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Trogir
23-25.09.2021

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2021 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

Trogir, 23-25.09.2021

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY
University of Rijeka Faculty of Medicine

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Dear Friends and Colleagues,

Hereby I would like to welcome you all to the annual meeting of the Croatian Immunological Society. After almost two years of isolation, it is almost unreal to meet each other once again face to face. We have long hesitated whether a real-live meeting would be advisable. However, the primary role of our society is to bring Croatian immunologists together, to share scientific discovery and to work together to generate better results through synergy. This is something you simply cannot emulate from behind a computer screen. Besides, I am sure that we all have had our fill of online meetings. Of course, we must be careful, but we believe that with the appropriate measures, we can have both a safe and stimulating congress.

If the COVID-19 pandemic has taught us anything, it is the resilience of Croatian immunology. From our ramparts, we have risen to the challenge provided by the virus and have performed both excellent science and have tried to play our part in education of the public. Over the last year, HID members have published findings in some of the highest impact journals, including **Immunity**, **J Exp. Med.** and **PLoS Biol.** Moreover, we are proud to say that our **Prof. Bojan Polić** has been elected as **president elect of EFIS**, an organization that unites 35 immunological societies, representing over 14.000 researchers in Europe. Finally, it was hard to turn on the news and to not spot a HID member explaining the science behind viruses, the immune system or vaccines. I think that we can rightfully be proud on our collective effort of our members to use the knowledge we have to help our country. At the same time, we are faced by an unprecedented challenge. Scepsis against vaccination is at an all-time high, making many people refuse the very therapy that might save their lives. Media, politicians, and even highly seated scientists spread misinformation and doubt about the safety and efficacy of vaccines. As scientists, we are very aware of the limitations of our knowledge, also with regards to vaccines. Nevertheless, as scientists we should openly communicate what we do and do not know, without going into battle with people that make unfounded claims. In the coming year us immunologists in particular will therefore face some big challenges, not only scientifically, but also in how we let the public benefit from our knowledge.

These three days, however, we can rejoice in a ‘classical’ congress, full of science, friendship, and entertainment. We have done our best to set up a stimulating program that emphasizes the beauty of immunology and being an immunologist. We hope that you will enjoy it.

I wish you all a splendid meeting!

Felix M. Wensveen, President

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THURSDAY September 23rd 2021

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15:00-15:15 OPENING CEREMONY

Felix M. Wensveen, president of the Croatian Immunological Society

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15:15-15:45 INVITED LECTURE:

Chairs: Dora Višnjić & Mariastefania Antica

Beata Halassy

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

`*SARS-CoV-2 neutralization assay as a key premise for implementation of COVID-19 serotherapy in Croatia*`

15:45-16:45 SELECTED ORAL PRESENTATIONS – SESSION 1

Chairs: Dora Višnjić & Mariastefania Antica

15:45 **Maša Filipović**, University of Zagreb
Notch 1 inhibition increases osteoclast progenitor activity in the mouse model of rheumatoid arthritis

16:00 **Tina Ružić**, University of Rijeka
Characterization of M116.1p, a murine cytomegalovirus protein required for efficient infection of mononuclear phagocytes

16:15 **Dino Šisl**, University of Zagreb
What is the role of canonical Notch signalling pathway in liver fibrosis?

16:30 *Sponsored Lecture*

16:45-17:15 INVITED LECTURE:

Chairs: Dora Višnjić & Mariastefania Antica

Alenka Gagro

Croatia

`*Multisystem inflammatory syndrome in children*`

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09:00-09:30 INVITED LECTURE:

Chairs: Alenka Gagro & Ilija Brizić

Vanda Juranić Lisnić

Medical Faculty, University of Rijeka, Croatia

'Cytomegalovirus infection of ovaries and fertility maintenance.'

09:30-10:30 `BRIGHT SPARKS` ORAL PRESENTATIONS – SESSION 2

Chairs: Danka Grčević & Ilija Brizić

09:30 **Jelena Materljan**, University of Rijeka
Immunization against SARS-CoV-2 using cytomegalovirus as a vaccine vector

09:50 **Blanka Roje**, University of Split
Gut microbiota carcinogen metabolism causes distant tissue tumors

10:10 **Maja Lenartić**, University of Rijeka
IL-17A producing innate-like T cells drive the progression of MAFLD

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11:00-11:30 INVITED LECTURE:

Chairs: Stipan Jonjić & Ivana Munitić

Marco Colonna, PhD

Department of Pathology and Immunology, Washington University School of Medicine in St.Louis.

`*Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders*`

11:30-12:30 SELECTED ORAL PRESENTATIONS – SESSION 3

Chairs: Stipan Jonjić & Ivana Munitić

11:30 **Marina Babić Čać**, University of Berlin
The pro-inflammatory role of NKG2D during experimental autoimmune encephalomyelitis

11:45 **Marko Šustić**, University of Rijeka
Cytomegalovirus vector expressing NKG2D ligand generates superior CD8 T cell response with distinct phenotypical and functional features

12:00 **Ena Sorić**, University of Zagreb
Convalescent plasma as a therapeutic modality in hematological patients with COVID19 pneumonia - a review of the results of patients treated in University Hospital Dubrava

	12:15 **Sanja Mikašinović**, University of Rijeka *Glucose promotes viral replication after conversion into lactate by inhibiting type-I interferon production*
12:30-15:00	LUNCH & LEASURE TIME
15:00-16:00	EFIS-IL LETURE AWARD CEREMONY: Chairs: Felix Wensveen & Janoš Terzić **Henrique Veiga-Fernandes** Champalimaud Research Director. Champalimaud Foundation, Portugal `*Neuronal regulation of immune fitness*`
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Chairs: Bojan Polić & Marina Babić Čač

Marc Schmidt-Supprian,

Department of Immunopathology and signal transduction, Technical university of Munich, Germany

'Timing invariant T cell fate decisions: how do effector lineages develop in absence of infection?'

09:30-10:30 SELECTED ORAL PRESENTATIONS – SESSION 4

Chairs: Bojan Polić & Marina Babić Čač

09:30 **Christina Stehle**, University of Berlin
T-bet and RORα control lymph node formation by regulating embryonic innate lymphoid cell differentiation

09:45 **Josip Peradinović**, University of Rijeka
Neuroimmune characterization of optineurin insufficiency mouse model

10:00 **Barbara Tomić**, University of Zagreb
Cytarabine induces monocytic differentiation via Chk1 activation

10:15 **Fran Krstanović**, University of Rijeka
Cytomegalovirus infection and dissemination in the developing brain

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10:30-11:00 COFFEE BREAK & HOTEL CHECK OUT

11:00-11:30 INVITED LECTURE:

Chairs: Danka Grčević & Ines Mrakovčić Šutić

Alberto Mantovani,

Humanitas University. President of Fondazione Humanitas per la Ricerca

`*Innate immunity and inflammation: from cancer to COVID-19*`

11:30-12:00 INVITED LECTURE:

Chairs: Alenka Gagro & Ines Mrakovčić Šutić

Felix M Wensveen

University of Rijeka, Faculty of medicine

`*Why we get sick; interactions between the immune and endocrine systems in context of viral infection*`

12:00-12:15	AWARD CEREMONY
12:15-12:30	CLOSING WORDS **Felix M. Wensveen**, president of the Croatian Immunological Society
12:30-13:00	LUNCH
13:00	END

INVITED LECTURES

SARS-COV-2 NEUTRALIZATION ASSAY AS A KEY PREMISE FOR IMPLEMENTATION OF COVID-19 SEROTHERAPY IN CROATIA

Beata Halassy

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

Passive immunotherapy is a century-old practice of administering antibodies from an exposed convalescents or vaccinated persons to patients susceptible to the disease in question. Specific immunoglobulins, some even from animal sources, have an important role in the treatment of various clinical conditions, including viral diseases (hepatitis A and B, rabies, varicella, infections with respiratory syncytial virus, cytomegalovirus, measles). In situations where vaccines and specific drugs are not available, such as during emerging infections and pandemics (influenza, SARS-CoV-1, MERS, Ebola), convalescent plasma is being collected from donors who have recovered from the disease, and used to treat different pathogens. Experience from prior outbreaks with other coronaviruses (SARS-CoV-1) shows that such convalescent sera contain neutralising antibodies against relevant virus, and that their use was beneficial in the treated patients. Therapy with antibody-laden plasma of those who have survived an infection has nowadays been used and investigated worldwide. Although convalescent plasma therapy has been considered generally beneficial, scientific medical community lacks definitive proof of its effectiveness coming from carefully designed randomized clinical trials.

Croatian approach to establish premises for COVID-19 convalescent plasma (CCP) usage in a scientifically sound way, enabling also comparison of Croatian to international practice, will be presented. The approach included several steps: (i) development of a reproducible SARS-CoV-2 neutralisation potency assay in a single biosafety level three facility in Croatia; (ii) establishment and continuous usage of an anti-SARS-CoV-2 in-house reference whose stability was monitored throughout the period of the assay lifetime; (iii) search for the best fitting correlation function between results of *in vitro* commercial assays used by Croatian transfusion centres and the results of neutralisation assay, enabling that the neutralization potency of plasma doses used in the whole country could be expressed in the same way and in the same units; (iv) and finally, and most importantly, we were able to express all neutralization potencies of plasma used in Croatia in relation to the first WHO international standard, by calibrating the assay's internal reference to this international standard once it was established and available to the scientific community.

Collection of COVID-19 convalescent plasma (CCP) at Croatian Institute of Transfusion Medicine (CITM) started in July 2020, the development of SARS-CoV-2 neutralization assay started in September 2020 and first unit for clinical use was issued in December 2020. Clinicians in Croatia started using CCP in second wave of pandemics, mostly for patients with hematological malignancies.

The research has been financed by Croatian Science Foundation (grant IP-CORONA-04-2053) and by European Regional Development Fund, grant number KK.01.1.1.01.0006, "Strengthening the capacity of CerVirVac for research in virus immunology and vaccinology".

MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN

Alenka Gagro

Children's Hospital Zagreb, School of Medicine, University of Zagreb, Zagreb
Medical Faculty Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Multisystem Inflammatory Syndrome in Children (MIS-C) manifests as a severe and uncontrolled inflammatory response with multiorgan involvement that occurs after primary SARS-CoV-2 infection in communities with high COVID-19 rates.

Patients with this condition recognized first in April 2020 have some overlapping signs and symptoms with those of Kawasaki disease, toxic shock syndrome and hemophagocytic lymphohistiocytosis /macrophage activation syndrome. Three diagnostic criteria of MIS-C has been proposed, however, the World Health Organization's (WHO) definition is preferred, as it is more precise, while encompassing most cases. MIS-C is defined by the WHO as an illness in children 0 to 19 years old, with a fever for ≥ 3 days, with at least two clinical signs of multisystem involvement, elevated markers of inflammation, with no other obvious microbial cause of inflammation, and evidence of a SARS-CoV-2 infection. However, this new condition is heterogeneous and at least three subtypes have been identified based on the severity of symptoms.

The pathogenesis of MIS-C is under intense investigation but so far remains undefined. The possible triggers of hyperinflammation in MIS-C include viral persistence in gastrointestinal or other sites, superantigen potential of spike protein, autoantibodies of pathogenic potential as well as monogenic inborn errors of immunity. Current treatment with immunomodulatory agents has mainly been derived from previous experience treating Kawasaki disease and other hyperinflammatory disorders and includes intravenous immunoglobulin, corticosteroids, and biologics.

The aim of the presentation is to analyze critically the novel evidence related to the pathogenesis of MIS-C and to provide the interpretation of these findings based on experience with children with MIS-C diagnosed and treated at our hospital in collaboration with COVID Human Genetic Effort.

CYTOMEGALOVIRUS INFECTION OF OVARIES AND FERTILITY MAINTENANCE

Vanda Juranić Lisnić

University of Rijeka faculty of Medicine, Rijeka, Croatia

Viral infections during pregnancy are recognized as a significant cause of adverse outcomes and birth defects. Interestingly, in many instances, the underlying mechanisms are poorly understood. Among those, cytomegalovirus (CMV) infection is the most common intrauterine infection in humans, causing devastating congenital CMV disease and is a putative cause of early pregnancy loss.

To study the impact of CMV on fertility and pregnancy maintenance, we employed the murine CMV model. While pregnant mice successfully controlled the CMV infection, we observed a highly selective and strong infection of corpora lutea (CL) cells within their ovaries and exclusion of the virus from follicles. High densities of virus infected cells indicated a failure of immune control within CL resulting in progesterone insufficiency and pregnancy-loss. Restriction of CMV to CL and stroma was also observed in non-pregnant mice, even in highly virus sensitive IFN$\gamma^{-/-}$ mice.

Follicles are structures that house oocytes and as such are very important for fertility maintenance. As such, understanding mechanisms governing their resistance to wide-spread pathogen like CMV, might help us understand etiology of unexplained infertility. We thus further investigated the mechanisms that mediate follicular resistance to CMV infection and uncovered multiple, overlaying layers of protection including abundance of gap-junctions, absence of vasculature, strong type I IFN responses and interaction of innate immune cells. Of those, IFN I–mediated responses provided the greatest protection. This research is, to our knowledge, one of the first describing CMV pathogenesis in the ovaries and highlighting the importance of INF I protection of fertility.

HETEROGENEITY OF MENINGEAL B CELLS REVEALS A LYMPHOPOIETIC NICHE AT THE CNS BORDERS

Marco Colonna

Washington University School of Medicine in St.Louis.

NEURONAL REGULATION OF IMMUNE FITNESS

Henrique Veiga-Fernandes

Champalimaud Research Director, Champalimaud Foundation, Portugal

Innate lymphoid cells (ILC) are the most recently defined cell family to be included to the increasingly complex atlas of the immune system. ILC have a lymphoid morphology, lack rearranged antigen receptors and are abundantly present at mucosal surfaces. The combined expression of lineage-specific transcription factors with discrete cytokine profiles led to the identification of distinct ILC subsets. ILC development and function have been widely perceived to be programmed. However, emerging evidence indicates that ILC are also controlled by complex environmental signals. Here, we will discuss how ILC perceive, integrate and respond to their environment, notably to nutritional and neuronal cues.

TIMING INVARIANT T CELL FATE DECISIONS: HOW DO EFFECTOR LINEAGES DEVELOP IN ABSENCE OF INFECTION?

Marc Schmidt-Supprian

Technical university of Munich, Germany

Innate-like T cell populations expressing conserved TCRs play critical roles in immunity through diverse effector functions. These functions are acquired as intrinsic part of their development in absence of infection or other external challenge. However, many aspects of this process remain unclear and controversial. We generated a differentiation roadmap obtained by the temporal analysis of a genetically induced developmental wave of NKT cells, a prototypical innate-like T lineage. We define the precise timing of positive selection, lineage commitment, acquisition of cytokine secretion potential, proliferation and thymic egress. We find that a short period of homogenous TCR signaling triggers highly synchronous and uniform NKT cell development, strongly arguing against widely favored models of TCR-instructed effector subset diversification. These effector subsets emerge simultaneously from highly proliferating progenitors but follow dramatically different fates. Our results indicate that differences in NKT cell generation rates can influence steady state effector subset composition, offering an alternative interpretation of experimental results involving genetically altered NKT cell differentiation.

INNATE IMMUNITY AND INFLAMMATION: FROM CANCER TO COVID-19

Alberto Mantovani,

Humanitas University. President of Fondazione Humanitas per la Ricerca

The immune system is an extremely complex orchestra. The immune system and the central nervous system are the two most complex set of cells, connections and mediators in our body. Alterations of immunity and inflammation represent a metanarrative of modern medicine, spanning from infectious diseases to cardiovascular pathology to cancer.

Inflammatory cells and mediators are a key component of the tumor microenvironment. A change in paradigm and dissection of accelerators and brakes of immunity have spearheaded the birth of cancer immunotherapy. COVID-19 has highlighted how little we know of immunity to microbial challenges. As for cancer, a better understanding of the interaction of immunity with SARS-CoV-2 is likely to pave the way to new diagnostic and therapeutic tools.

WHY WE GET SICK;

INTERACTIONS BETWEEN THE IMMUNE AND ENDOCRINE SYSTEMS DURING VIRAL INFECTION

Felix M. Wensveen

University of Rijeka, Faculty of medicine

Being sick makes us miserable. Following infection with a pathogen we lose apetite, get a temperature and feel weak. We experience these feelings as pathology, but in fact they are a carefully orchestrated physiological response. Upon infection, the immune and endocrine system directly communicate to change systemic metabolism and induce a state that we experience as 'being sick'. The purpose of this state is to impair replication of the invading pathogen and at the same time generate an optimal environment for immune cell function. The underlying molecular mechanism of this process have long remained unknown, but recent advances have made clear how the immune system mediates changes in endocrine function upon infection. In the context of pre-existing metabolic disease, this system derails and may promote development of pathologies such as diabetes mellitus type 2 (DM2). Importantly, patients with metabolic disease fail to induce the immune-mediated anti-viral changes in systemic metabolism, which predisposes them to severe disease outcome following infection with pathogens such as SARS-CoV-2. Indeed, DM2 is one of the biggest risk factors for morbidity and mortality in the context of COVID-19. In this lecture, our recent discoveries on immune-endocrine interactions in the context of infection will be discussed.

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ORAL PRESENTATIONS
Session 1

NOTCH 1 INHIBITION INCREASES OSTEOCLAST PROGENITOR ACTIVITY IN THE MOUSE MODEL OF RHEUMATOID ARTHRITIS

Maša Filipović[1,2], Alan Šućur[1,2], Darja Flegar[1,2], Zrinka Jajić[3], Marina Ikić Matijašević[4], Nina Lukač[1,5], Nataša Kovačić[1,5], Tomislav Kelava[1,2], Dino Šisl[1,2], Katerina Zrinski Petrović[1,5], Vedran Katavić[1,5], Danka Grčević[1,2]

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2. Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia
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4. Clinical Hospital "Sveti Duh", Department of Clinical Immunology and Allergology, Zagreb, Croatia
5. Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia

Background: Osteoclast progenitor cells (OCPs) are susceptible to regulation through Notch signaling. We previously identified an increased frequency of OCPs expressing Notch receptors in arthritic mice. We aimed to determine the effects of Notch receptor signaling inhibition on OCP activity in murine collagen-induced arthritis (CIA).

Methods: Periarticular bone marrow (PBM) and spleen (SPL) were harvested from mice with CIA, additionally treated by i.p. injections of anti-Notch 1 neutralizing antibodies (1mg/kg). FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF/RANKL), in Jagged (JAG)1 or Delta-like (DLL)1 coated wells, with or without anti-Notch 1 antibodies. The research was approved by the Ethics Committee.

Results: Seeding OCPs on DLL1-coated wells increased, whereas seeding on JAG1-coated wells decreased the number of TRAP+ osteoclasts and expression of osteoclast differentiation genes. Addition of anti-Notch 1 antibodies to ligand-stimulated OCPs resulted in an increased number of TRAP+ osteoclasts, partially reversing Jag1 inhibition. In vivo treatment with anti-Notch 1 antibodies did not affect total OCP frequency, but increased the expression of Notch 4 both in PBM and SPL as seen by flow cytometry. Additionally, anti-Notch 1 treatment stimulated Notch transcription factors HES and HEY. Both PBM and SPL cultured OCPs from anti-Notch 1 treated mice produced a higher number of large TRAP+ osteoclasts and exhibited increased expression of osteoclast differentiation genes.

Conclusion: Both in vitro and in vivo anti-Notch 1 neutralizing antibodies enhanced osteoclastogenesis in CIA model, implying an inhibitory role of Notch 1 signaling in osteoclast differentiation.

Acknowledgments: Funding by Croatian Science Foundation projects IP-2018-01-2414, UIP-2017-05-1965 and DOK-2018-09-4276.

CHARACTERIZATION OF M116.1P, A MURINE CYTOMEGALOVIRUS PROTEIN REQUIRED FOR EFFICIENT INFECTION OF MONONUCLEAR PHAGOCYTES

Tina Ružić[1], Vanda Juranić Lisnić[1,2], Hana Mahmutefendić Lučin[3], Tihana Lenac Roviš[1], Jelena Železnjak[1,2], Maja Cokarić Brdovčak[1,2], Ana Vrbanović[1,2], Deni Oreb[1], Daria Kveštak[1,2], Kristina Gotovac Jerčić[4,5], Fran Borovečki[4,5], Pero Lučin[3], Barbara Adler[6], Stipan Jonjić[1,2], Berislav Lisnić[1,2*]

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4. Department of Neurology, University Hospital Center Zagreb, Zagreb, Croatia
5. Department for Functional Genomics, Center for Translational and Clinical Research, University of Zagreb, School of Medicine and University Hospital Center Zagreb, Zagreb, Croatia
6. Max von Pettenkofer Institute & Gene Center, Virology, Faculty of Medicine, LMU Munich, Munich, Germany

Human cytomegalovirus (HCMV) is a species-specific herpesvirus that causes severe disease in immunocompromised individuals and immunologically immature neonates. Murine cytomegalovirus (MCMV) is biologically similar to HCMV and it serves as a widely used model for studying the infection, pathogenesis and immune responses to HCMV. We have previously identified *M116* ORF as one of the most extensively transcribed regions of MCMV genome, indicating that it must play an important role for the virus' life cycle. Our molecular characterization revealed two 5' co-terminal spliced transcripts in M116 region. We have further shown that a glycosylated protein named M116.1p is expressed from this region with late kinetics and have generated a monoclonal antibody that specifically recognizes it. Additionally, we have shown that M116.1p is localized within the virion assembly compartment and it interacts with gH, one of the entry-complex proteins of MCMV. These characteristics are shared between M116.1p and its homologs; HCMV UL116 and RCMV R116, demonstrating yet again that MCMV is an excellent model for studying various aspects of HCMV biology. By comparing *in vitro* replication kinetics of ΔM116-MCMV and WT-MCMV, we observed comparable kinetics in primary mouse embryonic fibroblasts. However, ΔM116 was attenuated in mononuclear phagocytes. Finally, we have shown that M116.1p is affecting the spread of MCMV when administered via natural route of infection, intranasally. This study, therefore, expands our knowledge about virally encoded glycoproteins that play important roles in viral infectivity and tropism.

WHAT IS THE ROLE OF CANONICAL NOTCH SIGNALLING PATHWAY IN LIVER FIBROSIS?

Dino Šisl[1,2], Sanja Novak[4], Ivo Kalajzić[4], Maša Filipović[1,2], Darja Flegar[1,2], Alan Šućur[1,2], Nataša Kovačić[1,3], Danka Grčević[1,2], Antonio Markotić[1,] Tomislav Kelava[1,2]

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3. Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia
4. University of Connecticut Health Center, Farmington, USA

Hepatic fibrosis is a common feature of various liver diseases characterized by activation of hepatic stellate cells (HSC), a principal source of alpha smooth muscle actin (αSMA) liver myofibroblasts. The pathophisiological role of Notch activation has been well established, but the role of Notch pathway in activated HSCs is still not sufficiently investigated. In the present research we first used two common murine models of liver fibrosis, carbon tetrachloride (CCL4) treatment for 6 weeks and 0.1% DDC-supplemented diet for 4 weeks to analyse expression of Notch-related genes. In CCL4 model, PCR analysis showed an upregulation of Notch2, Hey1, HeyL, and Jag2, while DDC-induced fibrosis was associated with increased expression of Notch2, Notch3, Hey1, Hes1, HeyL, Jag1 and Jag2. In the next set of experiments we used double transgenic SmaCre∆Rbpjκ∆ and SmaCreNICD1 mice in which Notch signaling pathway was specifically inhibited or activated in myofibroblasts by tamoxifen injections during the fibrosis development. However, Notch inhibition did not change significantly the degree of fibrosis, as evidenced by similar histological Sirius red liver staining and similar tissue expression of COL1A1 and ACTA2 between the control (SmaCre-∆Rbpjκ∆) and Notch inhibited (SmaCre+∆Rbpjκ∆) mice. Furthermore forcefull activation of Notch in myofibrroblasts did not change the degree of liver foibrosis. So far, our data do not support conclusion that Notch signaling in myofibroblasts contribute to liver fibrosis development in CCL4 and DDC model.

ORAL PRESENTATIONS
Session 2 – Bright Sparks

IMMUNIZATION AGAINST SARS-COV-2 USING CYTOMEGALOVIRUS AS A VACCINE VECTOR

Jelena Materljan[1], Marko Šustić[1], Maja Cokarić Brdovčak[2], Tina Ružić[2], Sanda Ravlić[3], Maja Lang Balija[3], Beata Halassy[3], Dubravko Forčić[3], Luka Čičin-Šain[4], Berislav Lisnić[2], Astrid Krmpotić[1], Stipan Jonjić[12]

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3. Center for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia
4. Department of Vaccinology and Applied Microbiology, Helmholtz Center for Infection Research, Braunschweig, Germany

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current worldwide COVID-19 pandemic, with over 200 million people infected so far. To combat the pandemic, several vaccines that elicit successful protective immune response have been developed and approved. Two main types of these vaccines include messenger RNA (mRNA) based technology and viral vectors. Although these vaccines proved to be very efficient, the longevity of protective immune response is still undefined. Cytomegaloviruses (CMVs) are β-herpesviruses with great potential to be used as viral vectors. CMVs establish latency from which periodic reactivation occurs, boosting the specific immune response to viral antigens. Their key characteristic is the induction of a large pool of functional antigen-specific $CD8^+$ T cells, which accumulate over time. We have constructed several murine CMV (MCMV) vaccine vectors expressing S (spike) and M (membrane) proteins of the SARS-CoV-2. Immunization with these vectors led to outstanding $CD8^+$ T cell response and the generation of neutralizing antiviral antibodies. Importantly, not only immunization via systemic route but also via intranasal application of vectors, resulted in induction of protective immune response accompanied with induction of tissue resident memory $CD8^+$ T cells in the lungs. Overall, our results demonstrate that herpesviruses are promising vaccine vectors against SARS-CoV-2 due to their capacity to induce exceptional and long-lasting antibody and cellular immune response.

GUT MICROBIOTA CARCINOGEN METABOLISM CAUSES DISTANT TISSUE TUMORS

Blanka Roje[1], Boyao Zhang[2], Eleonora Mastrorilli[2], Ana Kovačić[3], Lana Sušak[1], Elena Ćosić[1], Katarina Vilović[4], Emilija Lozo Vukovac[4], Antonio Meštrović[4], Željko Puljiz[4], Ivana Karaman[4], Michael Zimmermann[2], Janoš Terzić[1]

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2. Laboratory for metabolic host-microbiome interactions, EMBL, Heidelberg, Germany
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Exposure to environmental toxins is a well-recognized risk factor for cancer development. Furthermore, microbiome composition was recently shown to influence carcinogenesis in the gut, liver, and lungs. One of the proposed mechanisms of the microbiome's effect on cancer development is its impact on the toxicokinetics of carcinogens. In the present study, we investigated the role of the gut microbiota in urinary bladder tumor development using a nitrosamine-induced bladder cancer model in mice. We found that antibiotic depletion of the gut microbiota significantly reduces the cancer burden in the bladder, which we then causally link to gut microbial metabolism affecting the toxicity and tissue distribution of the nitrosamine. Further, we tested microbial gut, lung, and oral cavity communities and isolates from different human donors to demonstrate that microbial nitrosamine metabolism strongly varies between individuals. Altogether, these results suggest microbiome carcinogen metabolism is an important contributing factor for chemical-induced carcinogenesis and could potentially open new avenues for microbiome-based risk assessment and prevention of urinary bladder and other types of cancer.

IL-17A PRODUCING INNATE-LIKE T CELLS DRIVE THE PROGRESSION OF MAFLD

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Metabolic dysfunction associated fatty liver disease (MAFLD) is the primary liver disease in Western countries. Typically, MAFLD is only associated with relatively benign steatosis, but in some patients, it progresses to steatohepatitis, which may lead to fibrosis, cirrhosis and is a high risk factor for development of hepatocellular carcinoma. Recently, we uncovered that metabolically stressed hepatocytes induce expression of NKG2D, which drives IL-17A production by local immune cells. Which cells are responsible for NKG2D-induced IL-17A production in context of MAFLD is unknown.

Using our own adapted dietary mouse model (steatosis-steatohepatitis diet, SSD), we identified γδ T cells as a main population of of IL-17A. Hepatic γδ T cells expressed high levels of NKG2D and animals genetically deficient for these cells showed a significant reduction in liver fibrosis following SSD feeding. TCR$\delta^{-/-}$ mice displayed higher levels of fibrosis than NKG2D-deficient animals. Furthermore, levels of profibrogenic IL-17A cytokine were comparable between TCR$\delta^{-/-}$ and wild-type mice upon SSD, indicating the involvement of a second population of cells. Indeed, both TCR$\alpha^{-/-}$ and CD4^{Cre}NKG2D^{Flox} mice showed a reduction in liver fibrosis compared to WT controls, indicating a role for αβ T cells. Ncr1^{Cre}NKG2D^{Flox} mice showed the same liver pathology as WT animals following SSD feeding, excluding a role for NK cells. Detailed phenotyping of TCR$\delta^{-/-}$ mice revealed MAIT cells as major source of IL-17A. MAIT cells highly expressed NKG2D and RORγt molecules with even more pronounced expression in the absence of γδ T cells. We observed no colocalization of IL-17A+ with either CD4+ nor γδ TCR+ cells, suggesting a prominent role for MAIT cells in human liver pathology in NASH.

Our study identifies γδ T cells and auxiliary innate-like T cells as primary mediators of early pathogenesis in MAFLD progression through NKG2D-mediated activation and IL-17A secretion in humans and mice. Our findings may be of great importance for the early identification and treatment of liver pathology in MAFLD.

ORAL PRESENTATIONS

Session 3

THE PRO-INFLAMMATORY ROLE OF NKG2D DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Current medical science puts great effort into elucidating the basis of chronicity and suggesting appropriate treatments for inflammatory and autoimmune diseases, however, the mechanisms driving aberrant immune responses are mostly unknown and deserve further study. The identification of lymphocyte subsets with non-overlapping effector functions as well as their unique features is crucial for the development of targeted therapies in immune mediated inflammatory diseases.

We performed single cell transcriptome analysis of CD4+ T cell pool from spleen and CNS of mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. Our data suggest that CD4+ T cells in the CNS form a transcriptional continuum with distribution skewed by the expression of key cytokines and activation markers. One of the prominent features of CNS CD4+ T cells compared to a splenic pool was the expression of innate receptors, particularly Klrk1, coding for Natural Killer Group 2, Member D (NKG2D). NKG2D is a potent activator of the immune system, known as a sentinel for “induced-self” ligands, i.e., cellular danger signals presented by cells being exposed to an inflammatory cytokine milieu, undergoing tumor transformation, endoplasmic reticulum (ER) stress, cell death or viral infection. During EAE, antigen-specific CD4+ T cells from mice with Klrk1-deficiency in the T cell compartment (Klrk1CD4) were impaired in the production of inflammatory cytokines, particularly IFN-□ and GM-CSF, as well as in the recruitment of inflammatory myeloid cells. Importantly, we could demonstrate that Klrk1CD4 mice show significant resistance to EAE when compared to their littermate controls (Klrk1FLOX).

Altogether, our findings suggest that NKG2D represents an important checkpoint target for helper T cell-mediated inflammatory diseases.

CYTOMEGALOVIRUS VECTOR EXPRESSING NKG2D LIGAND GENERATES SUPERIOR CD8 T CELL RESPONSE WITH DISTINCT PHENOTYPICAL AND FUNCTIONAL FEATURES

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The twentieth century saw a huge expansion in the number of vaccines used with great success in combating diseases, especially the ones caused by viral and bacterial pathogens. Despite this, several major public health threats, such as HIV, tuberculosis, malaria and cancer, still pose an enormous burden in both humanitarian and economic terms. As vaccines based on the induction of protective, neutralizing antibodies have not managed to effectively combat these diseases, in recent decades the focus has increasingly shifted towards cellular immune response. Live replicating viral vectors, genetically engineered to express foreign epitopes, can generate potent and long-lasting cellular immunity against both infectious agents and malignant cells. In this respect, cytomegaloviruses (CMVs) represent particularly attractive viral vectors due to their large genome and numerous immunomodulatory genes which can be manipulated in order to modulate their vaccine properties. In addition, CMVs induce strong antigen specific CD8 T cell response with gradual accumulation of these cells in latently infected hosts. We have constructed a murine CMV vector expressing an NKG2D ligand RAE-1γ (RAE-1γMCMV). RAE-1γMCMV proved to be highly attenuated compared to the control vector yet induced and maintained several-fold higher CD8 T cell response to vectored foreign epitope. These epitope-specific CD8 T cells had a terminally differentiated, effector-like phenotype, expressing low levels of Tcf1, CD62L and CD127 and high levels of KLRG1. During priming, CD8 T cells activated with RAE-1γMCMV had much stronger TCR signaling and proliferated more abundantly than cells primed with the control vector. Overall, our studies indicate that small genetical changes of viral vectors can lead to gross differences in CD8 T cell expansion, phenotype, and function.

CONVALESCENT PLASMA AS A THERAPEUTIC MODALITY IN HEMATOLOGICAL PATIENTS WITH COVID19 PNEUMONIA - A REVIEW OF THE RESULTS OF PATIENTS TREATED IN UNIVERSITY HOSPITAL DUBRAVA

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During the COVID19 pandemic, University Hospital Dubrava treated, among others, hematological patients with COVID19 disease. Large proportion of hematological patients have overt secondary immunodeficiency mainly due to treatments that also target cells that are the base of humoral and cellular response. In COVID-19 it is manifested by diminished specific immunological response and prolonged disease course. Passive immunization with convalescent plasma in these setting may be helpful. Total of 40 patients (24 men, 16 women) received convalescent plasma (rFFP) as part of treatment. The mean age at hospitalization was 65 years (age range 28–88 years). The median time from the onset of symptoms or the first positive test to hospitalization is 11.5 days. The most common hematological disease was chronic lymphocytic leukemia (13 patients, 32.5%), followed by follicular lymphoma (5), mantle cell lymphoma (3), multiple myeloma (3), and acute myeloid leukemia (2). Thirty patients (75%) had a history of treatment for the underlying hematological disease, and 22 patients were currently being treated, of whom 11 were receiving rituximab. All patients were admitted for bilateral pneumonia. On admission, IgG antibodies to SARS-CoV-2 were tested in 34 patients, of whom only two had a positive result. Positive serum PCR SARS-CoV-2 was found in 15 patients (37.5%). The median number of rFFPs administered is 4 (range 1 -15), mostly 18.5 days after the onset of symptoms, or on day 4 of hospitalization. The majority of patients (30, 75%) were treated with oxygen therapy at the first dose of rFFP; 7 patients were treated with high-flow oxygen therapy and two were mechanically ventilated. 16 patients (40%) died, and hospitalization lasted an average of 26 days. In conclusion, passive immunization with rFFP may help control COVID-19 infection until patient's own immunological system recovers from consequences of previous immunosuppressive treatments.

GLUCOSE PROMOTES VIRAL REPLICATION AFTER CONVERSION INTO LACTATE BY INHIBITING TYPE-I INTERFERON PRODUCTION

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Viral infection has a major impact on systemic metabolism. Previously, we showed that strong viral infection results in relative hypoglycemia (RHG). However, the molecular mechanism(s) underlying the RHG and its beneficial effect remain unclear.

Recently, lactate was shown to impair IFN-I production in response to viral infection by inhibiting RIG-I. We observed *in vivo* that viral titers are higher in fasted mice that were drinking glucose-laced water in comparison to animals drinking normal water. To determine whether glucose availability regulates IFN-I production through a lactate-dependent mechanism, infected mouse embryonic fibroblasts (MEF) and seminal vesicle epithelial cells (SVEC) were cultivated under low- or high glucose concentrations and supplemented with sodium oxamate, a specific LDHA inhibitor. Low glucose concentrations, as well as sodium oxamate, significantly reduced the level of viral replication in both SVEC and MEF. In addition, we supplemented the low glucose medium with different nutrients such as citrate, acetate, and galactose, yet none of them managed to compensate for the effect of limited glucose. Finally, infected cells cultured under high-glucose conditions in the presence of oxamate showed significantly increased IFN-β production as determined by ELISA. To summarise, we showed that sodium oxamate suppresses the level of viral replication *in vitro* by reducing lactate levels.

Our results confirm that glucose promotes viral replication through direct inhibition of IFN-I secretion by lactate, rather than by promoting catabolic metabolism. Overall, these findings bring us one step closer towards elucidating the metabolic pathway through which RHG promotes IFN-I production.

ORAL PRESENTATIONS
Session 4

T-BET AND RORA CONTROL LYMPH NODE FORMATION BY REGULATING EMBRYONIC INNATE LYMPHOID CELL DIFFERENTIATION

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The generation of lymphoid tissues during embryogenesis relies on group 3 innate lymphoid cells (ILC3) displaying lymphoid tissue inducer (LTi) activity and expressing the master transcription factor RORγt. Accordingly, RORγt-deficient mice lack ILC3 and lymphoid structures, including lymph nodes (LN). Whereas T-bet affects differentiation and functions of ILC3 postnatally, the role of T-bet in regulating fetal ILC3 and LN formation remains completely unknown. Using multiple mouse models and single-cell analyses of fetal ILCs and ILC progenitors (ILCP), here we identify a key role for T-bet during embryogenesis and show that its deficiency rescues LN formation in RORγt-deficient mice. Mechanistically, T-bet deletion skews the differentiation fate of fetal ILCs and promotes the accumulation of PLZF^{hi} ILCP expressing central LTi molecules in a RORγt -dependent fashion. Our data unveil an unexpected role for T-bet and RORγt during embryonic ILC function and highlight that RORγt is crucial in counteracting the suppressive effects of T-bet.

NEUROIMMUNE CHARACTERIZATION OF OPTINEURIN INSUFFICIENCY MOUSE MODEL

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor neuron loss, chronic neuroinflammation and proteinopathy. Mutations in 50+ genes, including the optineurin (*OPTN*) gene, can cause ALS. OPTN is an adaptor protein that plays a role in many processes, including inflammatory signalling and autophagy. To understand the role of OPTN in neurodegeneration, we analysed a mouse model carrying $OPTN^{470T}$ truncation, which lacks ubiquitin-binding domain, thus mimicking some ALS patient mutations and leading to protein insufficiency. Contrary to the initial findings in cell lines, in primary cells from $OPTN^{470T}$ mice, we showed that in response to Toll receptor stimulation OPTN was dispensable for NF-κB activation but required for optimal TBK1 activation and IFN-β production. Since IFN-β was shown to potentiate autophagy and phagocytosis, we tested phagocytic potential of macrophages, but found that OPTN was dispensable for elimination of apototic neurons. Since ALS occurs late in life, we conducted neurological tests in two-year-old $OPTN^{470T}$ mice. $OPTN^{470T}$ mice did not show motor impairment but showed decreased memory in novel object recognition tests. Perhaps surprisingly, upon induction of experimental autoimmune encephalomyelitis (EAE), $OPTN^{470T}$ exhibited lower clinical score and less weight loss compared to WT mice. Therefore, OPTN is dispensable for NF-κB activation and phagocytosis, but indispensable for proper TBK1 activation and IFN-b production. Our experiments with EAE and ageing suggest that OPTN insufficiency may be either neuroprotective or neurotoxic, depending on other risk factors. We are currently developing other two-hit ALS models to elucidate this bimodal effect of optineurin.

CYTARABINE INDUCES MONOCYTIC DIFFERENTIATION *VIA* CHK1 ACTIVATION

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Our recent research demonstrated that 5-aminoimidazol-4-carboxamide ribonucleoside (AICAr) inhibits UMP synthase and induces cellular differentiation via ataxia telangiectasia and RAD3-related (ATR)/checkpoint kinase 1 (Chk1)-mediated signaling pathway due to pyrimidine depletion, similarly to brequinar, a dihydroorotate dehydrogenase (DHODH) inhibitor. Cytarabine is a well-known chemotherapeutic which interferes with the process of DNA synthesis exerting not only cytotoxic effects, but also monocytic differentiation. Nevertheless, the mechanism responsible for cellular differentiation in response to cytarabine remains unclear. Therefore, this study is aimed to test for the role of Chk1 DNA-damage signaling pathway in differentiation of leukemia cells and to compare the effects of cytarabine to the effects of AICAr and brequinar. This study was conducted on human monocytic cell lines U937 and THP-1, as well as on non-adherent mononuclear cells from bone marrow samples of five acute myeloid leukemia (AML) patients. Cytarabine dose-dependently decreased cell viability and induced the expression of differentiation markers CD11b and CD64 in AML cell lines. Moreover, cytarabine dose-dependently increased the level of activating Ser-345 phosphorylation of Chk1, and increased the level of inhibitory Thyr-15 phosphorylation of cyclin-dependent kinase 1 (Cdk1), a possibly important downstream target of Chk1 in cell cycle arrest. Torin2 and VE-821, pharmacological inhibitors of ATR/Chk1 signaling pathway, as well as transfection of U937 cells with siRNA targeting Chk1 reduced differentiative effects of cytarabine, AICAr and brequinar. Furthermore, cytarabine dose-dependently induced the expression of differentiation markers in two primary AML samples responsive to inhibitors of de novo pyrimidine synthesis. Therefore, our results suggest that cytarabine induces differentiation of AML cells by activating Chk1 and shares the same mechanism as pyrimidine synthesis inhibitors.

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CYTOMEGALOVIRUS INFECTION AND DISSEMINATION IN THE DEVELOPING BRAIN

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Congenital cytomegalovirus (cCMV) infection is a leading viral cause of mental retardation and sensorineural hearing loss in infants and children. Despite its importance, pathogenesis of congenital CMV infection (cCMV) remains poorly understood. Upon entering the central nervous system (CNS), human cytomegalovirus (HCMV) targets all resident cells, consequently leading to the development of widespread histopathology and inflammation. To elucidate the mechanisms of brain infection during cCMV infection, we are using a murine model. We have first confirmed that mouse cytomegalovirus (MCMV) lacks cell tropism during brain infection, efficiently infecting astrocytes, microglia and neurons. To study the dissemination of the virus in the brain, we utilized cell-type-specific virus labeling system. Our data suggest that MCMV enters the CNS in both cell-free and cell-associated form. Astrocytes, microglia and neurons support productive MCMV infection in vivo, with astrocytes being initial targets and major virus producing cell type. Microglia also significantly contributed to virus production during the peak of infection. In contrast, contribution of neurons to brain virus production during early and peak phase of infection was negligible. However, during the late phase of infection, when immune control of infection is established, neuron derived virus was dominant in brain. These data argue that immune control of MCMV in neurons is not efficient and that neurons are potential site of CMV persistence. Finally, our data shows that upon entering the CNS, MCMV does not disseminate back to the periphery. Altogether, we provide new insights into the pathogenesis of cCMV infection.

ABSTRACTS

STRONG VIRAL INFECTION CAUSES γδ T CELL MEDIATED RELATIVE HYPOGLYCEMIA WHICH PROMOTES THE INNATE ANTI-VIRAL IMMUNE RESPONSE

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Viral infection has a major impact on systemic metabolism. In humans, severe infection may lead to hypoglycemia, but how this is regulated on a molecular level is unknown, and how this response benefits the host is also unclear. We have recently shown that mild viral infection alters endocrine regulation of systemic blood glucose without causing dysglycemia.

Here, we investigated how severe infection impacts regulation of blood glucose homeostasis. We show that infection of mice with high, non-lethal titres of mCMV or LCMV causes transient, relative hypoglycemia. This effect depends on IFNγ secreted by γδ T cells, as TCRδ-/- mice and animals treated with IFNγ-neutralizing antibodies fail to develop hypoglycemia upon infection. Infection-induced IFNγ causes specific insulin resistance in muscle, but not in liver, leading to increased insulin secretion by the pancreas. Consequently, hepatic glycogenolysis and liver glucose output were reduced, leading to the decrease in systemic glucose levels. Limited glucose availability amplified cellular stress response of infected cells, leading to higher production of type-I interferons which reduced viral replication. When glucose levels were increased, artificially or due to diabetes, viral loads were strongly increased because of an impaired type-I interferon response. This effect was present both *in vitro* and *in vivo*.

Our findings indicate that reduction of blood sugar levels during infection is a well-regulated part of the body's natural anti-viral response. This response is derailed in diabetes leading to increased susceptibility to infections.

GLIAL CELL ADAPTATION TO LATENT VIRUS INFECTION IN THE CNS

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Congenital cytomegalovirus infection is a leading infectious cause of neurodevelopmental defects and hearing loss. Using a murine model of congenital cytomegalovirus infection, it was previously shown that infection with mouse cytomegalovirus (MCMV) is associated with a strong host inflammatory response in the brain, which leads to pathological damage. Following the resolution of productive infection, the virus establishes latency. Virus-specific T cells are retained in the brain and control reactivating virus. Whether these permanent changes in brain homeostasis affect resident glial cells is not known. To answer this question we have performed single-cell transcriptomic analysis of microglia and astrocytes from latently infected mice. Our analysis revealed that latent MCMV infection drastically changes the composition of microglia at the single-cell level, while astrocyte homeostasis is minimally affected, indicating differential homeostatic features of these glial cells following infection. Infection induced novel subpopulations of microglia, characterized by the expression of different pro-inflammatory gene sets. Microglial subpopulations associated with MCMV latency have highly expressed genes encoding for MHC I and II molecules, and genes involved in response to interferon type I and II (*Cxcl9*, *Cxcl10*). These changes were not due to virus latency in microglia, since we did not detect viral genomes in these cells. Antiviral treatment administered early during acute infection can reduce the impact of infection on microglia, however, such treatment during latency is not effective. Altogether, our results show that latent CMV infection in the brain leads to permanent perturbation of microglial homeostasis and drives persistent neuroinflammation.

CHANGES IN SERUM LEVELS OF INFLAMMATORY BIOMARKERS IN PATIENTS WITH ACUTE AND CHRONIC CORONARY SYNDROME CONSUMING N-3 POLYUNSATURATED FATTY ACID ENRICHED HEN EGGS - A RANDOMIZED STUDY

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There is a strong potential of n-3 polyunsaturated fatty acids (n-3 PUFAs) consumption to reduce cardiovascular risk and to prevent adverse outcomes in existing cardiovascular diseases. This study aimed to test if dietary supplementation of n-3 PUFAs in form of enriched hen eggs may modulate serum lipid and fatty acid profile and inflammatory biomarkers in patients with coronary artery disease (CAD). Forty CAD patients participated in this study; 20 patients had acute CAD (Ac-CAD) and 20 patients had chronic CAD (Ch-CAD). Control group (N=20) ate three regular hens' eggs/daily (249mg n-3 PUFAs/day), and n-3 PUFAs group (N=20) ate three n-3 PUFAs enriched hen eggs/daily (1053mg n-3 PUFAs/day) for 3 weeks. Serum n-3 PUFAs concentration significantly increased (in all CAD patients), while total cholesterol and LDL cholesterol and IL-6 (in Ac-CAD patients), as well as total cholesterol and LDL, hsCRP and IL-1a (in all CAD patients) significantly decreased in n-3 PUFAs group. Consumption of three n-3 PUFAs enriched hen eggs for three weeks have favorable effect on fatty acids profile (lower n-6/n-3 PUFAs ratio) and mild anti-inflammatory effect. Since consumption of both regular and n-3 PUFAs eggs had no negative effects on any of the measured parameters, results of the present study also indicate that eggs can be safely consumed in the daily diet of patients with coronary artery disease.

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THE QUANTIFICATION OF SIALIC ACIDS RELEASED FROM GUINEA PIG PRIMARY CELL CULTURES FOR INVESTIGATION OF MUMPS VIRUS ENTRY AND INFECTION

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Mumps virus (MuV), an aerosol-transmitted human pathogen, has two envelope glycoproteins, hemagglutinin neuraminidase (HN) and a fusion (F) protein, which engage in receptor binding and mediate membrane fusion to the target cells. MuV-HN specifically recognize sialic acid (SA) containing structures of glycoconjugates present on the host cells, preferring unbranched α2,3-sialylated glycans. SAs are negatively charged monosaccharides found on the non-reducing termini of glycans which are involved in many biological interactions. A diverse range of SAs are found in nature, but N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are the most frequent SAs in mammals.

Guinea pigs are resistant to robust, symptoms-involving MuV infection. One of hypothesis is that this is due to lack of receptors for MuV on guinea pig cells, thus preventing virus entrance and infection. The aim of our work was to investigate whether cells isolated from guinea pig organs have properly glycosylated surface proteins, and whether the primary cultures prepared from these organs are susceptible to MuV infections. For this purpose we developed HPLC method for identification and quantification of fluorescently labelled SAs which are released by treatment guinea pigs primary cells with 2 different sialidases. The HPLC analysis showed the presence of both α2,3-linked Neu5Ac and α2,6-linked Neu5Ac on the surface of all analysed primary cell cultures. The α2,3-linked Neu5Ac was more abundant in the all analyzed cells in comparison to α2,6-linked Neu5Ac. In according to this results we detected the high susceptibility of the all analysed primary cells to infection with different mumps virus strain.

EXTREME ANAEROBIC EXERCISE IMPAIRS CYTOTOXICITY AND ENHANCES CYTOKINE PRODUCTION – A FLOW CYTOMETRY STUDY OF HUMAN BLOOD LYMPHOCYTES

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Exercise is well known to have beneficial effects for our body. However, exercise is not universally beneficial for the immune system and can become detrimental at high intensity. Little is known about the underlying mechanism of increased susceptibility to infection under conditions of intense physical strain. Freedivers, people who dive to high depths on a single breath, perform extreme exercise under anaerobic conditions. In this study, we investigated the impact of freediving on the cytotoxic arm of the immune system. At rest, elite freedivers did not display changes in their immunological profile compared to non-diving controls. In contrast, after a freedive, granzyme B and IL-2 production were impaired, whereas IFNγ and TNF secretion were increased by cytotoxic immune cells. Using in vitro models mimicking freedive conditions, we could show that hypoxia in combination with stress hyperglycemia had a negative impact on Granzyme B secretion. IL-2 production was inhibited by stress hormones. Our findings suggest that in response to extreme stress, cytotoxic immune cells transiently change their functional profile to limit tissue damage. This work was supported by a University of Rijeka Support grant (19-41-1551) and the Croatian Science Foundation (IP-2016-06-8027, IP-CORONA-2020-04-2045) to FMW and (IP-2020-02-7928) to TTW.

PERINATAL CYTOMEGALOVIRUS INFECTION EXTENSIVELY RESHAPES THE TRANSCRIPTIONAL PROFILE AND FUNCTIONALITY OF NK CELLS

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Infections during early life can have substantially different outcomes and consequences than infections occurring in adulthood. In this study, we have used newborn mice infected with mouse cytomegalovirus (MCMV) to investigate the immunomodulatory effects of a perinatal beta-herpesvirus infection on NK cells. We found that MCMV infection causes a significant shift of NK cell population towards the terminally mature phenotype and severely compromises their functionality, as demonstrated by the reduced ability of NK cells from infected mice to produce cytokines. Such extensive reshaping of NK-cell phenotype occurred only in mice infected during early life and required active virus replication. Remarkably, even infection with heavily attenuated MCMV strains induced NK cell hyporesponsiveness, suggesting that NK cell dysfunction is not due to impaired control of the virus in newborn mice. Mechanistically, the infection caused suppression of principal transcription factors governing NK cell fate and function, such as TCF-1 and Eomes, and resulted in dysregulation of numerous genes and impairment of NK cell function. Altogether, our data indicate that perinatal cytomegalovirus infection can have profound adverse effects on the functional abilities of NK cells.

INFLUENCE OF PRODUCTION CONDITIONS ON IGG-BASED SNAKE ANTIVENOMS' STABILITY PROPERTIES

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Antivenoms, having pure animal IgGs or their fragments as an active drug, are the only specific medicines against envenoming due to venomous animals' bites. However, such products are of low sustainability for many reasons resulting in constant shortages all over the world. Stability of the product is one of them contributing not only to sustainability but it's safety as well. It has been hypothesized that roughness of conditions to which IgGs are exposed during downstream purification disturbs more or less their conformation, making them consequently more prone to aggregation to varying degree, particularly after secondary stress exposure. The aim of this research was to investigate the impact of five commonly applied biochemical principles for IgG extraction from plasma on stability properties of pure IgGs. For that purpose, equine IgGs were purified from unique sample of hyperimmune plasma by two mild condition operational procedures (anion-exchange chromatography (AEX) and caprylic acid precipitation (CAP)) and three harsher ones (ammonium sulphate precipitation (ASP), cation-exchange chromatography (CEX) and affinity chromatography (AC)). Their stability was studied under non-optimal storage conditions (42 °C, transient lowering of pH) by monitoring the changes of aggregate content and thermal stability of pure IgG preparations. We found that gentle protocols initially generate IgGs with lower aggregate content in comparison to harsher ones. Their tendency for further aggregation was proportional to the initial aggregate share. Thermal stability of IgG molecules inversely correlated to the aggregate content in refined samples. We can conclude that mild condition purification protocols indeed generate more stable IgGs.

GENETIC STABILITY OF RECOMBINANT MUMPS VIRUSES GENERATED BY REVERSE GENETICS TECHNOLOGY

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Reverse genetics technology enables recovery of infectious, replication-competent RNA virions from plasmids with cloned complementary DNA (cDNA). Among nonsegmented negative strand RNA viruses, viruses produced using reverse genetics have been based mostly on measles virus or vesicular stomatitis virus. The ability to manipulate mumps (MuV) genome by reverse genetics systems has been used as a tool for investigation of MuV biology and to develop MuV-based recombinant viruses with varying insert lengths (additional transcription units).

In our work, we established a rescue system for MuV based on the consensus sequence of L-Zagreb vaccine. RNA viruses produced by rescue methodology are often referred to as viruses derived from infectious clone, implying that high genetic consistency of viral populations can be achieved. The goal of our research was to characterize the level of population diversity during the rescue processes; seven different recombinant MuVs were rescued.

The analysis of deep sequencing results showed that plasmids used in rescue are genetically homogenous, while viral populations in primal rescue stocks contain variants present mostly at low percentages. One substitution was observed in all 7 primary rescue stocks: C9660T leading to the amino acid change Pro408Leu in L protein. Interestingly, plasmid used in rescue codes for proline at this position (a mutation which was introduced during cloning), while original L-Zagreb vaccine strain, as well as all publicly available mumps sequences code for leucine, indicating this reversion could be important for function and/or structure of L protein.

COVID-19 CONVALESCENT PLASMA AS LONG-TERM THERAPY IN IMMUNODEFICIENT PATIENTS?

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The patients with hematological malignancies are a vulnerable group to COVID-19, due to the immunodeficiency resulting from the underlying disease and oncological treatment that significantly impair cellular and humoral immunity. Here we report on a beneficial impact of a passive immunotherapy with convalescent plasma to treat a prolonged, active COVID-19 in a patient with a history of nasopharyngeal diffuse large B-cell lymphoma treated with the therapy inducing substantial impairment of particularly humoral arm of immune system. The specific aim was to quantify SARS-CoV-2 neutralizing antibodies in a patient plasma during the course of therapy. Besides the standard of care treatment and monitoring, neutralizing antibody titers in patient's serum samples, calibrated according to the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human), were quantified in a time-dependent manner. During the immunotherapy period peripheral blood flow cytometry immunophenotyping was conducted to characterize lymphocyte subpopulations. The phases of clinical improvements and worsening coincided with transfused neutralizing antibodies rises and drops in the patient's systemic circulation, proving their contribution in controlling the disease progress. Therapeutic approach based on convalescent plasma transfusion transformed a prolonged, active COVID-19 into a partly manageable chronic disease.

CYTOMEGALOVIRUS-BASED VECTORS AS CANDIDATES FOR CD8 T CELL-BASED VACCINES

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Research during recent years identified cytomegalovirus (CMV) as an attractive vaccine vector against infectious diseases and tumors. CMV encodes numerous non-essential immuno-evasion genes. The deletion of those genes results in virus attenuation in vivo, which enables us to dramatically manipulate its virulence and the immune response. Additionally, CMV infection in human and mice is lifelong and induces an atypical CD8 T cell response characterized by expansion and maintenance of effector memory T cells in peripheral tissues, a process termed memory inflation. As inflationary T cells are highly functional, CMV-based vaccines have gained substantial interest for vaccination purposes. The exact mechanisms underlying inflation of these CMV-specific CD8 T cell populations are still poorly understood. In this study we investigated the contribution of costimulatory molecules in CD8 T cell response upon mouse CMV (MCMV) infection and their role in CD8 T cell inflation. We infected mice with WT MCMV or recombinant MCMV viruses lacking viral proteins that negatively regulate the expression of different CD8 T cell costimulatory molecules and recombinant viruses expressing cellular ligands for CD8 T cells costimulatory receptor NKG2D and followed CD8 T cell response over time. Our results show that upon infection with recombinant MCMV expressing NKG2D ligands, as well as with most of the MCMV mutants lacking genes that regulate expression of the costimulatory molecules, a higher frequency of MCMV-specific memory precursor effector cells is established early during infection, and we will investigate whether this effector memory pool serves as a source of inflationary cells in peripheral tissues.

UNCOVERING SARS-COV-2 PROTEOME: DEVELOPMENT OF A HIGHLY SPECIFIC ANTI-SARS COV-2 MONOCLONAL ANTIBODIES

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In early 2020, pandemic of COVID-19, triggered health and economy crisis worldwide, and to date, great efforts have been invested into studying this novel coronavirus. Advancement in research requires development of quality molecular tools. Our aim is to develop recombinant SARS-CoV-2 proteins and monoclonal antibodies raised against entire SARS-CoV-2 proteome. Here, we present characterization of mouse monoclonal antibodies targeting Spike/RBD, Nucleoprotein and several non-structural proteins (nsp16, nsp10, etc.). Briefly, we produced recombinant SARS-CoV-2 proteins for immunization in either eukaryotic or prokaryotic expression systems. Following protein purification, mice were immunized and used to develop monoclonal antibody producing hybridoma cell lines. In addition to using recombinant proteins for mAb generation, we also used them to develop in-house ELISA to perform serosurveillance for the presence of COVID-19 antibodies amongst healthcare professionals from Clinical Hospital Center of Rijeka. Demographic and clinical data were collected at baseline, including self-reported prior laboratory-confirmed COVID-19, when applicable. Using ELISA, we checked their blood samples for antibodies against N protein at different time points. Our preliminary findings suggest 19% of baseline seroprevalence for N antigen, supporting the use of serological assays for identification of COVID-19 positive patients. Development of SARS-CoV-2 tools and their application in research will contribute to the better understanding of the biology of SARS-CoV-2 virus. This work has been fully supported by Croatian Science Foundation under the project IP-CORONA-04-2073.

ACCURACY OF CONVENTIONAL CELL CULTURE POTENCY ASSAYS FOR MUMPS VIRUS

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Viral titer is an important parameter for virus characterisation in virology and vaccinology. Most frequently used methods for titer determination are the plaque assay, based on the cytopathogenic effect (CPE) in form of plaques, and the 50% cell culture infectious dose ($CCID_{50}$) assay, based on detection of CPE in 50% of the infected cell cultures.

In these assays, the viral titer is determined after macroscopic plaque counting or after CPE detection using light microscopy. Both processes are prone to operator's subjectivity, which can lead to inaccurate determination of titer. The aim of this research was to compare viral titres in both assays using the two ways of titar determination: a) conventionally, using the light microscopy or macroscopic plaque counting and b) by using fluorescence microscopy.

In this study, we prepared recombinant mumps viruses, MRV3 and Vdeopti-MRV3, by inserting the enhanced green fluorescent protein gene into the consensus sequence of L-Zagreb strain. We detected significant difference in results of the plaque assay, because both viruses had poorly visible plaques for macroscopic plaque counting. The proportion of these plaques can lower the viral titer for 0.3-0.4 log plaque forming units in comparison to the viral titer obtained by using fluorescence microscopy and lead to inaccurate titer determination. Viral titres in the $CCID_{50}$ assays were comparable.

For viruses with poorly visible plaque morphology, $CCID_{50}$ assay is a better choice for titer determination.

COMPARISON OF PRODUCTION- AND PURIFICATION- RELEVANT PROPERTIES OF MURINE AND HUMAN CYTOMEGALOVIRUS

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The impact of human CMV (HCMV) infections on public health is significant while affecting the most vulnerable groups, immunocompromised individuals and congenitally infected infants. Thus, a vaccine to reduce the incidence and severity of HCMV infection is a public health priority. Moreover, cytomegalovirus has a number of features that makes it a very interesting vector platform for gene therapy. In both cases, preparation of highly purified virus is a prerequisite for safe and effective application. Murine CMV (MCMV) is by far the best studied model for HCMV infections with regard to the principles that govern the immune surveillance of CMVs. The transfer of knowledge from MCMV and mice to HCMV and humans could face challenges not only because of differences in the immune systems of these two species, but also because of differences in the biological and biophysical properties of the two viruses. We carried out a detailed investigation of the MCMV and HCMV growth kinetics as well as stability under the influence of clarification, different storage conditions and ultracentrifugation. We also investigated possibilities to purify both viruses by ion-exchange chromatography. The effectiveness of the procedures was monitored using $CCID_{50}$ assay, Nanoparticle tracking analysis (NTA), ELISA for host cell proteins, and quantitative PCR assay for host cell DNA. MCMV generally proved to be more robust in handling and despite its greater sensitivity, HCMV was efficiently (100% recovery) purified and concentrated by anion-exchange chromatography using QA monolithic support. These results provide important data for research on all upstream and downstream processes on these two viruses regarding biotechnological production and basic research.

NKG2D LIGAND RECOGNITION BY γδ T CELLS DRIVES STEATOHEPATITIS AND FIBROSIS IN MAFLD

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Metabolic-associated fatty liver disease (MAFLD) is considered to be the hepatic manifestation of metabolic syndrome. It encompasses a plethora of liver abnormalities ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). What triggers hepatitis in MAFLD is of particular interest because inflammation is the underlying cause of liver fibrosis. However, most investigations focus on the later stages of disease pathogenesis when changes in the liver may be permanent. About the initial triggers of liver inflammation in context of MAFLD, little is known.

To investigate the role of early immuno-metabolic sensing in the development of NASH, we have developed a dietary mouse model (steatosis-steatohepatitis diet, SSD). Upon 16 weeks on SSD, mice develop steatosis and fibrosis with observed immunopathology. Increased IL-17A production was observed as an early immunopathogenic event in SSD mice, with innate-like T cells as a major source. Direct signaling of IL-17A to hepatocytes appeared to be an important underlying cause of liver fibrosis in our model of MASH as Alb^{Cre}IL-$17R^{fl/fl}$ mice showed impaired immunopathology and alleviated fibrosis after 16 weeks of SSD feeding. Immunohistochemical staining of human livers revealed increasing abundance of IL-17A producing cells with the disease severity and staging. We observed that IL-17A production was most prominent in T cells expressing high levels of the activating receptor NKG2D. Indeed, metabolic stress upon SSD caused an upregulation of NKG2D stress ligands on the surface of hepatocytes. Furthermore, immunohistological staining of human liver biopsies revealed increased NKG2DL expression in steatotic areas of the liver of patients with MAFLD. Using NKG2D deficient (*Klrk1*$^{-/-}$) mice, we showed that deficiency of NKG2D receptor on immune cells reduces immunopathology and alleviates fibrosis after 16 weeks on SSD. Importantly, innate-like T cells showed decreased IL-17A secretory capability in *Klrk*$^{-/-}$ mice.

Our study shows that NKG2D-mediated activation of innate-like T cells drives IL-17A secretion, immunopathology and fibrosis during the earliest stages of MAFLD. Inhibition of this axis therefore appears to be a promising target for the prevention of MAFLD progression.

IMMUNOLOGICAL ROLE OF CELLULAR PRION PROTEIN (PRP^C) DURING VIRAL INFECTION

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PrP^C is a GPI-anchored glycoprotein predominantly expressed in the brain and then in other tissues, including immune cells. Its best-described physiological role is a neuroprotective effect. it has been shown that PrP^C is a significant factor in several mouse models of viral infections that mimic human conditions. Our goal is to examine the role of PrP^C protein in brain immunology, where this protein is most pronounced and where cytomegalovirus (CMV) has the most devastating consequences during brain development.

We have shown that CMV in different cell line and primary cell cultures affects the amount of PrP^C on the surface and inside the cells. After initial strong induction of PrP^C expression, CMV actively removes PrP^C from infected cells. The loss of PrP^C following infection is not the result of protein degradation pathways activation as the samples treated with degradation inhibitors show no difference from untreated samples. PrP^C is cleaved from the surface of infected cells by the ADAM10 protease through the process of shedding similar to what happens in HIV infection shown by a different group.

In our preliminary experiments, the amount of PrP^C on microglia cells isolated from CMV-infected newborn mice was significantly increased. By comparing PrP-KO newborn mice that have a mutation only in the Prnp gene and wild-type Black/6 newborns, we found that PrP-KO mice have lower virus titers in brain, spleen, and salivary gland.

Overall, presented data indicate that PrP^C is involved in immune response to viral infection.

VIRAL INFECTION OF THE OVARIES COMPROMISES PREGNANCY AND REVEALS INNATE IMMUNE MECHANISMS PROTECTING FERTILITY

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Viral infections during pregnancy are a considerable cause of adverse outcomes and birth defects, while the underlying mechanisms are poorly understood. Among those, cytomegalovirus (CMV) infection stands out as the most common intrauterine infection in humans, putatively causing early pregnancy loss. Herein, we employed murine CMV (MCMV) as a model to study the impact of virus infection of the ovaries on pregnancy outcome and fertility maintenance. Our data showed highly selective mMCMV infection in the ovaries, with strong infection of corpora lutea (CL) and no signs of infection in the follicles. High infection densities indicated complete failure of immune control in CL cells, resulting in progesterone insufficiency and pregnancy loss. While corpora lutea infection might lead to miscarriage, follicles infection may promote sterility. Thus, uncovering the follicular antiviral mechanisms is of ultimate importance. Our results reveiled that an abundance of gap junctions, absence of vasculature, strong type I interferon (IFN) responses, and interaction of innate immune cells fully protected the ovarian follicles from viral infection. These findings provides fundamental insights into the impact of CMV viral infection on pregnancy loss and mechanisms protecting fertility.

IFN-γ PRODUCED IN INFECTION DOWN-REGULATES PPAR-Γ TO MODULATE ADIPOSE TISSUE BIOLOGY

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Adipose tissue is a major lipid storage organ which releases and distributes lipids to maintain energy homeostasis. In context of metabolic disease, adipose tissue was shown to closely interact with the immune system as obesity drives inflammation in this organ which alters local and systemic regulation of metabolism. However, how immune cells interact with adipocytes in context of viral infection is largely unknown. Here, we investigated the impact of virus-induced activation of the immune system on adipose tissue metabolism and the underlying benefit of these changes to the organism. In an *in vitro* model of adipocyte differentiation, we could show that the pro-inflammatory cytokine IFN-γ significantly reduces cellular lipid content. High-throughput transcriptome analysis of these cells demonstrated that IFN-γ mediates down-regulation of PPAR-γ, a master regulator of adipocyte tissue metabolism, as well as many of its downstream targets, causing a net efflux of nutrients. Infection of mice with cytomegalovirus induced a striking reduction of adipocyte cell size and induced a change in the transcriptional profile of these cells corresponding with an IFN-γ imprint. Accordingly, infection caused a systemic increase of adipose tissue derived nutrients, such as free fatty acids in circulation. Importantly, our results indicate that these nutrients promote the acute lymphocyte response to viral infection. These findings suggest that cytokines produced in response to viral infection can modulate adipocyte and systemic metabolism to benefit the immune response to infectious disease. This project is founded by Croatian Science Foundation (HRZZ).

7-KETOCHOLESTEROL BINDS TOLL-LIKE RECEPTOR 4 ON SYNOVIAL TISSUE MACROPHAGES OF PATIENTS WITH OSTEOARTHRITIS AND SUPPORTS DOMINATION OF M1 CHEMOKINE PRODUCTION

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Introduction: We hypothesized that the oxidized lipid derivative, 7-ketocholesterol (7-KC), represents a danger signal in the synovial membrane of patients with osteoarthritis (OA). The aim was to investigate the influence of 7-KC on chemokine production in synovial tissue CD68+ macrophages of patients with OA in respect of lipopolysaccharide (LPS) stimulation, the prototypic M1 polarization stimulus.

Material and methods: Double immunofluorescence and immunohistology were performed in paraffin-embedded synovial tissue sections, which were obtained during the knee alloarthroplasty. Synovial mononuclear cells were isolated by enzymatic digestion. We analyzed the binding of 7-KC for Toll-like receptor (TLR)-4 and the viability of CD68+ cells, intracellular chemokine expression in 18 hour-stimulated CD68+ cells with 7-KC, 7-KC+LPS, LPS or medium only, using flow cytometry.

Results: 7-KC bound for TLR-4 on CD68+ cells, in a dose-dependent manner (3,125 μM -25 μM) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB)+CD68+ cells were found in synovial tissue. The increasing concentrations of 7-KC proportionally killed CD68+ cells *in vitro* and apoptotic protease activating factor (APAF)-1+CD68+ cells were sparse in synovial tissue. 7-KC and LPS independently increased expression of CC ligand (CCL)3, however, 7-KC increased CCL2 only in combination with LPS. Contrary to LPS, 7-KC decreased the frequency of CCL22, although both of them independently decreased frequency of CCL17 within the CD68+ population.

Conclusions: 7-KC binds to TLR-4 in a dose-dependent manner and in pharmacological dose supports type 1 macrophage chemokine production, followed by CD68+ cell-apoptosis *in vitro*.

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MCMV INDUCES ACTIVATION AND ACCUMULATION OF ARF6 GTPASE ON MEMBRANES OF VIRION ASSEMBLY COMPARTMENT

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Shortly after entering the host cells, murine cytomegaloviruses (MCMVs) reorganize Golgi and endosomal system of the infected cells and form the MCMV assembly compartment (AC) in order to enable production of the new virions. One of the features of the reorganized membrane organelles in AC is the extensive tubulation that in uninfected cells, among other GTPases from Ras family, is triggered by Arf GTPases, especially Arf6.

The aim of this study was to determine expression, localization, as well as degree of activation of Arf6 and its main regulators (GAPs and GEFs) during MCMV infection. In order to address this question, Balb 3T3 cells were infected with recombinant murine cytomegalovirus Δm138-MCMV and expression of Arf6, its regulators, and MCMV viral proteins was followed up to 30 hours post infection (30 hpi) by Western blot and by confocal microscopy. Degree of Arf6 activation was determined by pull-down and wound healing assays. Viral replication was monitored in cells infected with C3X-GFP MCMV.

Up to 30 hpi, there are no significant changes in Arf6 expression, although its intracellular localization and activation are altered by MCMV infection, as are the levels and intracellular localization of its major regulators.

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TOWARDS NANOBIOSENSOR FOR CORONAVIRUS (COVID-19) DETECTION: STRUCTURAL CHARACTERIZATION OF GOLD NANOPARTICLES FUNCTIONALIZED WITH MONOCLONAL ANTI-SARS-CoV-2 ANTIBODIES

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Unique characteristics of the nanomaterials (NMs) and their cost- and time-effective production protocols have enabled application of new nanobiosensors with high precision and great sensitivity in molecular detection of various biomarkers. Amongst many NMs, gold nanoparticles (AuNPs) have attracted extensive attention due to their unique functional and surface plasmon resonance properties, which may significantly enhance diagnostic features. By careful design, novel antibody-functionalized AuNPs may aid in rapid testing and improve the accuracy and sensitivity of diagnostic technology for COVID-19 caused by SARS-CoV-2 virus.

This study demonstrates the development of three different conjugation strategies of AuNPs for more efficient binding of monoclonal anti-SARS-CoV-2 antibodies: a) direct conjugation of antibodies by electrostatic interactions or physical adsorption on nanosurface, b) conjugation mediated by glycopeptide using peptidoglycan monomer GlcNAc-MurNAc-L-Ala-D-*iso*Gln-*meso*DAP(NH2)-D-Ala-D-Ala (PGM) and c) covalent conjugation using EDC chemistry. The AuNPs-antibodies conjugates were characterized by UV-Vis, DLS and TEM techniques. Preliminary results showed that covalent conjugation using EDC/NHS chemistry enabled the most stable nano-enabled conjugate with high potential for use in SARS-CoV-2 virus detection.

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ANTIVIRAL ACTIVITY OF RIBAVIRIN AGAINST MUMPS VIRUS

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Mumps virus (MuV) is an important aerosol-transmitted human pathogen causing epidemic parotitis, meningitis, encephalitis, orchitis and deafness. Mumps is prevented by vaccination, although vaccine efficacy and safety are being re-examined in the last decades. Specific treatment for mumps does not exist.

In our work we have treated different MuV strains with a range of concentrations of ribavirin (nucleoside analog) in two different cell lines (Vero and LLC-MK2). Dose-dependent titer decrease was present proving a strain-independent antiviral effect. Results obtained from deep sequencing show increase in population diversity for ribavirin treated virus, as well as increase in ribavirin specific mutations. We have also conducted consecutive passages of virus treated with ribavirin in different concentrations in Vero cells to determine long-term effect of mutagen on MuV. Initial decrease in titer was present after the first passage. For the lower concentrations of mutagens, titer started to rise from that point forward. Higher concentrations of ribavirin resulted in no detectable virus titer after 5 passages. We conducted three additional blind passages in the control medium that have not resulted in revival of the virus.

Our results suggest that lower concentrations of ribavirin lead to mutagen resistance. We did not determine if this is a property of a whole viral population or whether it is dependent on a specific viral variant. Nonetheless, higher concentrations of ribavirin seem to cause virus extinction proposing lethal mutagenesis for mumps. This finding opens up possibilities for treatment after the infection has already taken place.

COMPREHENSIVE PHENOTYPIC AND FUNCTIONAL ANALYSIS OF MEMORY CD8 T CELL RESPONSES AFTER SARS-COV-2 INFECTION AND COVID-19 VACCINATION

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Infection with SARS-CoV-2 induces both a potent cellular and humoral immune response. Unfortunately, various mutants ofthis virus have emerged that manage to mostly escape antibody recognition. Therefore, it is critically important to understand ifSARS-CoV-2 convalescent individuals develop functional memory CD8 T cells that are capable of protection from subsequentinfections. Here we performed a comprehensive phenotypic and functional analysis of antigen-specific CD8 T cells in SARS-CoV-2–infected individuals 3 and 6 months' post infection and in vaccinated individuals. Mice with a humanized immunesystem using cells from SARS-CoV-2 convalescent donors were infected with mCMV-strains carrying dominant SARS-CoV-2or Influenza epitopes, to assess the in vivo recall capacity of antigen-specific cells. We demonstrate that both SARS-CoV-2infection and vaccination elicit potent antigen-specific memory CD8 T cell response. However, the overall magnitude of theanalyzed antigen-specific response was higher after vaccination. Importantly, convalescent individuals developed SARS-CoV-2-specific memory T cells that persisted for at least six months. Expression of CD3, CD57 and NKG2D was lower in theindividuals vaccinated against COVID-19 compared to SARS-CoV-2–infected individuals whereas expression of CD27 washigher after COVID-19 vaccination. In addition, we observed distinct phenotypic profiles of SARS-CoV-2 and Influenza-specificmemory CD8 T cells. Our findings indicate that both infection and vaccination induce a potent memory CD8 T cell responseagainst SARS-CoV-2, but that there are key functional difference between these methods of memory induction.

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COMPARISON OF PRECLINICAL PROPERTIES OF SEVERAL AVAILABLE ANTIVENOMS IN THE SEARCH FOR EFFECTIVE TREATMENT OF *VIPERA AMMODYTES* AND *VIPERA BERUS* ENVENOMING

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Snakebites in Europe are mostly caused by *Vipera ammodytes*, *Vipera berus* and *Vipera aspis*. Among eight available antivenoms, only Zagreb antivenom, Viperfav and ViperaTAb have been used almost exclusively for decades. Zagreb antivenom and Viperfav are considered clinically efficient against envenoming caused by all three medically relevant species, while ViperaTAb is indicated for the treatment of *V. berus* bites solely. When the production of Zagreb antivenom was discontinued and a shortage of Viperfav occurred, other potentially suitable antivenoms were implemented into clinical practice, but without comparative assessment of their eligibility. The aim of our work was to identify at preclinical level a high-quality antivenom that might ensure successful treatment of envenoming caused by both *V. ammodytes* and *V. berus*.

A thorough preclinical analysis of the safety-related properties and efficacy of a panel of anti-*Vipera* spp. antivenoms that are currently available, or in development for the European market, was performed in a comparative manner. Emphasis was placed on their physicochemical properties, primarily purity and aggregate content, and *in vivo* protective efficacies.

As Zagreb antivenom is no longer available on the European market, Viperfav emerged as the highest-quality product and the only one whose neutralisation potency against *V. ammodytes* and *V. berus* venoms was above regulatory requirements.

Although monitoring of effectiveness is of utmost importance in the decision-making process, the presented findings may serve as a starting point for guidance to clinicians when choosing the most appropriate antivenom for the treatment of envenoming in Southeastern Europe.

MODULATION OF MHC I EXPRESSION BY M04 AND MATP1 MCMV PROTEINS AND THE EFFECT OF MHC I-M04-MATP1 COMPLEX ON NK AND CD8+ T CELL RESPONSE

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Human cytomegalovirus (HCMV) is a widespread β-herpesvirus. While it does not cause significant disease in healthy immunocompetent individuals, immunosuppressed patients or newborns with immature immune system are at high risk for complications, multi-organ disease and even death. No vaccine or effective therapy has yet been found, and one of the reasons is certainly our insufficient understanding of mechanisms of viral immune evasion. Since HCMV cannot infect experimental animals, mouse cytomegalovirus (MCMV), a closely related and similar virus, is often used as a model to investigate pathogenesis and immune responses to human CMV. One of the mechanisms CMVs employ to evade the immune response is the modulation of MHC I molecules. We have previously found that viral m04 and MATp1 proteins form a tri-molecular complex with host MHC I molecules which can then bind activating and inhibitory Ly49 receptors of NK cells and modulate their responses. Since MHC I molecules are also important for CD8 T cell responses, we are currently investigating the impact this viral immunoevasive strategy has on CD8 T cell response. m04 and MATp1 could help in evasion of CD8 T cells by engaging inhibitory Ly49 receptors on CD8 T cells and/or by modulating the presented peptides.

THE INFLUENCE OF ANTI-INFLAMMATORY DIET ON INNATE AND ACQUIRED IMMUNE RESPONSE IN OBESE POPULATION

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Introduction: One of the greatest clinical and public health challenges of the 21st century is obesity, which may be associated with more chronic disseases, such as diabetes type 2, hypertension and atherosclerosis. Obesity represents a chronic low-grade inflammation with consequently activation of immune system and may have a key role in the pathogenesis of obesity-related metabolic disorders. The Dietary Inflammatory Index (DII) was developed and validated as a scoring algorithm of 45 food parameters to investigate the inflammatory potential of an individual's diet.

Objective: The aim of study was to determine changes in immune status of obese patients after 24 weeks of nutritional intervention based on anti-inflammatory diet.

Subjects and Methods: Participants were divided into group with nutritional intervention based on anti-inflammatory diet (intervention subjects IS) and into control group (CG) with the KBC Rijeka standard education protocol and energy and nutritional restriction of the diet.
Human peripheral blood mononuclear cells (PBMNC) were analysed on flow cytometer. Inflammatory status was assessed by concentration of hs-CRP, IL-6 and TNF-α.
The inflammatory potential of the diet was assessed by the Dietary Inflammatory Index (DII).
Data were analyzed using Statistica for Windows.

Results: The percentage of innate immune cells (NKT and Treg cells) is significantly decreased after antiinflammatory diet in comparisson to standard diet. In both studied groups markers of inflammation: hs-CRP, IL-6 and TNF-α were statistically significantly reduced.

Conclusion: The use of anti-inflammatory diet has been shown to be effective in the treatment of obesity.

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THE ROLE OF INNATE IMMUNE CELLS IN AUTOIMMUNE THYROID DISEASE (AITD) DURING PREGNANCY AND POSTPARTUM

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Background: Autoimmune thyroid disease (AITD) is very common in women in reproductive age. The pregnancy and postpartum period affect the regulation of thyroid gland and conversely thyroid disorders may influence conception and the course of pregnancy. Hormonal changes and Th1/Th2 cytokine balance with Th2 predominance has been seen as a very important mechanism determining the maintenance of pregnancy.

Aim: was to investigate the changes of NKT and T regulatory cells (Tregs) in pregnant and postpartum women with AITD and compare the results to normal pregnant and postpartum women.

Material and methods: The study included 185 pregnant women; 111 in 1. Half of pregnancy, 74 in 2. Half of pregnancy and 77 women in postpartum period (3 weeks-9 months after delivery). Peripheral blood and sera obtained from women was screened for thyrotropin (TSH), free thyroxine, free triiodothyronine level, titers of anti-thyroid peroxidase antibody (TPO), thyroglobulin (TgAbs) antibody levels and TSH receptor stimulating antibodies. The subpopulations of innate immune cells were determined by flow cytometric analysis.

Results: The percentage of innate cells was significantly higher in 1. and 2. half of pregnancy with subclinical or clinical hypothyroidism and hyperthyroidism compared to control pregnancy and non-pregnant control women. The percentage of NKT cells was significantly higher in postpartum women with subclinical and clinical hypothyroidism and hyperthyroidism compared to control postpartum women.

Conclusion: innate immunity is very important in immunomodulation in pregnancy and is responsible for balancing the self-tolerance and homeostasis and mediates maternal tolerance to fetus.

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GENE EXPRESSION AND CYTOKINE SECRETION PROFILES OF PRIMARY MONOCYTES IN RESPONSE TO ORTHOHANTAVIRUS INFECTION

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We aimed to investigate gene expression and cytokine secretion of primary monocytes in response to orthohantavirus infection in a timely manner. For this purpose, we analyzed the gene expression dynamics of selected immune and lineage genes, as well as the secretion patterns of selected cytokines and chemokines. Primary monocytes were isolated from six healthy donors and infected with pathogenic (PUUV) or low pathogenic (TULV) orthohantavirus.

Monocytes infected with PUUV, TULV or mock control (MOCK) were cultured for up to seven days post infection and at the indicated time points the cells were lysed. qPCR array for 19 genes (*CD68, TNF, IL6, IL1B, IL1RN, CD40LG, CXCL10, CXCL8, CCL4, IL27, IL37, CCR2, CCR5, CXCR4, CCL13, STAT1, STAT3, MRC1, IFITM3*) was performed. The gene expressions were normalised to MOCK and compared between PUUV, TULV and MOCK infection.

Concentration dynamics of 21 cytokines and chemokines (IL-1 beta, IL-1RA, IL-6, IL-10, IL-17F, IL-27, IL-37, IFN-gamma, MIF, CCL2, CCL3, CCL4, CCL5, CCL22, CD40L, CXCL8, CXCL10, M-CSF, GM-CSF, TNF-alpha, TGF-beta1) in supernatants of PUUV-, TULV- or MOCK-infected cells, at indicated time points, were measured using magnetic bead-based immunoassays with reads on Luminex 200 analyzer.

Orthohantavirus infection triggers early proinflammatory response of primary human monocytes. Prolonged infection further induces differentiation of primary human monocytes into macrophages, shaping the "M2-like"polarization profile. Significant differences between viruses regarding their pathogenicity were seen at both gene and cytokine and chemokine release levels.

THYMUS AS A SOURCE OF ADULT STEM CELLS FOR REGENERATIVE THERAPY

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The thymus has still many developmental and regeneration features in the adulthood that are a matter of controversy. Indeed, the thymus gets discarded as medical waste during heart surgical interventions in infants. The heart surgery itself is a life saving and urgent intervention, whereas thymus deficiency is not easily visible at once. There is growing evidence of the thymus importance in the adulthood, especially regarding viral diseases and aging.

The main objective of our work is to regenerate the thymus function by clonal expansion of tissue-specific stem cells, especially thymic epithelial stem cells (TESC). Current solutions are mostly based on transcription-factors activation or iPSC that can be differentiated into thymus epithelial cells. These methods have a number of disadvantages and cannot be appropriately applied. We propose an alternative solution developing an in vitro 3D system adapted for a specific task to induce and support clonal expansion of TESC and reversibly block their differentiation into mature cells. These tasks depend on the composition of culture media applied specifically for TESC clonal expansion in vitro, and the multicellular environment composed of epithelial and lymphoid cells as well as the heart condition of the thymus donor. Our goal is the development of conditions that can drive thymus regenerative therapy in vivo. Together, it will enable us to achieve autologous thymic tissue transplantation and immune rehabilitation of patients who were subject for partial or total thymectomy during cardiac surgery or who have impaired thymus function due to aging or iatrogenic causes.

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