatment to assess immune populations and OCP subsets. Sorted OCPs were cultured in osteoclastogenic conditions (M-CSF/RANKL) to evaluate differentiation into TRAP-positive osteoclasts. CX3CR1CreERT2/Ai9fl/+ transgenic mice were used for in vivo lineage tracing of TdTomato-labeled OCPs. Bone resorption was quantified via serum TRAP5b and CTX-I immunoassay.

STZ-treated mice developed marked hyperglycemia (11.2 [7.8–15.9] mmol/L vs CTRL 7.7 [5.7–10.8], $p<0.001$) and showed expansion of myeloid/monocyte and B cell populations. Both BM and SPL OCPs were increased, with elevated CCR2 and CX3CR1 expression and higher TRAP5b levels (37.4 [32.9–55.4] vs 29.9 [27.2–39.7] U/L, $p<0.05$). STZ-derived OCPs generated more multinucleated TRAP+ osteoclasts in vitro, and TdTomato+ osteoclasts were enriched in tibial sections in vivo.

These findings demonstrate OCP expansion and enhanced osteoclastogenic potential in T1D, warranting further investigation into mechanisms driving their activation and recruitment.

P4 Optimal Diabetes Management is Associated with Decreased Cytokine Production by Cytotoxic Lymphocytes

Dora Gašparini[1], **Inga Kavazović[2]**, Felix M. Wensveen[2], Tamara Turk Wensveen[1,3,4]

[1]Center for Diabetes, Endocrinology and Cardiometabolism, Special Hospital for Medical Rehabilitation of the Heart and Lung Diseases and Rheumatism Thalassotherapia Opatija, Croatia; [2]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia; [3]Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Croatia; [4]Department of Endocrinology, Diabetes and Metabolic Diseases, Clinic of Internal Medicine, Clinical Hospital Center Rijeka, Croatia

Type 2 diabetes mellitus (T2D) is associated with a chronic low-grade inflammation, which is thought to be an important underlying risk factor for many of its life-threatening complications such as atherosclerosis, cancer and stroke. Modern oral anti-diabetic drugs have been associated with a reduced cardiovascular risk profile. Previously, we have shown that T2D causes a hyperinflammatory profile of the anti-viral immune system. Here we investigated whether oral anti-diabetic drugs ameliorate inflammation in patients with T2D. Patients with uncontrolled T2D (HbA1c>6.5) were recruited and treated with metformin and SGLT2 inhibitors alone or in combination with GLP1 mimetics. Cytokine production was analyzed before and 6 and 12 months after treatment. HbA1c levels were improved from 8.6 ±1.1% to 6.5 ±1.1%. Importantly, IFNγ and TNF production by these cells was significantly reduced, which was similar for all treatment groups. Changes in cytokine profiles were not associated with alterations in effector and memory CD8 T cell subsets. Sequencing results showed that CD8 T cells of people with T2D had reduced expression of SOCS1 and SOCS3, suggesting that their hyperresponsive phenotype is the result of impaired inhibitory signaling. Treatment with anti-diabetic drugs caused an increase in anti-inflammatory signaling molecules. Our findings provide clues why people with T2D have a hyperinflammatory profile and how treatment with modern anti-diabetic drugs may ameliorate this phenotype. Future therapy may therefore be directed towards enhancing the anti-inflammatory impact of these medications.

P5 Role of Immune Cells in Regulating Ketogenesis

Vedrana Jelenčić, Felix M. Wensveen, Bojan Polić

Department of histology and embryology, Faculty of Medicine, University of Rijeka, Croatia

Fasting is considered a voluntary abstinence from food and drink, which can be short-term (up to 24 hours) or long-term (up to 72 hours). Recently, fasting and ketogenic diets have been used for therapeutic purposes in diseases such as epilepsy and tumors. The body utilizes its nutrient stores to provide the necessary energy to maintain organ function during fasting. A key fasting mechanism is converting free fatty acids released by adipose tissue into ketones in the liver. Until recently, hepatocytes were considered the only cell type responsible for recognizing the need to increase ketogenesis during fasting. In this study, we examine the role of immune cells, with a focus on innate immune cells, as key mediators capable of rapidly sensing and responding to environmental changes. Our findings suggest that macrophages and γδ T cells play a role in regulating ketogenesis during fasting.

P6 Peripheral Cues Shape CNS Autoimmunity: Dietary Modulation of Immunity and Disease Outcome in EAE

Lucija Mušak[1], Christoforos Dimitropoulos[2], Chiara Romagnani[2], Marina Babić[1]

[1]*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*
[2]*Institute of Medical Immunology, Charité Unversitätmedizin Berlin, Germany*

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system involving immune-mediated demyelination and neurodegeneration. Central to its pathogenesis is the dysregulation of lymphocytes, particularly autoreactive T and B cells, and myeloid cells, which are activated in the periphery and infiltrate the CNS, driving neuroinflammation. Key immunological signals such as cytokines, chemokines, and co-stimulatory molecules coordinate immune cell trafficking and effector functions. Recent research highlights the critical role of peripheral cues, such as diet, systemic inflammation, and infections, in modulating immune cell behavior and disease susceptibility.

Our research investigates how dietary interventions impact immune responses during neuroinflammation, focusing on $CD4^+$ T cells and myeloid cell dynamics. Using the experimental autoimmune encephalomyelitis (EAE) model, we assessed the effects of different dietary regimens on disease outcome. Preliminary findings show that mice on a high-fat diet (HFD) unexpectedly exhibit improved clinical outcomes compared to those on a standard diet (SD), while an alternating diet yields an intermediate phenotype. Notably, intermittent fasting with SD does not provide protection.

These results suggest that both diet composition and consistency influence immune modulation. Our ongoing studies aim to define how peripheral metabolic and infectious environments shape immune phenotypes, particularly autoreactive cells and antigen-independent bystander $CD4^+$ T cells, in EAE/MS pathology.

Altogether, our findings support the concept that dietary variability, or "noise", may reduce therapeutic efficacy, whereas stable dietary patterns may better modulate chronic inflammation. This has potential clinical implications for dietary strategies in autoimmune disease management.

P7 Immune Surveillance of Latent Virus in the Brain

Andrea Mihalić[1#], **Fran Krstanović**[1#], Daria Kveštak[1], Berislav Lisnić[1], Vanda Juranić Lisnić[1], Isabell Bochow[2], Shirin Hosseini[2,3], Katarzyna M. Sitnik[4,5], Luka Čičin-Šain[4,6], Stipan Jonjić[1], Ilija Brizić[1]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia; [2]Department of Cellular Neurobiology, Zoological Institute, Technische Universität Braunschweig, Germany; [3]Helmholtz Centre for Infection Research, Research Group Neuroinflammation and Neurodegeneration, Braunschweig, Germany; [4]Department of Viral Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany; [5]Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Austria; [6]Centre for Individualized Infection Medicine, a joint venture of the Helmholtz Centre for Infection Medicine and Hannover Medical School, Hannover, Germany

[#]authors contributed equally to this work

Herpesviruses establish lifelong latency in host tissues. Here, we employed cytomegalovirus (CMV) infection to investigate the immune surveillance of latent infection in the brain. We show that following perinatal infection of mice, mouse CMV (MCMV) can persist in neurons and that CD4+ T-cell-derived interferon-gamma is critical in restricting active viral replication in this cell type. Simultaneously, chronic MCMV infection in the brain drives a persistent state of microglial priming, characterized by IFN-γ-dependent microglia activation and extensive transcriptional reprogramming at the single-cell level. Primed microglia provide enhanced control of latent infection and superior recall responses, but are associated with excessive loss of synaptic dendritic spines mediated by primed microglia. Together, these findings reveal a coordinated but double-edged immune strategy in the CMV-infected brain: CD4+ T cells, IFN-γ, and microglial activation act in concert to contain latent virus, yet their persistent engagement disrupts neuronal structure and function.

P8 Psoriatic Arthritis and Ankylosing Spondylitis are Driven by Functionally Different Monocyte Phenotypes

Ivo Krešić[1], Pavao Planinić[1], Sara Aničić[3,4], Marta Radošević[3,4], Sara Priselac[4,5], Tomislav Balen[4,5], Katerina Z. Petrović[4], Danka Grčević[3,4], Tomislav Kelava[3,4], Nataša Kovačić[4,5], Marina I. Matijaševi[2], Ivan Ćavar[1], Alan Šućur[3,4]

[1]Department of Physiology, School of Medicine, University of Mostar, Bosnia and Herzegovina; [2]"Sveti Duh" University Hospital, Zagreb, Croatia; [3]Department of Physiology, School of Medicine, University of Zagreb, Croatia; [4]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia; [5]Department of Anatomy, School of Medicine, University of Zagreb, Croatia

Introduction: Monocytes play a crucial role in the pathogenesis of spondyloarthropathies (SpA), such as ankylosing spondylitis (AS) and psoriatic arthritis (PsA), by differentiating into key inflammatory cells. Among these, monocyte-derived dendritic cells (moDCs) are of particular interest due to their close interaction with T-cells via the IL-12-23/IL-17 axis. This study aims to analyze the expression of DC markers on blood monocytes, evaluate changes upon their differentiation into DCs, and test their subsequent functionality.
Methods: PBMCs were isolated from patients with active PsA, AS, and healthy controls. Using flow cytometry, we analyzed a panel of markers for monocyte lineage (CD14, CD16), DC differentiation (CD1a, CD1c, CD141, CD206, CD209), antigen presentation (CD40, MHCII), and costimulation (CD80, CD86). Sorted classical monocytes were cultured with DC-inducing factors, IL-17, or both; a control group underwent spontaneous differentiation. Phagocytic ability was tested via pHrodo assay, and antigen-presenting capacity was assessed in a moDC:T-cell co-culture by measuring cytokine production.
Results: Fresh PsA monocytes expressed higher levels of CD206, CD209 and CD1a. In vitro, spontaneously differentiated AS monocytes showed lower CD141 and higher CD40. AS-derived moDCs had heightened phagocytosis but produced less IL-12/23, resulting in lower T-cell IFN-γ and TNF-α production. Conversely, PsA-derived moDCs showed higher expression of costimulatory (CD86) and dendritic (CD1c) markers. Across all groups, IL-17 supplementation increased CD209 while decreasing CD141 and CD1a expression.
Conclusion: Our findings reveal distinct pathological pathways in SpA. In psoriatic arthritis, monocytes and moDCs show a phenotype skewed towards heightened antigen presentation. In contrast, cells in ankylosing spondylitis display a profile characterized by enhanced phagocytosis but a reduced capacity to stimulate T-cell responses, suggesting different primary mechanisms of immune dysfunction between these two diseases.

P9 Ribonucleotide Reductase Knockdown Enhances Monocytic Differentiation in Response to Metabolic Perturbation but not Genotoxic Stress

Alojzija Brčić[1], Hrvoje Lalić[1], Vilma Dembitz[1], Tomislav Smoljo[1], Romana Penker[2], Giovanny Rodriguez Blanco[2], Dora Visnjic[1]

[1]*Department of Physiology and Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia*
[2]*Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria*

Disruption of nucleotide metabolism can trigger monocytic differentiation in acute myeloid leukemia (AML) cells. We previously showed that metabolic stressors such as 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) and brequinar promote differentiation through depletion of nucleotide pools and induction of replication stress. Cytarabine (AraC), a nucleoside analog used in AML therapy, also induces monocytic differentiation but via direct incorporation into DNA causing genotoxic replication stress. Here, we examined the contribution of ribonucleotide reductase (RNR), a key regulator of deoxynucleotide pool homeostasis, to AML differentiation driven by these inducers. Metabolomic profiling confirmed nucleotide depletion following AICAr and brequinar treatment, whereas AraC increased nucleotide levels. Notably, all three agents, AICAr, brequinar, and AraC, upregulated the RNR subunit RRM2 in U937 cells, consistent with a replication stress response. SiRNA-mediated knockdown of RRM2 enhanced differentiation in response to AICAr and brequinar. In contrast, RRM2 knockdown had no effect on AraC-induced differentiation, indicating mechanistic divergence. Supplementation with uridine reversed differentiation induced by AICAr and brequinar, but not AraC. However, supraphysiological levels of deoxyribonucleosides inhibited differentiation by all three agents, suggesting that overall nucleotide imbalance, not a specific nucleoside, underlies the differentiation response. These findings indicate that RNR downregulation sensitizes AML cells to replication stress driven by metabolic, but not genotoxic, perturbation. Modulating nucleotide metabolism in combination with RNR inhibition may enhance differentiation therapy in AML.

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P10 Novel Antibodies Reveal Expression Patterns of Soluble and Tissue PVR in Urogenital Cancers

Ema Bellulovich[1] *, Paola Kučan Brlić*[1] Mijo Golemac [1], Ante Jakšić[2], Dean Markić[2], Karmela Miklic[1], Suzana Malic[1], Emina Babarović[3], Gordana Đorđević[3], Berislav Lisnić[1], Pini Tsukerman[4], Guy Cinamon[4], Stipan Jonjić[1], Tihana Lenac Roviš[1]

[1]*Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia*
[2]*Department of Urology, Clinical Hospital Center Rijeka, Croatia*
[3]*Clinical Department of Pathology and Cytology, Clinical Hospital Center Rijeka, Croatia*
[4]*Nectin Therapeutics LTD, Jerusalem, Israel*

The poliovirus receptor (PVR), a member of the nectin-like family of proteins, is an adhesion molecule involved in various cellular processes such as migration, proliferation and contact inhibition. Furthermore, PVR functions as an immune checkpoint, limiting the immune response through its interaction with inhibitory and activating immune cell receptors, which is one of the key reasons it is being investigated as a potential therapeutic target. PVR is highly expressed in various tumor tissues, and its overexpression in tumors correlates with a poorer survival prognosis. In contrast, its expression in healthy tissues remains low. PVR exists in two main forms: membrane-bound PVR, which is anchored to the plasma membrane, and secreted PVR, produced through alternative mRNA splicing that omits the transmembrane domain. Additionally, proteolytic cleavage of the extracellular domain, known as "shedding," has been demonstrated for other immune checkpoints but has not yet been explored for PVR. To explore this further, we analyzed PVR expression in tissue biopsies and serum samples from 500 patients. Using immunohistochemistry we assessed its distribution, interpatient heterogeneity, and its association with clinicopathological parameters to determine whether PVR could be a potential therapeutic target for these cancers. We have developed antibodies and established ELISA protocols capable of distinguishing between the cleaved and secreted forms in patient serum, aiming to assess whether secreted or shed PVR could serve as a potential biomarker for urogenital tumors.

P11 *Development of Novel Monoclonal Antibodies Targeting Viral Proteins*

Maja Cokarić Brdovčak[1], Paola Kučan Brlić[1], Iva Vladić[1], Lucija Šakota[1], Karmela Miklić[1], Suzana Malić[1], Leonarda Mikša[1], Anto Vrdoljak[2], Ilija Brizić[1], Tihana Lenac Roviš[1]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia
[2]Vaxxinova Zagreb d.o.o., Zagreb, Croatia

In this study, we present the development and comprehensive characterization of a novel panel of monoclonal antibodies (mAbs) directed against five distinct viral glycoproteins of major poultry viral pathogens. These include hemagglutinin (HA) from avian influenza virus (AIV), VP2 from infectious bursal disease virus (IBDV), glycoproteins gI and gD from infectious laryngotracheitis virus (ILTV), and the fusion (F) protein from avian metapneumovirus (AMPV). These antigens were selected based on their critical roles as primary targets of virus-neutralizing antibodies. To generate these mAbs, recombinant forms of the selected viral glycoproteins were first expressed and purified using optimized protocols. These purified antigens were then used to immunize mice, thereby eliciting antigen-specific immune responses. Following immunization, hybridoma technology was employed to isolate and establish monoclonal antibody-producing cell lines. The resulting mAb clones were systemically screened to assess their binding affinity and specificity toward the corresponding recombinant glycoproteins.
This newly developed set of monoclonal antibodies represents a valuable resource for virological research. Owing to their high specificity and reliability, these antibodies 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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P12 *Quality Attributes of Mumps Virus and Vesicular Stomatitis Virus Preparations Developed for the Oncolytic Virotherapy Application*

Beata Halassy[1], Maja Jagušić[1], Adela Štimac[1], Sanda Ravlić[1], Jelena Ivančić Jelečki[1], Sanja Mateljak Lukačević[1], Tanja Košutić Gulija[1], Tihana Kurtović[1], Renata Jug[1], Ana Vučenović Mijatović[2], Maja Lang Balija[1], Dragan Jurić[3], Petra Gulan[1], Dubravko Forčić[1]

[1]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Croatia
[2]Institute of Immunology, Zagreb, Croatia; [3]Croatian Institute of Public Health, Zagreb, Croatia

Introduction: The mumps virus (MuV) is a human RNA virus from the Paramyxoviridae family. The vesicular stomatitis virus (VSV) is an animal RNA virus from the Rhabdoviridae family. Both viruses demonstrate a potent oncolytic activity in different preclinical but also early clinical studies. Due to its stringent host specificity, MuV seems as an ideal candidate for use in veterinary medicine since it does not cause disease, but successfully replicates in different animal cell cultures, tissues and organs. Due to the lack of pathogenicity in humans, VSV seems as an ideal candidate for use in human medicine, since it replicates in different human cell culture, tissues and organs.
Objectives: We aim to develop an appropriate methodology for reliable and precise characterization of MuV and VSV preparations, produced from supernatants of virus-infected Vero cell cultures. Another goal is to find the longest time frame and storage conditions in which properties of stabilizer-free oncolytic virus preparations do not significantly change.
Materials and methods: Compositional properties of several batches of either MuV or VSV samples were monitored: infective virus quantity (CCID50 assay); total particle (viruses and extracellular vesicles) quantity and size (NTA); host cell DNA (qPCR) and protein (ELISA) content; chromatographic fingerprint in the form of SEC chromatograms generated by the usage of UV, refractive index, fluorescence and light scattering detectors (HPLC), genomic composition of viral population (NGS) and microbial purity.
Results: Several successive MuV and VSV batches have been reproducibly prepared. MuV contains on average 107.7 CCID50/mL of infective virus and 109,9/mL total particle concentration ranging in size from 135 to 320 nm with the median size of dominant particle population being 208 nm. VSV infectivity was higher - 108.9 CCID50/mL in 1010.2/mL total particles ranging in size from 118 to 179 nm, with the median size being 140 nm. VSV is purer sample containing on average 24 μg/mL host cell proteins and 25 ng/mL host cell DNA, in contrast to MuV sample having 51 μg/mL and 450 ng/mL, respectively. NGS analyses of MuV and VSV batches indicates genomic stability of viral populations during the production process. Short-term storage of both virus samples at +4 °C and lower than -70 °C resulted in a detectable drop of infectivity, which was less pronounced at +4 °C.
Conclusion: The methodology developed and the data generated will contribute to the decision-making process in the developmental pathway of these two preparations for oncolytic virotherapy.

P13 Spontaneous Canine Mammary Tumors as a Platform for Human Breast Cancer Virotherapy Development

Maja Lang Balija[1], Maja Jagušić[1], Erika Gamulin[1], Renata Jug[1], Ivan Butković[2], Sanja Mateljak Lukačević[1], Tihana Kurtović[1], Dražen Vnuk[3], Andrea Gudan Kurilj[4], Lidija Medven Zagradišnik[4], **Beata Halassy**[1], Dubravko Forčić[1]

[1]Center for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Croatia; [2]Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Croatia; [3]Clinic for Surgery, Orthopedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Croatia; [4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, Croatia

According to the WHO, human breast cancer (HBC) is the most common malignant disease in women. In Europe, 557,532 new cases are reported each year, accounting for 26.4% of all new cancer diagnoses in women. In dogs, canine mammary tumors (CMT) account for up to 70% of all tumors in intact females, and approximately half of these cases are malignant.

HBC and CMT share many biological and molecular similarities. Because dogs develop tumors spontaneously, they represent a more realistic model than mice or other genetically engineered animals. Dogs share the same environment with humans, display same/similar intratumoral heterogeneity, and exhibit natural drug resistance. Oncolytic virotherapy has attracted attention in both human and veterinary medicine as a new treatment strategy for many types of cancer. The mumps virus (MuV) and Newcastle disease virus (NDV), both belonging to the Paramyxoviridae family, show strong oncolytic potential and are considered promising candidates for future cancer virotherapy.

The aim of this study is to investigate the oncolytic potential of MuV and NDV vaccine strains in various cultures of primary canine tumor tissue obtained through surgical extraction as well as in continuous human and canine breast cancer cell lines. We focus particularly on tumor-selective viral replication and its cytotoxic effects.

Both MuV and NDV exhibited strong replication across all in vitro model systems used with clear difference between viruses in terms of speed and intensity of their replication. We also found that both viruses are able to significantly decrease cell viability and induce apoptosis in both human and canine tumor cells.

Conclusion: MuV and NDV act as potent tumor cytolytic agents in various 2D and 3D cell cultures prepared from primary canine tumor tissue as well as from continuous human and canine breast cancer cell lines. These initial findings represent an important step toward the development of novel therapeutic strategies for hard-to-treat cancers in both species.

P14 Monitoring the Viral Samples' Composition During the Wet-Lab Procedure for RNA-Seq

Tanja Košutić Gulija, Maja Jagušić, Jelena Ivančić Jelečki, Dubravko Forčić, Tihana Kurtović, Sanja Mateljak Lukačević, Beata Halassy

Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Croatia

The wet-lab procedure for viral RNA sequencing (RNA-seq) consists of two main steps: RNA sample preparation and library construction.

Sample preparation involves the degradation of DNA and the extraction of RNA. When sequencing a virus obtained from the supernatant of infected cell cultures, the starting sample contains nucleic acids from cells that have been damaged due to viral budding, the formation of cytopathic effects, and natural cell aging and death. RNA isolated from such a sample contains mainly ribosomal RNA (rRNA). Depleting this rRNA is crucial, as its presence interferes with obtaining a sufficient number of viral RNA sequencing reads, which is particularly important when investigating sub-genomic variability of the virus.

According to the official protocol, to prepare high-quality samples for RNA-seq, it is essential to know the input of total RNA that is free from DNA contamination and to assess the library quality from the Bioanalyser DNA electropherogram.

The samples examined in this study were preparations of the mumps virus or vesicular stomatitis virus (VSV) obtained from the supernatant of infected Vero cell cultures. To ensure the quality and quantity of viral RNA required for successful downstream viral RNA-seq, we included monitoring of sample composition using in-house RT-qPCR or qPCR for detecting mumps virus, VSV, and 28S rRNA targets. Our final workflow procedure includes RT-qPCR monitoring at three key points: assessing the efficiency of DNA degradation step, evaluating the effectiveness of rRNA depletion, and checking the composition of the library.

qPCR results demonstrated the effectiveness of cell DNA degradation using the Denerase enzyme applied directly to the viral samples before RNA isolation. The depletion protocol successfully reduces the 28S rRNA target concentration to nearly undetectable levels with only a slight decrease of both viral RNA targets. qPCR data were compared against the viral titer of the starting sample, concentration of isolated total RNA, and concentration of residual DNA. qPCR results indicate that library composition can predict the quality of statistical data from RNA-seq analyses, which was not evident from the DNA electropherogram.

P15 HCMV Induces Senescence and Paracrine Inflammation in Human Endothelial Cells

Stefano A. Garagnani[1,2], Federica Castiglioni[1], Francesca Pezzoni[1], Anna Gerbi[1], Valeria Caneparo[1], Marta Catozzo[2], Irene Lo Cigno[2], Marisa Gariglio[1,2], Marco De Andrea[1,3]

[1]Intrinsic Immunity Unit, CAAD - Center for Translational Research on Autoimmune and Allergic Disease, University of Eastern Piedmont, Novara, Italy; [2]Molecular Virology Unit, Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy; [3]Viral Pathogenesis Unit, Department of Public Health and Pediatric Sciences, Turin, Italy

Human cytomegalovirus (HCMV) is an opportunistic pathogen that causes severe disease in immunocompromised hosts. Replication of its large DNA genome profoundly alters cellular homeostasis, inducing features reminiscent of replicative senescence. Senescent cells release a set of factors, collectively known as the senescence-associated secretory phenotype (SASP), which exert immunomodulatory effects. We previously showed that HCMV infection drives a senescence program in renal proximal tubular epithelial cells (RPTECs), where the SASP, largely sustained by IL-6, induces senescence in neighboring uninfected cells.

Here, we investigated the impact of HCMV infection in endothelial cells and its contribution to hyperinflammation and vascular dysfunction. Infected cells displayed clear virus-induced senescence (VIS) features, including reduced EdU incorporation and increased expression of p16INK4a. IL-6 levels were markedly elevated, accompanied by IL-8, another canonical senescence marker. To further define this phenotype, we analyzed NF-κB activation, γH2AX and p16INK4a in co-staining with viral proteins to discriminate infected from uninfected cells. We detected nuclear NF-κB in both infected cells and a subset of surrounding uninfected cells, while γH2AX and p16INK4a were exclusively present in infected cells, consistent with DNA damage and stable cell cycle arrest.

Finally, conditioned medium from HCMV-infected cells significantly decreased EdU incorporation in fresh HUVECs compared to controls, indicating a paracrine effect, as previously observed in RPTECs.

Overall, our results support a model in which HCMV induces cellular degeneration with senescence features in endothelial cells. These processes may vary across cell types, ultimately shaping organ-specific outcomes and contributing to HCMV-driven inflammatory and vascular pathologies.

P16 Unveiling the Role of PTX4 in Viral Infections: Insights from Murine Models

Francesca Vaino[1,3], Francesco Scavello[1], Domenico Supino[1], Virginia Cianchi[2], Jelena Železnjak[4], Irene Di Ceglie[1], Giovanni Pezzone[1], Anna Rigatelli[1], Marika Ventura[1], Giovanna Grieco[1], Silvia Carnevale[1], Elena Magrini[1], Sébastien Jaillon[1,2], Cecilica Garlanda[1,2]

[1]IRCCS Humanitas Research Hospital, Milan, Italy; [2]Department of Biomedical Sciences, Humanitas University, Milan, Italy; [3]Department of Medical Biotechnology, University of Siena, Italy; [4]Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia.

Pentraxins are a phylogenetically conserved family of proteins. Some members contribute to the humoral arm of innate immunity, acting as functional ancestors of antibodies by mediating agglutination, complement activation, and opsonization. They are classified as short (CRP, SAP) or long (PTX3, NP1, NP2) pentraxins.

In 2010, our group identified PTX4, a novel long pentraxin showing high structural homology with PTX3, a key component of humoral innate immunity (Martinez de la Torre Y, et al., 2010). Given the limited knowledge of PTX4's biological role, we hypothesized that it may contribute to the regulation of innate immune responses during viral infections.

To test this hypothesis, PTX4-deficient ($PTX4^{-/-}$) and wild-type (WT) mice were treated at multiple time points with Poly I:C (8 mg/kg i.p.), an agent that mimics viral infection. Samples were analyzed by ELISA and flow cytometry. In parallel, we assessed the responses to murine cytomegalovirus (MCMV) and influenza A/PR/8/34 (H1N1) infections using immunophenotyping and clinical monitoring.

Following Poly I:C stimulation, $PTX4^{-/-}$ mice showed a rapid increase in plasma CXCL10 levels, and elevated splenic lymphocytes compared with WT controls. In the MCMV infection model, immunophenotyping revealed no differences between experimental groups, but in vitro NK cells from $PTX4^{-/-}$ mice exhibited reduced cytokine production compared to WT after stimulation in the presence of Brefeldin. Finally, $PTX4^{-/-}$ mice infected with PR8 exhibited greater body weight loss.

Overall, these preliminary data suggest a potential role for PTX4 as a modulator of innate immune activation during viral infections, providing a basis for future studies to elucidate its biological function.

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