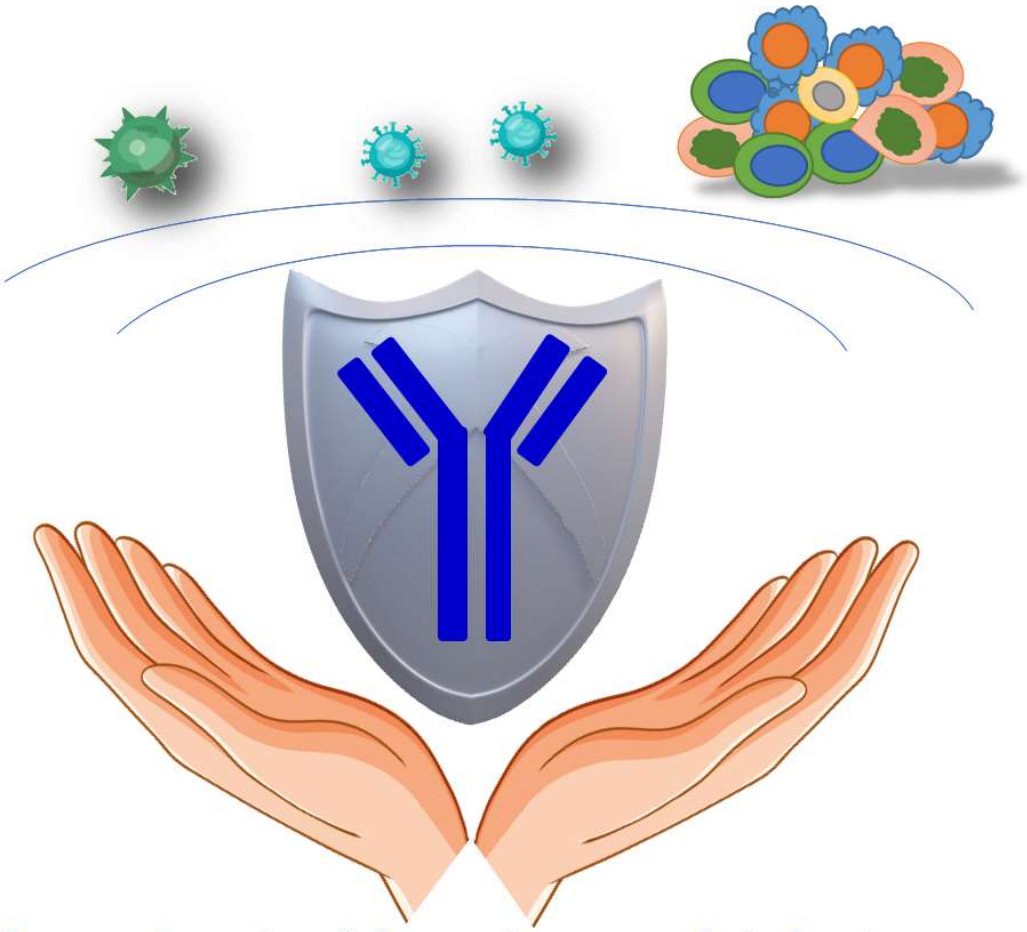

The Annual Meeting of the Croatian Immunological Society 2023

Dates: from 5th until 7th October 2023.

Venue: Hotel Neptun/Istra, Brijuni, Croatia

2023 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

Brijuni, 05 - 07.10.2023.

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY
Bijenička cesta 54, Ruđer Bošković Institute, Zagreb

President: Mariastefania Antica, Zagreb
Vice-President: Vanda Juranić Lisnić, Rijeka
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Dear Friends and Colleagues,

I would like to welcome you to the Annual meeting of the Croatian Immunological Society (HID) with international participation for 2023. This year it will be held in the Brijuni National Park, where scientists from Croatia as well as international invited speakers would have a great opportunity to discuss the major achievements of immunology through the last year in an atmosphere of a unique calmness and beauty. The Society board (Malo vijeće) worked diligently to continue our tradition of excellence in immunology and to plan three days of exceptional and cutting-edge sessions focused on research and education. We are happy to include lectures from renowned speakers as well as presentations of young scientists covering different fields of immunology, hoping we would all benefit from dynamic and diverse discussions. As an active member of the European Federation of Immunological Societies (EFIS), HID is grateful for the continuous EFIS support and has a number of activities under the general flag, but also under the focused task forces. We are proud to announce the EFIS-Immunology Letters Lecture Award that will be granted to Professor Burkhard Becher during the meeting. Through the year, we participated in a number of EFIS activities, including Day of Immunology celebration (DOI 2023), Young Immunologist Task Force (yEFIS) webinars, EFIS Gender and Diversity Task Force discussions and many others. We are grateful to all our members who are contributing with almost 40 abstracts of innovative immunology research as well as educational and technical contents, making this meeting meaningful for establishing new connections and collaborations. For the first time, we are introducing a new session for young group leaders, who will give us an insight in their future projects and immunology groups expected to emerge in the years to come.

As a recently elected president, I am exceptionally proud and excited to share with you the meeting events in a friendly and productive atmosphere. I would like to thank all the members of the Society board for the help in preparing the meeting, especially our secretary Lidija Milković for her hard work. We also thank our sponsors and hope you will all enjoy the stimulating and interesting talks, wishing you to widen your knowledge and collaborations in this challenging and expanding field of science.

With my best wishes,

Mariastefania Antica, President

THURSDAY, October 5th 2023

14:00-24:00	**HOTEL CHECK-IN**
14:30-15:30	**REGISTRATION**
15:30-15:40	**OPENING CEREMONY** **Mariastefania Antica**, president of the Croatian Immunological Society
15:40-16:20	**INVITED LECTURE** **Chairs: Danka Grčević & Jurica Arapović** **Cormac Gahan** University College Cork, Ireland ***"Bile acids as signalling molecules in microbiota-host interactions"***
16:20-17:20	**SELECTED ORAL PRESENTATIONS – SESSION 1– "YOUNG SCIENTISTS – NEW PROJECT LEADERS"** **Chairs: Danka Grčević & Jurica Arapović**
16:20	**Špela Konjar** - Universidade de Lisboa, Lisboa, Portugal ***"Metabolic reprogramming of IELs and their dependence on metabolites during activation"***
16:40	**Maja Cokarić Brdovčak** - University of Rijeka, Rijeka, Croatia ***"Immunization against SARS-CoV-2 using alternative viral vector vaccines and alternative routes"***
17:00	**Vilma Dembitz** - Queen Mary University of London, London, UK ***"SCD inhibition preferentially eradicates AML displaying high de novo fatty acid desaturation and synergizes with chemotherapy"***
17:20-18:00	**INVITED LECTURE** **Chairs: Danka Grčević & Jurica Arapović** **Stipan Jonjić** Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia ***"New player in the cancer immunotherapy: anti-PVR mAb with multiple modes of action"***
19:00	**DINNER**

FRIDAY, October 6th 2023

9:00-9:40 **INVITED LECTURE**

Chairs: Stipan Jonjić & Asja Stipić Marković

Ian Humphreys

Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom

"T cells in cytomegalovirus chronicity: the dark side"

9:40-10:25 **SELECTED ORAL PRESENTATIONS – SESSION 2**

Chairs: Stipan Jonjić & Asja Stipić Marković

9:40 **Mia Krapić** - University of Rijeka, Rijeka, Croatia

"NK cell derived IFN-γ causes free fatty acid release by adipocytes to promote B cell responses during mCMV infection"

9:55 **Ilija Brizić** - University of Rijeka, Rijeka, Croatia

"Perinatal cytomegalovirus infection reshapes the transcriptional profile and functionality of NK cells"

10:10 **Vanna Imširović** - University of Rijeka, Rijeka, Croatia

"Largely preserved functionality after the combined loss of NKG2D, NCR1 and CD16 demonstrates the remarkable plasticity of NK cell responsiveness"

10:25-10:40 **SPONSORED LECTURE BD – GOREA**

"See what you sort & sort what you see. A novel technology from BD Biosciences for High-speed fluorescence image-enabled cell sorting"

10:40-11:20 **COFFEE BREAK (SPONZORED BY GOREA)**

11:20-12:00 **INVITED LECTURE**

Chairs: Alenka Gagro & Vanda Juranić Lisnić

Marija Jelušić

University of Zagreb School of Medicine, University Hospital Centre Zagreb, Department of Paediatrics, Zagreb, Croatia

"New Insights in IgA Vasculitis and IgA Vasculitis With Nephritis"

FRIDAY, October 6th 2023

12:00-12:45	**SELECTED ORAL PRESENTATIONS – SESSION 3 – BRIGHT SPARK**
	Chairs: Alenka Gagro & Vanda Juranić Lisnić
12:00	**Karlo Mladenić** - University of Rijeka, Rijeka, Croatia
	"NKG2D-mediated detection of metabolically stressed hepatocytes by innate-like T cells is essential for initiation of NASH and fibrosis"
12:15	**Darja Flegar** - University College Cork, Cork, Ireland
	"Dietary chitosan related changes to the microbiome and course of Listeria monocytogenes infection in high-fat diet fed mice"
12:30	**Fran Krstanović** - University of Rijeka, Rijeka, Croatia
	"IL-12 – CD4 T cell – IFNy axis mediates suppression of cytomegalovirus replication and establishment of latency in cortical and hippocampal neurons"
12:45-13:00	**SPONSORED LECTURE – MEDIC**
	Michal Maj - Application Specialist, Cytek Biosciences
	"Advancing your immunological research with Full Spectrum Profiling and high resolution Imaging Flow Cytometry"
13:00-14:30	**LUNCH**
14:30-15:00	**GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY**
15:00-16:00	**INVITED LECTURE – EFIS-IL LECTURE AWARD CEREMONY**
	Chairs: Bojan Polić & Mariastefania Antica
	Burkhard Becher
	Institute of Experimental Immunology, University of Zurich, Switzerland
	"IL-12 and IL-23: the paradox of simultaneous pro/anti inflammatory properties"
16:00-17:00	**POSTER SESSION**
19:30	**GALA DINNER**

SATURDAY, October 7th 2023

9:00-9:30	**INVITED LECTURE** **Chairs: Tomislav Kelava & Felix Wensveen** **Vanda Juranić Lisnić** Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia ***"Characterizing NK and ILC1 responses to cytomegalovirus in adrenal glands and ovaries"***
9:30-10:45	**SELECTED ORAL PRESENTATIONS – SESSION 4** **Chairs: Tomislav Kelava & Felix Wensveen**
9:30	**Božo Šušak** - Faculty of Medicine, University of Mostar, Mostar, BIH ***"The impact of pre-existing immunity on the course of SARS-CoV-2 infection in health-care workers"***
9:45	**Beata Halassy** - University of Zagreb, Centre for Research and Knowledge Transfer in Biotechnology, Zagreb, Croatia ***"What can neutralizing antibodies tell us about the quality of specific immunity in COVID-19 convalescents and vaccinees?"***
10:00	**Petra Svoboda** - Research Department, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia ***"Primary human monocytes differentiate into M2-like macrophages upon long-term orthohantavirus infection"***
10:15	**Sanja Mikašinović** - University of Rijeka, Rijeka, Croatia ***"An IFNg-dependent immune-endocrine circuit lowers blood glucose to potentiate the innate anti-viral immune response"***
10:30	**Tomislav Smoljo** - Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia ***"The bone marrow microenvironment reduces monocytic differentiation induced by low-dose cytarabine"***
10:45-11:15	**COFFEE BREAK**

SATURDAY, October 7th 2023

11:15-11:45	**INVITED LECTURE** **Chairs: Dora Višnjić & Alan Šućur** **Danka Grčević** Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia ***"Modulation of Notch signalling in bone cell progenitors: Venue for therapeutic intervention"***
11:45-12:15	**INVITED LECTURE** **Chairs: Dora Višnjić & Alan Šućur** **Felix Wensveen** Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia ***"Why we get sick; Interactions between the immune and endocrine systems during viral infection"***
12:15-12:45	**AWARD CEREMONY AND CLOSING REMARKS**

Bile acids as signalling molecules in microbiota-host interactions

Cormac G.M. Gahan[1,2,3]

[1] APC Microbiome Ireland, University College Cork, Cork, Ireland.

[2] School of Microbiology, University College Cork, Cork, Ireland.

[3] School of Pharmacy, University College Cork, Cork, Ireland.

Gut microorganisms (known collectively as the gut microbiota) have the capacity to significantly modulate local and systemic immune functions, gut barrier homeostasis, neuro-endocrine functions and circadian rhythm. However the molecular mechanisms that underpin bacteria-to-host signalling events remain to be fully established. Bile acids have emerged as important signalling factors that regulate cellular functions through interaction with specific bile acid receptors (including the FXR and TGR5 receptors) that are widely distributed in human tissues. The gut microbiota alters the signalling properties of bile acids through the production of specific microbial enzymes (including bile salt hydrolase (BSH) and 7-alpha-dehydroxylase) and thereby significantly influences the bile acid signature of the host. We have investigated the role of the bacterial enzyme BSH in microbe-host signalling events using monocolonised gnotobiotic mouse models and cell culture systems. The studies suggest a major role for this enzymatic activity in gut homeostasis, endocrine function & adiposity, and circadian rhythm. Recent work from our lab has also indicated a role for the enyme in gut developemnt in neonatal mice with bile acid signalling reducing epithelial cell proliferation and promoting differentiation. These data suggest an evolutionary paradigm whereby microbial BSH activity potentially influences bacterial colonization and in-turn benefits host gastrointestinal maturation. The microbial and host dependency upon BSH will be discussed along with potential roles of the enzyme in immune signalling, gut development and homeostasis.

New player in the cancer immunotherapy: anti-PVR mAb with multiple modes of action

Stipan Jonjić[1]; Paola Kucan Brlic[1]; Anas Atieh[2]; Akram Obeidat[2]; Keren Paz[2]; Guy Cinamon[2]; Lea Hirsl[1]; Marija Mazor[1]; Tihana Lenac Rovis[1]; Ofer Mandelboim[3]; Pini Tsukerman[2]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia;

[2]Nectin Therapeutics LTD, Jerusalem, *Israel;*

[3]Hebrew University, Jerusalem, Israel

Poliovirus receptor (PVR) has been gaining considerable scientific interest because of its intrinsic and extrinsic roles in tumor progression. The intrinsic functions of PVR in tumor cells promote tumor progression and metastasis, whereas its extrinsic functions involve interaction with the activating and inhibitory immune cell receptors. Therefore, targeting PVR by blocking monoclonal antibodies (mAbs) offers an attractive therapeutic approach for patients with cancer. Here we describe a first-in-class potent therapeutic blocking antibody to human PVR called NTX-1088, developed by Nectin Therapeutics LTD, that is being investigated for the treatment of solid tumors. The antibody blocks PVR interaction with inhibitory receptors TIGIT and CD96, interrupting their immunosuppressive signaling. Additional advantage of the NTX-1088 is manifested through its ability to block the interaction between PVR and the activating receptor DNAM1, preventing PVR-induced internalization of DNAM-1, leading to robust antitumor activation. Efficacy of the NTX-1088 was validated on human tumor cell lines both *in vitro* and in humanized murine models *in vivo*. *In vitro* data showed that NTX-1088 increases activation of CD8 T-cells and NK-cells. *In vivo*, NTX-1088 induces a robust tumor growth inhibition, accompanied by higher prevalence of $DNAM1^{+}$ $CD8^{+}$ tumor infiltrating cells. Altogether, NTX-1088 shows exclusive triple mechanism of action, whereby simultaneous and effective blockade of TIGIT and CD96 is complemented by the efficient restoration of DNAM1. This is a step change in antitumor immune activation, which is currently being validated in the clinic, in a Phase 1, First-in-Human Study of NTX-1088 in patients with advanced solid malignancies (NCT05378425).

T cells in cytomegalovirus chronicity: the dark side

Ian R. Humphreys

Systems Immunity University Research Institute/Division of Infection and Immunity

The beta-herpesvirus Human cytomegalovirus (HCMV) establishes lifelong infection. Although infection in immunocompetent hosts is typically asymptomatic, infection of the immunological immature (congenital infection) and the immune suppressed can cause severe disease. Data derived from clinical studies and experimental models highlight an important role for T cells in control of CMV replication, dissemination and disease. However, herein, I will discuss findings from our laboratory that highlight the detrimental impact that T cells can have during CMV infection *in vivo*.

$CD4^+$ T cells are pivotal in the control of CMV replication in mucosal tissues. However, I will first discuss how CMV infection induces the development of inhibitory T cells in the mucosa that can suppress antiviral immunity during chronic infection. I will describe the mechanisms through which these cells facilite CMV peristence and dissemination, and discuss the clinical implications of these processes. A unique feature of CMV infection is that it induces unusually high frequencies of polyfunctional $CD8^+$ T-cells that are maintained over time. This process, termed 'memory inflation', is accompanied by the formation of mucosal-resident T-cell responses in peripheral tissues. I will discuss unpublished data derived from the mouse CMV (MCMV) infection model that demonstrates that CMV triggers the accumulation of viral-specific $CD8^+$ T-cells in the brain, and these responses are implicated in a decline of neuronal cell function and a concurrent acceleration of congnitive decline. Thus, overall, our data highlight a role for CMV-induced T cell in facilitating virus persistence and driving tissue damage during virus chronicity.

New insights in IgA vasculitis and IgA vasculitis with nephritis

Marija Jelušić

Department of Paediatrics, University of Zagreb School of Medicine, University Hospital Centre Zagreb, Zagreb, Croatia

We have provided a comprehensive overview of the current understanding of IgA vasculitis, the most common systemic vasculitis in children, covering epidemiology, pathogenesis, genetics, diagnostic methods, and treatment. Among the acute complications associated with IgA vasculitis, those involving the gastrointestinal system are most common, while the predominant chronic complication and the primary determinant of morbidity and mortality in children with IgA vasculitis is the renal involvement, referred to as IgA vasculitis nephritis (IgAVN), making it a crucial prognostic factor. The incidence of IgA vasculitis and IgAVN exhibits global variation, likely influenced by a complex interplay of genetic and environmental factors. Besides HLA class II genes, there is emerging evidence suggesting the involvement of various non-HLA genes in the etiopathogenesis of the disease. Moreover, factors beyond immune complexes containing Gd-IgA1 may contribute to the multi-hit pathogenesis of IgA vasculitis. While renal biopsy remains the gold standard for diagnosing IgAVN, it's important to consider that tubulointerstitial changes could serve as significant predictors of adverse outcomes, leading to alternative histologic classifications such as the revised Oxford classification (MEST-C score) being considered. Elevated serum levels of Gd-IgA1 consistently emerge as a reliable biomarker in IgA vasculitis patients, though non-invasive methods for confirming nephritis are still under investigation. Given the lack of high-level evidence from randomized controlled trials, treatment recommendations, such as those provided by the SHARE guidelines, have been developed. Patients with IgA vasculitis, particularly those with IgAVN, require long-term monitoring, even after achieving disease remission, to detect and manage potential complications effectively.

EFIS-IL LECTURE AWARD

IL-12 and IL-23: the paradox of simultaneous pro/anti inflammatory properties

Burkhard Becher

Institute of Experimental Immunology, University of Zurich, Switzerland

The IL-12 superfamily encompasses several heterodimeric cytokines. Among the best-studied members are IL-12 and IL-23 which have been well established to play major roles in the immunity against cancer and in preclinical models of autoimmunity. There are however several paradoxical findings which to this day remain unresolved. IL-12 has emerged as a powerful trigger to remodel the tumor microenvironment and to elicit strong and lasting anti-tumor immunity. However, in contrast to it's well-documented proinflammatory function, in preclinical models of autoimmunity, IL-12 consistently shows immunomodulatory and tissue protective properties. IL-23 on the other hand appears to be a crucial player in the inflammatory cascade to initiate inflammatory lesions, but is unexpectedly tumor promoting in both mice and cancer patients. To address these long-standing paradoxes, we generated a series of reporter mice and conditional mutants to manipulate the IL-12R and IL-23R complexes in autoimmunity and cancer and I will present and discuss these thus far unpublished data at the annual meeting of the Croatian Immunological Society.

Characterizing NK and ILC1 responses to cytomegalovirus in adrenal glands and ovaries

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

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[2] Department of Histology and Embryology, Faculty of Medicine, University of Rijeka B. Branchetta 20, 51000 Rijeka, Croatia

Cytomegalovirus (CMV) is a highly relevant and widespread human pathogen and an excellent model virus for studying antiviral immune responses. Due to its wide tropism, CMV infects nearly every tissue, making it an excellent model for studying organ and tissue-specific immunity as well. However, our knowledge about antiviral immune responses is often restricted to large and readily accessible organs, such as the spleen, liver and lungs. We have recently shown overwhelming infection of ovaries and adrenal glands by murine CMV, with virus kinetics indicating strong involvement of innate immune system in the virus control. In ovaries, a highly tissue-specific infection has been observed, with pervasive infection of corpora lutea resulting in progesterone insufficiency and pregnancy loss. At the same time, ovarian follicles that house oocytes were protected from the infection. We demonstrated that NK and ILC1 cells play a role in the follicular resistance to infection. In adrenal glands, NK/ILC1 depletion results in stronger infection and delayed virus clearance. NK and ILC1 cells in these organs is largely unexplored. We have utilized multiparametric flow cytometry, various murine transgenic strains, adoptive transfers, and functional assays to characterize phenotype, functionality and roles of these cells in virus control in ovaries and adrenal glands.

Modulation of Notch signalling in bone cell progenitors: Venue for therapeutic intervention

Danka Grčević

Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia

Notch pathway is critical for development and homeostasis of many tissues. It is a cell-cell communication system of Notch ligands and receptors, in which signal is transmitted by the release of Notch intracellular domain (NICD) that translocates to the nucleus, interacts with the DNA-binding protein CSL (CBF1/Suppressor of Hairless/LAG-1 or RBPJ) and Mastermind-like protein (MAML), and induces gene expression. Revealing the steps in Notch signaling opens the venue for its therapeutic modulation.

Our research group has been focused on identification of bone progenitors, which give rise to major bone cell lineages – bone forming osteoblast of mesenchymal origin and bone resorbing osteoclasts of hematopoietic origin. Using mouse models, we demonstrated that Notch pathway has significant impact on fate determination and maturation of bone cells.

In the fracture model, we identified alpha smooth muscle actin (aSMA) as a marker of mesenchymal progenitors with osteogenic potential. Increased Notch signaling in osteochondroprogenitors, driven by overexpression of NICD1, resulted in the expansion of osteoblasts and more mineralized callus, whereas anti-Notch1 antibody reduced callus bone mass and biomechanical strength.

Model of rheumatoid arthritis was used to study activation of CX3CR1-expressing myeloid progenitors with the ability to differentiate into macrophages, dendritic cells and osteoclasts. Although Notch signaling modulation affected the activity of all three lineages, the major effect was observed in osteoclasts, resulting in enhanced differentiation and function with inhibition of canonical Notch signaling.

Since current therapeutic approaches are focused primarily on the inhibition of Notch signaling, these skeletal effects warrant additional attention, as they may bring about unwanted responses in osteoblasts and osteoclasts. The work was supported by Croatian Science Foundation projects UIP-2017-05-1965 and IP-2020-02-2431.

Why we get sick; Interactions between the immune and endocrine systems during viral infection

Felix M. Wensveen

University of Rijeka faculty of medicine, Dept. of Histology & Embryology.

When we get sick, we feel miserable. We get a temperature, lose appetite, and become lethargic. Whereas we experience these sickness behaviors as a pathology, in fact they are a set of carefully regulated metabolic adaptations, mediated by the immune system, with the purpose to better fight infection. Surprisingly, much is still unclear about how this is regulated and why it is beneficial for the organism. Over the last decade, our group has investigated how viral infection impacts the availability of nutrients in circulation. We find that infection restricts the availability of glucose and increases levels of lipids in the blood stream. Glucose restriction promotes the systemic responsiveness to viral infection and enhances type 1 interferon responses, whereas lipids promote early B cell activation. Interestingly, our findings suggest that metabolic diseases such as type 2 diabetes (T2D) are partially the result of a derailed mechanism aimed at protecting the body from viral infection. Moreover, people with diseases such as T2D fail to accomplish the systemic metabolic adaptations required to optimally fight infection upon pathogen encounter and therefore suffer from more frequent and severe disease. In this presentation I will outline some of the molecular mechanisms of immune-endocrine interactions that underly sickness behavior in response to viral infection and will explain how these derail in context of metabolic disease.

Metabolic reprogramming of IELs and their dependence on metabolites during activation

Špela Konjar[1,2], Cristina Ferreira[1], Filipa Sofia Carvalho[1], Patrıcia Figueiredo-Campos[1], Julia Fanczal[1], Sofia Ribeiro[1], Vanessa Alexandra Morais[1] and Marc Veldhoen[1]

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The metabolic properties of immune cells are tightly regulated and can adapt to the demands imposed by the environmental niche and pathogenic challenges. The intestinal epithelial barrier is home to one of the largest T cell populations in the body, recognized as intestinal intraepithelial T lymphocytes (IELs). Because of their location in an environment that is colonized by gut microbiota and under the constant challenge by pathogens, dietary antigens, and toxins, IELs must have specific adaptations that enable them to mount an appropriate immune response. One of the functional adaptations of IELs is that they contain high levels of cytotoxic molecules such as granzymes and express activation marker CD69 and CD44, indicating their increased activation state. We have shown that IELs are capable of a unique metabolic response to challenge; IEL activation is metabolically faster than activation of circulating CD8+ T cells. Glycolysis and oxidative phosphorylation (OXPHOS) of IELs are regulated in a mutually dependent manner. This metabolic characteristic is not observed with circulating CD8 T cells. Additionally, we identified nutritional metabolites that sustain IELs metabolic adaptation and enhance their ability to clear an intestinal pathogenic infection through secretion of interferon-gamma. Importantly all the metabolic properties of IELs that we identified, enable them to perform tightly regulated immune response within the fragile and diverse environment of the intestinal epithelial barrier.

Immunization against SARS-CoV-2 using alternative viral vector vaccines and alternative routes

Maja Cokarić Brdovčak[1], Jelena Materljan[1,2], Marko Šustić[1], Sanda Ravlić[3], Tina Ružić[1], Berislav Lisnić[1], Karmela Miklić[1], Marina Pribanić Matešić[1], Beata Halassy[3], Federico Bertoglio[4], Maren Schubert[4], Luka Čičin-Šain[5], Stipan Jonjić[1], Astrid Krmpotić[2]

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Since its outbreak in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused COVID-19 pandemic with over 770 million infected cases and almost 7 million deaths. Although large-scale vaccinations significantly reduced the severity and death, they were less effective in preventing breakthrough infections, especially due to the emergence of new SARS-CoV-2 variants and suboptimal mucosal immunity at the site of virus entry. Currently the COVID-19 pandemic is classified as an established and ongoing health issue, which requires development of more effective vaccines.

Cytomegaloviruses are β-herpesviruses that are promising viral vector vaccine candidates due to their ability to induce long-lasting humoral and cellular immune responses. We have generated recombinant murine CMV (MCMV) vaccine vectors expressing S (spike) protein of SARS-CoV-2 with or without RAE-1γ, ligand for activating receptor NKG2D. Immunization with these vectors resulted in excellent IgG antibody response, strong neutralization capacity and potent long-lasting S-specific CD8 T cell response in mice. Importantly, alternative intranasal route of immunization with our vectors also induced protective immune response.

Additionally, we compared immunization routes of commercially available COVID-19 vaccines. While intramuscular immunization with adenoviral vector vaccines and mRNA vaccine induced excellent S-specific CD8 T cell response in the spleen, intranasal immunization with adenoviral vector vaccines and intramuscular immunization with mRNA vaccine elicited a superior mucosal antibody response, which efficiently neutralized SARS-CoV-2.

Overall, our results indicate that the intranasal route of vaccination and the use of different viral vectors could be promising strategies to achieve efficient control at the site of SARS-CoV-2 entry.

SCD inhibition preferentially eradicates AML displaying high de novo fatty acid desaturation and synergizes with chemotherapy

Vilma Dembitz[1,2], Hannah Lawson[1], Richard Burt[3,4], Céline Philippe[1], Sophie C. James[1], Samantha Atkinson[3,4], Jozef Durko[1], Lydia M. Wang[1], Joana Campos[1], Aoife M. S. Magee[1], Keith Woodley[1], Michael Austin[1], Ana Rio-Machin[5], Pedro Casado-Izquierdo[5], Findlay Bewicke-Copley[5], Giovanny Rodriguez Blanco[6], Diego Pereira Martins[7], Lieve Oudejans[7], Emeline Boet[8,9], Alex von Kriegsheim[6], Juerg Schwaller[10], Andrew J. Finch[11], Bela Patel[1], Jean-Emmanuel Sarry[8,9], Jerome Tamburini[12], Jan Jacob Schuringa[7], Lori Hazlehurst[13], John A. Copland, III[14], Mariia Yuneva[4], Barrie Peck[11], Pedro Cutillas[5], Jude Fitzgibbon[5], Kevin Rouault-Pierre[1], Kamil Kranc[1], Paolo Gallipoli[1]

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Identification of specific and therapeutically actionable vulnerabilities in acute myeloid leukaemia (AML) is needed to improve patients' outcome. These features should be ideally present in many patients independently of mutational background. Here we identify de novo fatty acid (FA) desaturation, specifically stearoyl-CoA desaturase (SCD) inhibition, as a therapeutic vulnerability across multiple AML models in vitro and in vivo. We use the novel clinical grade SCD inhibitor SSI-4 to show that SCD inhibition induces AML cell death via pleiotropic effects, and sensitivity is based on their dependency on FA desaturation regardless of mutational profile. SSI-4 efficacy is enhanced by driving FA biosynthesis in vitro while stroma confers protective effects that extend to in vivo models. SCD inhibition increases DNA damage and its combination with standard DNA damage-inducing chemotherapy prolongs survival in aggressive murine AML models. Our work supports developing FA desaturase inhibitors in AML while stressing the importance of identifying predictive biomarkers of response and biologically validated combination therapies to realize their therapeutic potential.

NK cell derived IFN-γ causes free fatty acid release by adipocytes to promote B cell responses during mCMV infection

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Adipose tissue (AT) is a major lipid storage organ which releases and distributes lipids to maintain energy homeostasis. In the context of metabolic disease, AT 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 AT metabolism and the underlying benefit of these changes to the organism. High-throughput transcriptome analysis of adipocytes in vitro demonstrated that IFN-γ mediates down-regulation of PPAR-γ, a master regulator of AT metabolism, causing a net efflux of lipids. Infection of mice with cytomegalovirus (mCMV) induced a striking reduction of adipocyte cell size and changes in the transcriptional profile of these cells which was IFN-γ dependent. This corresponded with a systemic increase of adipose tissue derived lipids in circulation. In adipose tissue, IFN-γ was abundantly produced by NK cells which directly targeted adipocytes to alter their metabolic profile. Loss of NK cells or deficiency of the IFN-γ receptor on adipocytes prevented the infection-induced loss of adipose lipid content.

Importantly, our results indicate that circulating free fatty acids promote the early B cell activation and their ability to activate T cells as a 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.

Perinatal cytomegalovirus infection reshapes the transcriptional profile and functionality of NK

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Infections in early life can elicit substantially different immune responses and result in different pathogenesis than infections in adulthood. In this study, we investigated the consequences of a common perinatal beta-herpesvirus infection on NK cells using newborn mice infected with the murine cytomegalovirus (MCMV). We found that MCMV infection of newborns severely compromised NK cell phenotype, maturation and functionality. This dramatic effect was observed only if the infection occurred early in life and required active virus replication, even if the virus strain used was severely attenuated and well-controlled, implying that observed NK cell dysfunction is not due to compromised virus control. Inflammatory responses to infection dysregulated the expression of major transcription factors governing NK cell fate, such as Eomes, resulting in impaired NK cell function. Most prominently, NK cells from perinatally infected mice had a severely diminished ability to produce IFN-γ due to the downregulation of long non-coding RNA Ifng-as1. Moreover, the bone marrow's capacity to efficiently generate new NK cells was reduced, explaining the prolonged negative effects of perinatal infection on NK cells. Strikingly, antiviral treatment was unable to prevent this effect. This study demonstrates that viral infections in early life can profoundly impact NK cell biology, including long-lasting impairment in NK cell functionality.

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Largely preserved functionality after the combined loss of NKG2D, NCR1 and CD16 demonstrates the remarkable plasticity of NK cell responsiveness

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Natural killer (NK) cells play an important role in the early defense against tumors and virally infected cells. Their function is thought to be controlled by the balance between activating and inhibitory receptors, which often compete for the same ligands. Several activating receptors expressed on virtually all NK cells lack an inhibitory partner, most notably CD16, NCR1 and NKG2D. We therefore hypothesized that a signal through at least one of these receptors is always required for full NK cell activation. We generated animals lacking all three receptors (TKO) and analyzed their NK cells. *In vitro*, TKO NK cells did not show reduced ability to kill tumor targets but displayed hyperresponsiveness to NK1.1 stimulation. *In vivo*, TKO animals had a minor reduction in their ability to control non-hematopoietic tumors and cytomegalovirus infection, which was the result of reduced NK cell activity. Together, our findings show that activating NK cell receptors without an inhibitory partner do not provide a 'master' signal but are integrated in the cumulative balance of activating and inhibitory signals. Their activity is controlled through regulation of the responsiveness and expression of other activating receptors. Our findings may be important for future development of NK cell-based cancer immunotherapy.

NKG2D-mediated detection of metabolically stressed hepatocytes by innate-like T cells is essential for initiation of NASH and fibrosis

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Metabolic-associated fatty liver disease (MAFLD) comprises a spectrum of clinical entities ranging from benign steatosis to cirrhosis. A key event in the pathophysiology of MAFLD is the development of non-alcoholic steatohepatitis (NASH) that may lead to fibrosis and hepatocellular carcinoma. Despite increasing evidence in the context of NASH immunopathology, early metabolic stress-specific signals which drive immune response in the liver remain unknown. We find that lipid accumulation in hepatocytes induces expression of ligands for the activating immune receptor NKG2D. Tissue-resident innate-like T cells, especially γδ T cells, are activated through NKG2D and secrete IL-17A as a response. IL-17A licenses hepatocytes to produce chemokines that recruit pro-inflammatory myeloid cells into the liver, causing NASH and fibrosis. Importantly, NKG2D receptor deficient $Klrk^{-/-}$ mice, $TCR\gamma\delta^{-/-}$ and Alb^{Cre} $IL\text{-}17R^{fl/fl}$ mice had reduced immunopathology and alleviated fibrosis in a dietary model for NASH. Our findings identify a key molecular mechanism through which stressed hepatocytes trigger inflammation in context of MAFLD.

Dietary chitosan related changes to the microbiome and course of *Listeria monocytogenes* infection in high-fat diet fed mice

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Background: Diets rich in fat (high-fat diet, HFD) impact microbiome and gut barrier function and trigger intestinal and systemic inflammation, potentially influencing susceptibility to a life-threatening foodborne pathogen Listeria monocytogenes. Chitosan is an insoluble fibre with muco-adhesive and antimicrobial properties, derived from chitin, an immunogenic polysaccharide abundant in fungi and arthropods.

Aim: To evaluate the impact of dietary chitosan on HFD-induced changes in microbiome and host inflammatory response before and after L. monocytogenes infection.

Methods: Female C57BL/6 mice were fed HFD or HFD containing 1% chitosan (HFDC group) for 24 days. Murinized L. monocytogenes strain EGD-e InlAm was administered oraly (3.2 x 109 CFU/200uL) at day 21. Groups were sacrificed at day 21 (pre-infection) or 24 (infected). Organ homogenisates were plated for CFU/organ bacterial count. Gene expression was measured by RT-qPCR. Faecal DNA was extracted for assessment of microbiome composition by 16s rRNA sequencing and Listeria shedding dynamics by qPCR.

Results: Prior to infection, HFDC mice gained significantly less weight and increased the relative abundances of fibre- and mucus-degrading strains (Akkermansiaceae, Clostridiaceae), the Firmicutes/Bacteroidetes ratio, ileal IL-10 and liver iNOS expression, and decreased liver IL-1β expression in comparison to HFD mice. Microbiome PCoA showed a clear separation of HFD and HFDC group pre-infection, which was lost post-infection. Infection perturbated microbial compositions causing a decrease in α-diversity and abolishing previous differences. Bacterial load in primary and secondary infection sites was equal, however faecal shedding was decreased in HFDC group.

Conclusions: Chitosan supplementation ameliorated detrimental consequences of HFD by shaping gut microbiome and immunity and reducing body weight, however no direct effect on Listeria pathogenicity was observed.

IL-12 – CD4 T cell – IFNy axis mediates suppression of cytomegalovirus replication and establishment of latency in cortical and hippocampal neurons

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Human cytomegalovirus (HCMV) infection is the leading cause of congenital viral infections, which can cause a wide range of neurological sequelae. Following the resolution of the acute infection, the virus remains in the central nervous system (CNS) in the state of latency. To elucidate the mechanisms of brain infection and immune control during congenital CMV infection, we are using a murine model. Here we show that murine cytomegalovirus (MCMV) efficiently infects neurons, astrocytes, and microglia, as reported for HCMV. To study viral dissemination in the developing murine brain, we have utilized a cell-type-specific virus labeling system and detected infectious virus produced by astrocytes, microglia, and neurons, albeit with different kinetics. While astrocytes are both initial targets and substantial cellular source of infectious virus in the brain, the microglia-derived virus does not spread to other cells, indicating an efficient microglial mechanism of virus containment. At later time points of acute infection, virus is cleared from microglia and astrocytes, and neurons become the main source of infectious virus, suggesting impaired immune control of the virus in neurons. Finally, we provide evidence that the IL-12 – CD4 T cell – IFNy axis is essential for the suppression of MCMV and the establishment of latency in cortical and hippocampal neurons.

The impact of pre-existing immunity on the course of SARS-CoV-2 infection in health-care workers

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Continuing to this day, Coronavirus disease 2019 (COVID-19) still remains at the forefront of modern science, sparking discoveries and shaping our understanding of infectious diseases for years to come.

Our study assessed the severity of symptoms, duration of infection and viral loads of health-care workers (HCWs) who tested positive for COVID-19 during Omicron's prevalence, in regard to vaccination and previous infection.

A total of 141 HCWs were included in the study, whose nasopharyngeal swabs were analyzed by reverse-transcription quantitative PCR (RT-qPCR), targeting four different genes: RdRP, E, N and nsp14. The participants were divided into four groups: unvaccinated/not previously infected (group 1); unvaccinated/previously infected (group 2); vaccinated/not previously infected (group 3); and vaccinated/previously infected (group 4).

Our results highlight that the vaccination had less substantial effect on symptomatic disease among HCWs, while fever and loss of smell or taste were considerably less likely to occur upon reinfection. Negativization period was the shortest among fully vaccinated and previously infected participants, followed by groups that were either vaccinated or previously infected, suggesting a faster viral clearance in people with vaccine-induced and/or infection-induced immunity. We found no significant difference between four groups regarding *Ct* (cycle threshold) value means of analyzed genes. However, our data points towards the E gene being detected in earlier cycles, in comparison to other genes, which is notable across all four groups.

Since viral loads and negativization periods do not seem to significantly vary, irrespective of pre-existing immunity, systemic vaccination and mask-wearing should still be considered among HCWs.

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What can neutralizing antibodies tell us about the quality of specific immunity in COVID-19 convalescents and vaccinees?

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Understanding SARS-CoV-2-specific immunity generated by infection, vaccination or both is critical in drawing reliable conclusions about the management and outcomes of the COVID-19 pandemic. We provide here the most comprehensive analysis to date of neutralizing antibody (NAb) responses in cohorts of volunteers with differently generated SARS-CoV-2-specific immunity, utilizing a standardized wild-type SARS-CoV-2 neutralization assay calibrated to the WHO international standard. A head-to-head comparison of NAb responses elicited by four vaccines used in Europe during 2021 (BNT162b2, mRNA-1273, ChAdOx nCoV-19, Ad26.COV2.S) and their comparison to NAb responses in convalescents showed that while the amount was comparable, NAbs induced by natural infection were of higher quality. The highly dispersed immune responses elicited by the four vaccines were equaled by booster mRNA vaccination, reaching the highest, plateau level of NAb in the population. Equally high NAbs were induced in convalescents after a single dose of either vaccine. The use of wild-type virus as a challenge in the NAb determination revealed that NAbs produced by disease were better activators of the complement system than NAbs induced by vaccination. The contribution of spike protein-specific IgGs to the SARS-CoV-2 neutralization was lower in convalescents compared to vaccinees, indicating that those who recovered from COVID-19 were armed with antibodies of additional specificities and/or classes that contributed to virus neutralization. These findings suggest that the greater stringency of public policy measures targeting individuals who had recovered from COVID-19, compared to those who had been vaccinated, may not have been fully justified. We also emphasize the importance of a standardized approach in assessing humoral immunity using the most relevant assays as early as possible in future epidemics.

Primary human monocytes differentiate into M2-like macrophages upon long-term orthohantavirus infection

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Innate immunity is the first line of defense against different pathogens and its response determines the outcome of infection and subsequent disease development. Primary human monocytes can be permissive or resistant to viral infection and replication. Although monocytes are important innate immune system cells, their function and role during orthohantaviruses infection is still not clarified. The aim was to examine the elements of the innate immune response of human primary monocytes in vitro infected with pathogenic Puumala orthohantavirus or apathogenic Tula orthohantavirus.

The results have shown that pathogenic as well as apathogenic orthohantaviruses stimulate monocytes' innate immune response, occurring within the first hour after infection with changes at the levels of gene expression, surface molecules/receptors and the synthesis of soluble biological response modulators. Differential immune response was detected upon pathogenic versus apathogenic orthohantavirus infection. Primary monocytes immune response activation after orthohantavirus entry and infection was independent of viral replication. In the early phase of infection, in the interaction with orthohantaviruses, monocytes responded with pro-inflammatory pheno- and geno-type, while in the late phase of infection change of their functional phenotype prevails with regulatory pheno- and geno-type and differentiation towards M2-like monocyte-derived macrophages. Our study identified primary monocytes as transport system for orthohantavirus dissemination in the organism.

An IFNg-dependent immune-endocrine circuit lowers blood glucose to potentiate the innate anti-viral immune response

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When a viral infection is sufficiently strong, we display well-known symptoms of sickness behavior such as fever, loss of appetite and fatigue. Whereas we experience these changes as pathological, they are in fact carefully regulated adaptations of systemic metabolism mediated by the immune system with the purpose to optimize our protection against the virus. Viral infection-induced sickness behavior commonly includes the induction of a fasting-like metabolic state and the regulation of blood sugar concentrations in particular appears to be subject to change. However, the purpose and underlying mechanism of this process are mostly unknown. Here we describe a new immune-endocrine circuit that transiently lowers blood glucose concentrations in response to strong, non-lethal infection, which potentiates innate anti-viral immune defense mechanisms. Following viral infection, we find that IFNγ produced by γδ T cells directly stimulates pancreatic β-cells to increase glucose-induced release of insulin. Subsequently, hyperinsulinemia lessens endogenous glucose output by reducing glycogenolysis in hepatocytes during fasting. Glucose restriction enhances type-I interferon production of cells by curtailing lactate-mediated inhibition of IRF3 and NF-κB signaling. Induced hyperglycemia constrained IFN-I production and increased viral loads upon infection. Our findings indicate that the reduction of blood sugar concentrations during strong infection is part of a physiological response aimed to bring the body into a heightened state of responsiveness to viral pathogens. This immune-endocrine circuit is disrupted in hyperglycemia, which provides an explanation why people with metabolic disease are more susceptible to viral infection.

The bone marrow microenvironment reduces monocytic differentiation induced by low-dose cytarabine

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The bone marrow (BM) microenvironment regulates normal and malignant myeloid differentiation. Previous research showed that the murine BM stromal cell line MS-5 attenuates cytarabine-induced killing of acute myeloid leukemia (AML) cells. However, the effects on differentiation induced by low-dose cytarabine (LDAC) are not investigated. Thus, this study aimed to investigate the impact of BM stromal cells on LDAC-induced differentiation of AML cell lines and primary samples. Results of this study showed that the presence of MS-5 stromal cells decreased LDAC-induced cell cycle arrest, DNA damage signaling, and differentiation of U937 and MOLM-13 AML cell lines. Stromal cells also reduced LDAC-induced differentiation in primary samples from AML-M4 and myelodysplastic syndrome/AML patients. Transcriptomic analysis of U937 cells showed that the stroma decreased the expression of genes involved in cytokine signaling and oxidative stress. Still, pharmacological inhibitors and neutralizing antibodies did not support the role of CXCL12, TGF-β1, or reactive oxygen species. In conclusion, the results of our study show that the presence of stroma inhibits LDAC-induced differentiation of AML cells, which suggests that the impact of the BM microenvironment on AML may be one of the reasons for the modest and/or rare differentiation effects observed in patients.

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P1. *I.m.* and *i.v.* antivenom-mediated neutralization of snake venom: Part 1. Interplay in the systemic circulation

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Antivenoms, consisting of venom-specific IgGs or their fragments, constitute the mainstay in the snakebite envenoming therapy. Standardized protocol for their application does not exist. Despite insufficient clinical data, *i.v.* administration of antivenoms has been recommended, based on the results from animal studies of the venom/antivenom interplay in the bloodstream, as a principle of harmonizing their pharmacokinetics to that of the target venom. *I.v.* administration should also eliminate the restraint associated with the *i.m.* route. Although *i.v.* antivenoms have primacy, those given *i.m.* are sometimes used in the field. However, discrepancy between proposed inferiority of *i.m.* administration in comparison to that performed *i.v.* and their comparable effectiveness has still remained unresolved. Recently, it has been demonstrated that neutralization in the lymphatic system, which serves as a gateway for the venom absorption and further distribution, might also be important for the clinical outcome. Our aim was to comparatively investigate both therapeutic principles by monitoring the antivenom's effect on bioavailability of *s.c.* injected venom and its quantity decrement in both body compartments. Sheep, as large animal model, was employed. The venom dosage corresponded to the amount injected in a typical envenomation. Antivenom was given via *i.m.* bolus or *i.v.* infusion. Lymph samples were collected by a continuous drainage from *d. thoracicus*. Blood sampling was performed at defined time points from *v. jugularis*. Venom and antivenom concentrations were measured with a respective in-house ELISA. As part of the research, differences between *i.m.* and *i.v.* antivenom-mediated neutralization in the systemic circulation will be presented.

P2. *I.m.* and *i.v.* antivenom-mediated neutralization of snake venom: Part 2. Interplay in the lymphatic system

Erika Gamulin[1], Maja Lang Balija[1], Sanja Mateljak Lukačević[1], Dražen Vnuk[2], Ana Smajlović[2], Beata Halassy[1], Tihana Kurtović[1]

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Envenoming induced by snakebites constitutes a significant public health burden. Commonly, venoms are introduced into the subcutaneous or muscle tissue and carried to the interstitial space for distribution. It is widely recognized that viper venoms are mainly absorbed through the lymphatic system before being transported into the systemic circulation. Even though a standardized protocol does not exist, parenterally administered antivenoms remain the mainstay in the snakebite therapy. Widely held belief that *i.v.* administration of antivenoms is more effective than *i.m.* application is based on the studies of venom/antivenom pharmacokinetics in the bloodstream. Lately, it has been demonstrated that *i.v.* antivenom-mediated neutralization not only in the systemic circulation, but also in the lymphatic system might be highly important for clinical outcome. The role of *i.m.* antivenoms in the elimination of lymph-absorbed venom might be even greater, but it has not been studied yet. Our aim was to comparatively investigate both therapeutic principles by monitoring the antivenom's effect on bioavailability of *s.c.* injected venom and its quantity decrement in both body compartments. Sheep, as large animal model, was employed. The venom dosage corresponded to the amount injected in a typical envenomation. Antivenom was given via *i.m.* bolus or *i.v.* infusion. Lymph samples were collected by a continuous drainage from *d. thoracicus*. Blood sampling was performed at defined time points from *v. jugularis*. Venom and antivenom concentrations were measured with a respective in-house ELISA. As part of the research, differences between *i.m.* and *i.v.* antivenom-mediated neutralization in the lymphatic system will be presented.

P3. Influenza A-induced hemophagocytic lymphohistiocytosis in a girl with a homozygous mutation in the gene LARS1

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Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially life-threatening hyperinflammatory syndrome. In younger children, primary HLH is mainly caused by mutations in genes that are key to the function of NK-cells or cytotoxic T-lymphocytes. Secondary or acquired HLH is mainly related to immunologic activation following infections, malignancies or, autoimmune/autoinflammatory diseases.
Biallelic mutations in the LARS1 gene encoding leucyl-tRNA synthetase1 necessary for protein synthesis, cause infantile liver failure syndrome type 1 (ILFS1), a rare autosomal recessive disorder which is often associated with abnormalities of growth, blood, nervous system, and musculature. Patients are more prone to infections, and those caused by influenza virus leads to a significant worsening of the disease.
Our patient, a 4-years old girl manifested an increase in transaminases during at least three previous febrile episodes, initially associated with EBV infection starting from the third year of life. At the age of 4 years, she had a new significant deterioration in which she had prolonged fever, leukopenia, low platelets, decreased hemoglobin, significantly higher AST compared to ALT, hyperferritinemia and hypofibrinogenemia, without the presence of hemophagocytes in bone marrow punctate. Nasal swab was positive for influenza A using PCR method. Considering the clinical presentation and findings, she was treated as an infection induced HLH with corticosteroids and oseltamivir with excellent clinical response. In further monitoring by upon discharge from the hospital, she had a permanent mild elevation of ALT and on two occasions, a liver ultrasound suggestive for fatty degeneration, possibly induced by corticosteroid therapy. In terms of age, earlier episodes of hepatitis of open etiology and HLH induced by influenza, genetic analysis was performed using whole exome sequencing (WES), which showed homozygous mutation in the LARS1 gene.
Conclusion: ILFS1 manifestations become more apparent or activated during infection. Clinical features are non-specific and have features of sepsis, other systemic diseases, HLH and inborn errors of metabolism. The example of our patient supports the key contribution of genetic analysis in children of early age who present with HLH.

P4. Modulation of CD8+ T cell response mediated by mouse cytomegalovirus immunoevasines MATp1 and m04 /gp34

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Cytomegaloviruses invest considerable efforts to evade cytotoxic immune cells. To evade CD8 T cells, CMVs downregulate MHC I molecules but complete removal of MHC I could render them sensitive to NK-cell mediated missing-self attach. Mouse CMV (MCMV) has circumvented this problem by utilizing two proteins: m04 and MATp1 that collaborate to return certain MHC I alleles that can engage inhibitory Ly49 receptors and prevent missing self. Deletion of either m04 or MATp1 results in no MHC I present on the cell surface and better control of such viruses via NK cells. The absence of MHC I as well as diminished virus titers should result in a weaker CD8 T cell response due to poorer direct presentation and less available antigen for cross-presentation. Interestingly, deletion of MATp1 results in just the opposite – equal or better CD8 T cell response in comparison with WT viruses. Our findings indicate an additional role for MATp1 in modulating peptides loaded into MHC I.

P5. Immunobiology of cytomegalovirus expressing the NKG2D ligand H60a

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NKG2D is a potent activating receptor expressed by the immune cells, whose importance in the immunosurveillance of cytomegalovirus (CMV) infection is illustrated by the fact that CMVs encode genes involved in the immune evasion of NKG2D-mediated immune control. CMV infection induces an inflationary $CD8^+$ T cell response consisting of highly functional CMV-specific CD8 T cells. We have previously shown that murine CMVs (MCMV) expressing RAE-1γ or MULT-1, two of the cellular ligands for the NKG2D receptor, although highly attenuated *in vivo,* induce a strong $CD8^+$ T cell response.

Here, we demonstrate that a recombinant MCMV expressing the third NKG2D ligand, H60a, (H60aMCMV) inserted in the place of its viral inhibitor m155, is dramatically attenuated *in vivo* in an NKG2D- and NK cell-dependent manner. Despite efficient control by the host innate immunity, H60aMCMV induces a potent and long-lasting virus specific $CD8^+$ T cell response comparable to, or even more efficient than the one induced by wild type MCMV (WT MCMV). Moreover, H60aMCMV is attenuated in immunologically immature newborn mice. H60aMCMV infection is associated with lower viral titres, less influx of inflammatory cells and fewer pathoanatomical lesions in the central nervous system of newborn mice as compared to WT MCMV infection. Furthermore, H60aMCMV infection induces production of anti-viral antibodies which, by passing the placental barrier, protect the offspring of H60aMCMV vaccinated mothers from challenge MCMV infection. Altogether, our study further supports the concept of CMV expressing NKG2D ligand as a promising model for a CMV vaccine or CMV-based vaccine vector.

P6. Bulk release of the MCMV virions is a rare event, as revealed by live-cell tracking of secondary envelopment and egress

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The production of new capsids in the nucleus and their final envelopment at membranous organelles in the cytoplasm represent the end of the cytomegalovirus (CMV) replication cycle. The enveloped virions are collected in large multiviral bodies (MViBs). These MViBs are released from the cell as a bulk pulse by poorly understood mechanisms. Cytoplasmic egress was investigated using recombinant murine CMV (MCMV) with fluorescently tagged small capsid protein (S-mCherrry-SCP MCMV). For long-term live recording, digital holotomographic microscopy (DHTM) was used in Balb 3T3, NIH 3T3, and IFN-beta receptor knockout fibroblasts. We monitored the number of infectious virions by plaque assay, viral genome copy number by qPCR, and high-resolution imaging by confocal microscopy. Fluorescent SCPs accumulated in the nuclei of approximately 50% of infected cells, and fluorescent capsids were visualized in large cytoplasmic structures corresponding to MViBs. MViBs release from MCMV began 20-24 hours after infection and continued over the next 15-20 hours. Release events were detected in only 2-3% of infected Balb 3T3 and in 10-11% of infected NIH 3T3 cells, which is consistent with the observed heterogeneity of cellular responses to DNA virus infection. The frequency of cells with cytoplasmic releases increased to 40-45% in fibroblasts with IFN-beta receptor knockout.

These results suggest that only a small proportion of infected cells are capable of releasing virions, due to cell-to-cell variability. Therefore, single-cell analyses are needed to investigate the pathways and mechanisms of CMV egress, and DHTM imaging could be a very useful tool for these analyses.

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P7. Ubiquitinilation of Rab5 guanine nucleotide exchange factor (GEF) Rabex5 and deubiquitinilation of Rab11FIP1 affects transferrin recycling in MCMV infected cells

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Murine cytomegalovirus (MCMV) initiates remodeling of cellular membrane compartments leading to the formation of the viral assembly compartment (AC). This process involves host cell proteins that regulate membrane trafficking, some of which may use protein ubiquitination as a regulatory mechanism.
Our previous research has shown that MCMV infection impairs endosomal recycling, including transferrin (Tf) recycling. Interestingly, in cells infected with M48^{C23S} MCMV (MCMV with a mutation in the active site of M48 MCMV deubiquitinilase), these recycling kinetics could be partially reversed.
The aim of this study was to investigate whether MCMV affects the function of early endosomal proteins (EE): Rab5 and its guanine nucleotide exchange factor (GEF), Rabex5.
Confocal imaging showed Rab5 and Rabex5 accumulation in AC, but Western blot analysis showed that MCMV infection did not significantly affect the expression of either protein. However, it is known that Rabex5 can be (auto)ubiquitinylated and released into the cytosol. When deubiquitinated, Rabex5 can recruit cargo at EEs, activate Rab5, and allow normal endosomal recycling. Therefore, we transfected NIH3T3 cells with constructs expressing ubiquitin-hemagglutinin (Ub-HA) fusion proteins and infected the cells with wtMCMV and M48^{C23S} MCMV. After immunoprecipitation of Ub proteins and Western blot analysis, we found that Rabex5 was more ubiquitinated in wt-MCMV-infected cells. Interestingly, ubiquitination of the Rab11FIP1 protein, which activates recycling from the endosomal recycling compartment when ubiquitinylated, was decreased in cells infected with wtMCMV but partially normalized in cells infected with M48^{C23S} MCMV.
These results suggest that inhibition of membrane recycling of EEs and ERC may be the result of increased ubiquitination of Rabex5 and deubiquitination of Rab11FIP1. This mechanism demonstrates the role of ubiquitination as a posttranslational modification in the control of membrane dynamics in MCMV-infected cells.
This work was supported by Croatian Science Foundation grants (IP-2020-02-2916, and IP-2019-04-3582), and by the UNIRI (uniri biomed-18-180, uniri-biomed-18-88, uniri-biomed 18-229).

P8. EHBP1 and Rabin8 GTPase recruit Rab10 GTPase in Murine cytomegalovirus assembly compartment

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The GTPase Rab10 belongs to the Rab family of small GTPases that regulate vesicular transport between early endosomes (EEs) and the endosomal recycling compartment (ERC). These cellular membranes are involved in the formation of the assembly compartment (AC) for the production of new viral particles during murine cytomegalovirus (MCMV) infection.

The aim of our study was to identify the proteins of the Rab10 interactome that are important for its recruitment to cell membranes that are remodeled into MCMV AC.
Therefore, we generated a NIH3T3 cell line with stable expression of the Rab10-BioID2-HA fusion protein for proximity-dependent biotin identification method, in which the enzyme from Aquifex aeolicus (BioID2) biotinylates proteins within a radius of 10-15 nm. After immunoprecipitation of biotinylated proteins from uninfected and MCMV-infected cells by neutravidin agarose and Western blot analysis, we showed that EHBP1, MICAL-L1, and Rab11FIP1 were localized in close proximity to Rab10. In addition, Rabin8, Rab8, ACAP2, AS160, EHD1, Vps34, Arf6, PACSIN2, Rabex5, SNX3, γ-adaptin, and β-actin were also identified. Because EHBP1, MICAL-L1, and Rabin8 are known to activate Rab10 in uninfected cells, we investigated their role in Rab10 recruitment to MCMV early AC(preAC). Using confocal microscopy, we confirmed the localization of Rab10, EHBP1, Rabin8, and MICAL-L1 in the inner part of AC in the early phase of MCMV infection. However, Western blot analysis revealed that their expression did not change significantly during infection. Importantly, siRNA depletion of EHBP1 and Rabin8 and partially of MICAL-L1 inhibited Rab10 loading into early AC(preAC). Finally, murine fibroblasts were transfected with constructs transiently expressing YFP PH(PLCδ1) and dominantly negatively loading phosphatidyl inositol(4,5)-bisphosphate (PIP2). Mobilization of EHBP1 on PIP2 positive membranes was prevented, but so was mobilization of Rab10.
This suggests that EHBP1 and Rabin8 interact with Rab10 and recruit it to the cellular membranes that would be remodeled into the MCMV assembly compartment.
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P9. Purification of vesicular stomatitis virus (VSV) by immunoaffinity and ion-exchange monolith chromatography for development of oncolytic virus purification platform

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Vesicular stomatitis virus (VSV) is a non-pathogenic, negative-strand RNA *Rhabdovirus* with potent oncolytic potential. It has a short replication cycle, does not integrate into the host genome and is a strong inducer of apoptosis in the infected cancer cells. The use of viruses for oncolytic viraotherapy requires high titers of pure, infectious virus particles, so we aimed to develop a gentle and efficient downstream purification strategy that would not impair the infectivity of the virus particles. Today, chromatography has been deemed the most promising technology for large-scale purification of viruses used as biomedicines.
The aim of our work was to investigate the possibilities of immunoaffinity and ion-exchange chromatography on monolithic columns in the purification of a high-quality VSV particles intended for development of a new oncolytic therapy modality. Yield of each chromatography method was calculated for infective virus particles (using $CCID_{50}$ assay), and for total particles (using Nanoparticle tracking analysis). Their efficiency in the removal of the host cell proteins was followed with in-house ELISA. VSV-specific immunoaffinity column was prepared by oriented immobilization of polyclonal anti-VSV IgGs isolated from the serum of oncolytic VSV-treated patient via the antibody's carbohydrate moiety on a hydrazide activated monolithic column. The dynamic binding capacity of the prepared column was determined and the native elution efficiency of the bound VSV with several high-molarity amino acid solutions at physiological pH was examined. Additionally, preliminary experiments based on cation- and anion-exchange chromatography were performed and the purification efficiency were compared with immunoaffinity chromatography.

P10. Monocyte-associated cytokine profiling in peripheral blood of patients with chronic graft-versus-host disease

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Chronic graft-versus-host disease (cGVHD) is a multisystemic allo- and autoimmune disease that occurs as a major long-term complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT). The aim of this study was to assess the relationship between the presence and clinical manifestations of cGVHD and monocyte-associated cytokine concentrations in the peripheral blood of individuals experiencing newly diagnosed (de novo) cGVHD, as well as those with previously established cGVHD. Furthermore, we aimed to perform a comparative analysis between these two cohorts, as well as between each of them and the control group who underwent allo-HSCT but without cGVHD. The study encompassed 62 adult cGVHD patients (de novo=31, previously established=31) and 31 controls at University Hospital Centre Zagreb between 2017 and 2023. We quantified 12 cytokines (IL-4, IL-2, CXCL10, IL-1β, TNF-α, MCP-1, IL-17A, IL-6, IL-10, IL-γ, IL-12p70, IL-8) in plasma samples using microsphere bead array technology on a flow cytometer. No significant age or sex differences were observed among groups. CXCL10 was markedly elevated in de novo cGVHD patients versus controls ($p<0.001$). Additionally, both CXCL10 ($p<0.001$) and immunosuppressive IL-10 ($p=0.049$) were higher in de novo patients compared to those with previously diagnosed cGVHD. Concomitantly, we identified that IL-6 was positively correlated with the global NIH cGVHD score ($\rho=0.448$, $p<0.001$) and negatively correlated with the Karnofsky score ($\rho=-0.524$, $p<0.001$) in all cGVHD patients, indicating IL-6's association with disease severity. Our preliminary findings confirmed CXCL10's potential as a cGVHD biomarker and underscored the link between IL-6 plasma levels and disease severity.
This work has been funded by Croatian Cooperative Group for Hematological Diseases Foundation and Croatian Science Foundation (project IP- 2016-06-8046).

P11. Microevolution of a Mumps Based Recombinant Virus with HCV Genes During Passaging in Vero Cells

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Hepacivirus C (HCV) infection is an important health problem and development of a prophylactic vaccine is necessary to control the transmission of the virus. One of approaches in the development of HCV vaccines is the production of recombinant viruses expressing main neutralization antigens of HCV, envelope proteins E1 and E2. If an RNA virus is used as a vector, particular focus during vaccine development phase should be put on population variability, because the viral quasispecies structure may be the key element of attenuation phenotype.

We have produced a recombinant mumps virus, vCE1E2-HCV-MRV2, containing HCV proteins core (C), E1 and E2 which was based on the L-Zagreb mumps vaccine strain as a vector. The aim of this research was to analyze genetic stability of vCE1E2-HCV-MRV2 during in vitro passaging in a vaccine production cell line. The stability of viral populations was determined in supernatants of infected cells using next-generation sequencing.

While viral population in master seed stock was quite homogeneous, serial passaging of the virus led to removal of large segments of inserted sequences from the viral genome, which was also evident on the protein level. There was an increase in the diversity of viral populations during early passages. A number of heterogeneous positions was found in inserted genes, as well as few different nonsense substitutions that were detected in viral minority variants.
The results presented here strengthen the need of close monitoring of genomic stability during the production of recombinant viruses, especially the ones intended to be used as biomedicals.

P12. Neurovirulence of recombinant mumps viruses-effects of infectious viral dose and data analysis methods

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The rat-based neurovirulence test (RNVT) has become the standard model for estimating the neurovirulence potential of mumps strains and many studies have used it to identify the factors that contribute to mumps' neurovirulence. However, the RNVT has some drawbacks like ambiguities in viral load, units used for viral titre determination, and choosing the appropriate method for data analysis. These issues can make it very difficult to compare RNVT results from different studies.
To assess the neurovirulence of recombinant mumps viruses and the effect of viral load on RNVT scores, we administered standard, low and high viral infection doses in RNVT, using both PFU and CCID50% titre units. Parametric and non-parametric statistics were used to analyze RNVT row data and results were presented as mean and standard error (SE) or median and interquartile range (IQR).
The results did not show an increase in the RNVT score for the animal groups that received an infectious dose up to 10 times higher than the standard dose, but the RNVT score was lowered by the dose 10 times lower than the standard dose. The RNVT results for the tested viruses presented as a mean and SE falls within the 5% limit allowed for attenuated vaccines, making them comparable to the results of many other studies. However, if the RNVT results are presented as medians and IQR, their value is significantly lower, but they cannot be compared with the results of numerous other studies. For statistical accuracy, we suggest displaying results as medians and IQR, along with the RNVT raw data table that includes data for each animal analyzed.

P13. The correlation of SARS-CoV-2 excretion in feces with immunological and clinical characteristics of COVID-19 patients - a hospital-based study

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Aim: The aim of this research was to determine the correlation between the immunological and clinical characteristics of the patients tested positive for SARS-CoV-2 RNA in the feces.
Study design: 251 participants admitted to the COVID department of the Mostar University Clinical Hospital (UCH) were included in this study between December 2021 and January 2022. RT-PCR from

nasopharyngeal swabs (NF) and feces as well as serological testing for SARS-CoV-2 specific anti-spike IgGs were conducted. Demographic and clinical data were collected from questionnaires and medical records.
Results: Of the patients with positive IgG antibodies, 41 (47.7%) were vaccinated and 45 (52.3%) were unvaccinated (p=0.666), with no statistical difference in regards to clinical manifestation or death outcome. Unvaccinated patients with a negative antibody titer had a higher incidence of acute respiratory insufficiency (78%, p=0.029) and Intensive Care Unit-admission (17.9%, p=0.026) than those with positive antibody titer. Of the 75 patients who provided a feces sample, 47 of them (62.7%) were positive for SARS-CoV-2 RNA (p=0.028). Regarding fecal SARS-CoV-2 RNA excretion, there were no statistical differences in death outcome (p=0.604), antibody status (p=0.628) and vaccination status (p=0.588) between positive and negative tested groups of patients.
Conclusion: COVID-19 patients with a negative IgG titer had more frequent adverse outcomes. Although the isolation of SARS-CoV-2 RNA from feces is a viable method for the detection of SARS-CoV-2 infection, it has no predictive value for adverse outcomes of COVID-19.

P14. Efficacy of SARS-CoV-2 monoclonal antibodies against major variants of concern using flow cytometry

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Monocytes are essential to the early immune response against SARS-CoV-2. Their functions include early detection of the virus, cytokine production, antigen presentation, phagocytosis, tissue recruitment, and immunomodulation. Understanding the role of monocytes in COVID-19 is essential for developing strategies to modulate the immune response and manage the disease effectively. An effective method of analysis is flow cytometry utilizing antibodies. MedRi produced *in house* monoclonal antibodies against Wuhan variant of SARS-CoV-2 for which limited information concerning specificity for SARS-CoV-2 variants of concerns (VoC) was available.
To clearly detect monocytes infected with VoC, we aimed to screen the collection of monoclonal antibodies against cell surface and intracellular proteins of Wuhan variant of SARS-CoV-2 using flow cytometry.
Vero E6 cells were infected with four VoCs: alpha (lineage B.1.1.7), beta (B.1.351), delta (B.1.617) and omicron (BQ.1.1) as well as SARS-CoV-2 strain ZG/297 (lineage B.1.1.1) (MOI 0,1) and were tested with monoclonal antibodies against nsp1, nsp7, N, Orf3a, and S SARS-CoV-2 proteins. Antibodies with the best resolution on Vero E6 model were selected and tested on CD14++ separated and *in vitro* infected monocytes (MOI 0,1) with above mentioned variants of SARS-CoV-2, 24 h and 7 days post-infection.
This approach enabled us to develop a robust model for determining the resolution of SARS-CoV-2 infected cells using specific antibodies. This model serves as a valuable tool for future research on the immune response of SARS-CoV-2 infected monocytes *in vitro*.

P15. Targeting members of the nectin family of proteins in glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most aggressive malignant form of brain tumor, with an average survival rate of 15 months. To date there is no sufficient clinical treatment, however, recently, new immunotherapeutic approaches such as use of antibody-drug conjugate, oncolytic polioviruses, chimeric antigen receptor T-cell and checkpoint inhibitors, are being extensively investigated. Poliovirus receptor (PVR) and Nectin-2 (CD112), members of nectin and nectin-like family of proteins, are adhesion molecules involved in various cellular processes, including regulation of immune system. Both PVR and Nec-2 are highly expressed in primary GBM tissue and in glioblastoma cell lines while their expression in healthy brain tissue is low or limited to certain cells. In other solid tumors, lower levels of PVR and Nec-2 are shown to be associated with a longer disease-free period. Furthermore, both molecules serve as immune checkpoints i.e. limit the immune response due to their interplay with inhibitory and activating immune cell receptors which is one of the main reasons why their targeting is being investigated as potential therapeutic option. To date, immune-checkpoint blockade showed little impact in GBM. To address that, we investigated their expression on GBM by FACS and established IHC protocols to allow better assessment of their distribution and heterogeneity. To determine the capacity of tumor control by blocking PVR and Nec-2 we established GBM PDOX model (patient-derived orthotropic xenograft) in NSG mouse. Altogether, we are assessing the antitumor effect of PVR and Nectin-2 targeting by in-house produced checkpoint inhibitor antibodies.

P16. Targeting and recognition of peptidoglycan monomer by N-acetyl glucosamine-specific lectins

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The aim of the present study was to investigate the interaction of peptidoglycan monomer (GlcNAc-MurNAc-L-Ala-D-isoGln-mesoDAP(εNH2)-D-Ala-D-Ala), disaccharide pentapeptide isolated from Brevibacterium divaricatum and model plant lectins which specifically recognized N-acetylglucosamine (GlcNAc). Peptidoglycan monomer (PGM) is a fragment of peptidoglycan which is the essential component of the bacterial cell wall and is recognized by the innate immune system through pattern recognition receptors (PRR). PGM shows strong immunostimulatory and immunomodulatory. In order to study the interaction of glycan moiety of PGM with model plant lectins, agglutination method and surface plasmon resonance (SPR) method were used.

Gold nanoparticles modified with PGM (AuNPs-PGM) were synthesized and their interactions with model lectins were evaluated by agglutination method. Agglutination is one of the early methods to monitor sugar-receptor interactions, so the specific lectin is added to the AuNPs-PGM and the hydrodynamic volumes of the resulting aggregates were measured by dynamic light scattering (DLS). On the other hand the WGA (wheat germ agglutinin) lectin was immobilized on gold biosensor and interaction with PGM was analyzed. Preliminary results demonstrated that WGA lectin most succesfully bind to AuNPs-PGM by recognizing GlcNAc and that the particle size increases immediately after WGA lectin is added to the AuNPs-PGM. The binding of UEA-II (Ulex europaeus) lectin to PGM was weaker compared to WGA lectin, while GS-II (Griffonia simplicifolia) lectin showed the lowest activity although a significant increase in AuNPs-PGM nanoparticle size was observed. The successful immobilization of WGA on a biochip allowed the establishment of the SPR method and after fine tuning of analysis conditions KD was estimated, KD = 9.3 × 10-4 M.

Investigation of interactions between GlcNAc specific lectins and PGM will provide novel insight into the mechanism of molecular recognition of biologically active glycopeptides and model lectins using nano-enabled biosensing methods.

P17. Optimal Treatment Reverts Hyperinflammation in Cytotoxic Lymphocytes among Type 2 Diabetes Patients

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Chronic systemic low-grade inflammation is an underappreciated symptom of type 2 diabetes (T2D). The inflammatory cascade is believed to be a causative factor in the development of several comorbidities associated with T2D, such as atherosclerosis, diabetic kidney disease, and fatty liver disease. However, the impact of T2D on the inflammatory state of the immune system is incompletely characterized. The aim of this study is to investigate whether T2D is associated with a pro-inflammatory profile within the anti-viral arm of the immune system and whether this can be reverted by antihyperglycemic treatment. After obtaining signed informed consents, peripheral blood mononuclear cells were isolated from patients with T2D and control subjects. The phenotype and cytokine production by cytotoxic lymphocytes were analyzed using multiparametric flow cytometry. Significantly increased production of tumour necrosis factor by CD8+ T cells and Granzyme B by NK cells and γδ T cells was observed in patients with diabetes in comparison to the control group. These observations were associated with age and diabetes duration. In a subcohort of patients with poorly-controlled diabetes, we optimized their antihyperglycemic treatment, and the analysis was repeated six months after succesful lowering of blood glucose levels. Strikingly, optimal antihyperglycemic treatment was able to decrease cytokine production by CD8 T cells, NK cells and γδ T cells. In summary, cytotoxic immune cells change their functional profile in the context of diabetes and may, therefore, contribute to the development and worsening of inflammation-driven diabetic complications. Our findings show that optimal antihyperglycemic treatment may revert these changes.

P18. Unveiling the immunological and non-invasive biomarker potential in MAFLD progression

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Metabolic-associated fatty liver disease (MAFLD) is a chronic liver disease characterized by benigh steatosis but can progress to a more severe inflammatory condition known as non-alcoholic steatohepatitis

(NASH), associated with increased risk of cirrhosis and liver failure. Therefore, the mechanisms behind MALFD progression to NASH are an active area of research. The gold standard for diagnosing MAFLD is an invasive liver biopsy pointing to a need for investigating alternative approaches.
Given that MAFLD is defined by metabolic stress, we conducted immunohistochemical staining of ligands for activating stress receptor NKG2D on collected liver biopsies from KBC Rijeka. We observed that the ligands exhibited upregulation, specifically in sections most affected by steatosis.
Furthermore, we noticed a positive correlation between the frequency of IL-17A expressing cells and the severity of the disease. Additionally, publicly available single-cell RNA sequencing data from human liver biopsy material showed that *Rorc*, the transcription factor for mediating IL-17A, is present in a T cell subpopulation that also expressed *Klrk1*, which we confirmed through *in vitro* stimulation.
Moreover, we explored whether alteration in the immunological profile of hepatic immune cells associated with NASH could be identified in the blood of MAFLD patients diagnosed using ultrasound techniques. Since we observed a correlation between liver stiffness and γδ T cells expressing the transcription factor Rorgt, we believe this simple blood test could serve as a reliable non-invasive biomarker for NASH in humans.

P19. Evaluation of concentration of selected inflammatory cytokines in transplant candidates with alcoholic end-stage liver disease

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Background and aims: Alcoholic liver cirrhosis is associated with inflammation, and the prognostic and diagnostic potential of pro-inflammatory cytokines is the focus of ongoing research. In the present study, we analyzed the concentration of 13 cytokines and their association with clinical parameters among liver transplant candidates.
Methods: Concentrations of 13 selected cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33) were determined in plasma (N = 80) of liver transplant candidates and control age and sex-matched non-liver patients. Flow cytometry analysis was performed on Aria II instrument (BD Biosciences) using the commercially available assay (LEGENDplex™ Human Inflammation Panel 1). Laboratory and clinical data were retrieved from medical records.
Results: In comparison to the control group, liver transplant candidates had a significantly higher concentration of ten cytokines; IL-1β, IFN-α2, IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-12p70, IL-23, and IL-33 ($p < 0.001$), whereas the concentration of MCP-1 was significantly decreased ($p < 0.05$). No significant differences in IL 17 and IL 18 were detected. A subgroup of patients with hepatocellular carcinoma (HCC) had significantly lower concentrations of IFN-γ ($p < 0.01$) and TNF-α ($p < 0.01$), whereas the concentrations of IL-6 ($p < 0.01$) and IL-8 ($p < 0.05$) were significantly increased compared to non-HCC patients. The concentration of IL-1β correlated moderately with AST levels ($r = 0.72$, $p<0.001$), creatinine levels ($r = 0.449$, $p < 0.05$), and MELD score (0.453, $p<0.01$).
Conclusion: Concentrations of pro-inflammatory cytokines are increased in patients with advanced alcoholic end-stage liver disease liver and are associated with the disease severity. HCC patients display a different cytokine pattern and may add information on the identification and management of HCC. A larger number of samples and follow-up of the patients is needed to explore the prognostic value of these markers.

P20. Effect of Notch signaling in human osteoclast progenitors

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Rheumatoid arthritis (RA) affects approximately 1% of the global population and leads to significant morbidity and disability. Despite advances in treatment options, many patients experience suboptimal responses to therapy. Inflammation-induced activation of osteoclasts, exclusive bone-resorbing cells of hematopoietic origin, mediates joint destruction in RA. We previously characterized highly osteoclastogenic osteoclast progenitors (OCPs) present in the inflamed periarticular tissues as well as among peripheral blood mononuclear cells (PBMCs). As Notch signaling is implicated in osteoclast differentiation and activation, we investigated the effect of Notch modulation on OCP activity in RA. Substantial proportion of OCPs ($CD45^+CD15^-CD3^-CD19^-CD56^-CD11b^+CD14^+$) within PBMCs and synovial fluid-derived cells (SFDCs) of RA patients express Notch receptors. Moreover, we observed negative association between OCP expression of Notch 1 and Notch 4 with RA activity score DAS28. Parallel analysis of sorted OCPs vs PBMCs or SFDCs by qPCR indicated enrichment of Notch expression in OCPs, with an increase of Notch3 and DLL1 in arthritis. Sorted OCPs, differentiated in vitro by RANKL and M-CSF, increasingly expressed Notch1, Notch3, Jag1 and DLL1 mRNA, specifically in RA. In vitro stimulation of osteoclastogenic cultures by Notch ligands showed that JAG1 and DLL1 inhibited osteoclast formation in a dose dependent manner, whereas neutralizing anti-Notch 1 antibodies partially ameliorated that inhibition. In conclusion, Notch axis is effective in the regulation of OCP differentiation and activity, therefore modulation of Notch signaling may be important complementary approach to reduce bone resorption in RA. The work was supported by Croatian Science Foundation projects IP-2020-02-2431, UIP-2017-05-1965 and DOK-2021-02-6365.

P21. Inhibition of Notch signalling increases osteoclast progenitor differentiation and bone resorption in murine collagen-induced arthritis

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Rheumatoid arthritis is a chronic autoimmune disorder primarily affecting the joints, characterized by both localized and systemic bone loss, which is mediated by increased activity of osteoclasts, specialized bone-resorbing cells. Osteoclast progenitors (OCP), derived from the myeloid lineage, are specifically increased in arthritis and express the CX3CR1 chemokine receptor. Emerging evidence emphasizes the significance of Notch signaling in osteoclast differentiation. Our study aimed to investigate the impact of modulating Notch signalling on OCP differentiation and arthritis-induced bone resorption in murine collagen-induced arthritis (CIA). Murine bone marrow and splenic OCPs, identified as CD45+Ly6G−CD3−B220−NK1.1−CD11b−/loCD115+ and CD45+Ly6G−CD3−B220−NK1.1−CD11b+CD115+ cells, respectively, express all four Notch receptors and are susceptible to regulation through Notch

signaling. In arthritis, we observed an increase in the frequency of OCPs expressing Notch 2 and 3. Stimulation with Notch ligands JAG1 and DLL1 results in inhibition of osteoclast differentiation, while treatment with neutralizing antibodies targeting Notch 1 and 2 resulted in increased osteoclast formation in vitro and an elevated frequency of OCPs in vivo. Additionally, we employed transgenic mice models with Notch signaling inhibition (CX3CR1CreERT2/RBP-J) or Notch 1 overexpression (CX3CR1CreERT2/NICD1) in OCPs. These experiments revealed enhanced osteoclastogenesis with Notch deletion and reduced osteoclast formation with Notch 1 activation. Arthritic mice with inhibition of Notch signaling in OCPs exhibited increased osteoclast differentiation and reduced talar bone volume. Collectively, our findings confirm the inhibitory role of Notch signaling in osteoclast differentiation during arthritis. The work was supported by Croatian Science Foundation projects IP-2020-02-2431, UIP-2017-05-1965 and DOK-2021-02-6365.

P22. Primary stem cells from human thymus

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Surgical thymectomy as well as congenital athymia at an early age in children with heart diseases have a clear impact on the immune system. Evidence of an increased risk in development of autoimmune processes, allergic reactions or even cancer has been found and we believe it is a long-term consequence of thymectomy. In order to protect the immune system in those patients, we present our research on thymic epithelial stem cell progenitors as the basis of organoids formation *in vitro* for human thymus regeneration. By means of immunohistochemistry, flow cytometry and confocal microscopy methods we compare histological sections of a newborn, 7 days old, and an 11 years old pediatric thymus for markers specific for stem cells.

We employed the in vitro 3D cultures of the thymic cells in low attachment conditions in order to evaluate the formation of thymospheres as a measure of their self-renewal capacities. We use the spheres as the founding elements of reaggregated organotypic cultures together with $CD34^+$ cells for T-cell development and differentiation.

In conclusion, we present human thymic cells that have progenitor/stem cell features and that can be further cultured or developed as the backbone of thymus regeneration studies in order to protect the immune system in congenital or iatrogenically caused thymic defects.

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