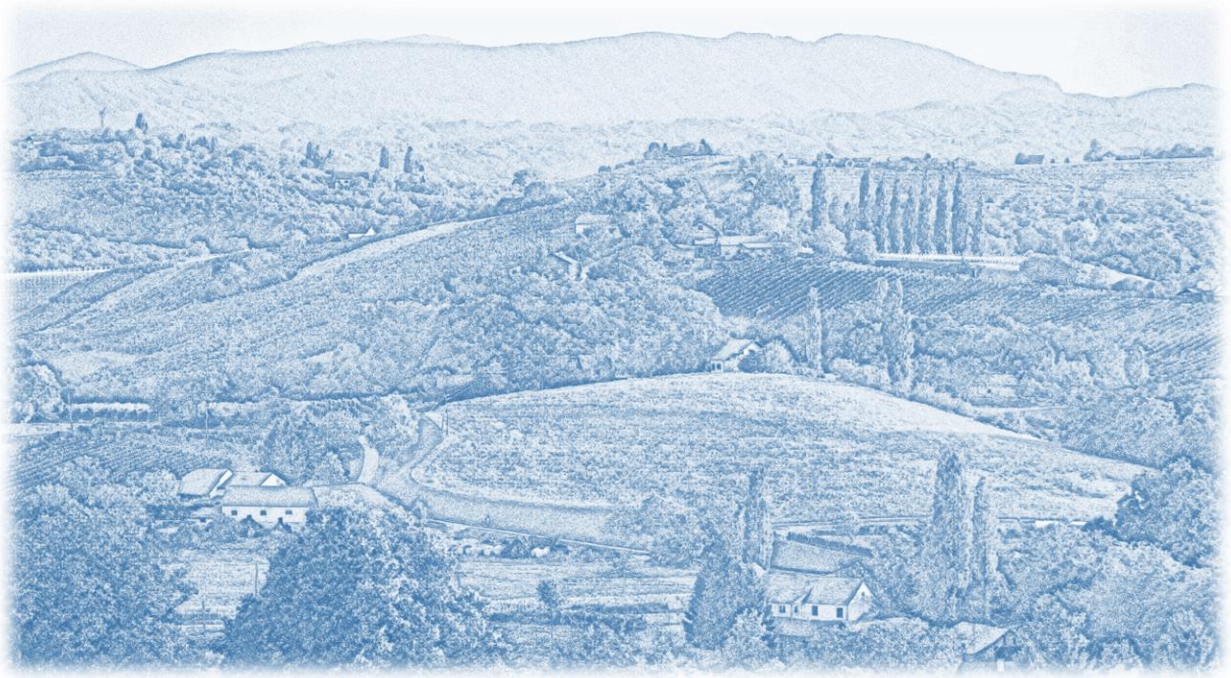
Annual meeting of the Croatian Immunological Society 2022



Sveti Martin na Muri

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2022 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

Sveti Martin na Muri, 06-08.10.2022

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY
University of Rijeka Faculty of Medicine

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Dear Friends and Colleagues,

Hereby I would like to welcome you all to the annual meeting of the Croatian Immunological Society. I am happy that after years of pandemic, we can once again meet face to face without epidemiological measures. This year, we welcome you in the beautiful hills of Međimurje.

One thing that the pandemic has made abundantly clear is the importance of research for the defeat of viral pathogens and immunology has once again shown to be of great importance. Not only have immunologists educated people in better understanding of how our body fights infection, but also its importance for the development of new vaccines. I am proud to say that members of our society have made a significant contribution to SARS-CoV-2 research, which is also illustrated by the many abstracts on this topic submitted to our meeting. Immunology in general is blossoming in Croatia and we have received a record number of 48 abstracts on a large variety of topics. Members of our society have published articles in top-ranking journals, such as ***Immunity***, the ***Journal of Experimental Medicine*** and ***Frontiers in Immunology***. Many of our members have won prestigious awards and I would particularly like to congratulate secretary of our society Inga Kavazović for winning the state award of best young scientist in the field of biomedicine. Considering the high quality of abstracts also this year, many of which of young researchers, I foresee a bright future for our field in the coming years.

This year is also the last year that I will be addressing you as president. After four years of me, we will elect a new person as a head of our society. I would like to thank our members for the opportunity to lead HID in the last four years and wish our new president all the best for the coming period. I would also like to thank my team, in particular Inga Kavazović, for all their hard work for HID in the last years.

What remains is for me to welcome you to our annual meeting. Once again, we have done our best to set up a stimulating program that emphasizes the beauty of immunology and allows for us to strengthen our bonds. We hope that you will enjoy it.

I wish you all a splendid meeting!

Felix M. Wensveen, President

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PROGRAM

THURSDAY October 6th 2022

14:00-24:00 HOTEL CHECK-IN

14:00-15:00 REGISTRATION & WELCOME

15:00-15:15 OPENING CEREMONY
Felix M. Wensveen, president of the Croatian Immunological Society

15:15-15:45 INVITED LECTURE:
Chairs: Danka Grčević & Mariastefania Antica

Alan Sučur

School of medicine, University of Zagreb, Zagreb, Croatia

`*Notch signal modulates phenotype and function of osteoclasts differentiated from common trilineage myeloid progenitor under inflammatory conditions*`

5:45-16:45 SELECTED ORAL PRESENTATIONS – SESSION 1
Chairs: Dora Višnjić & Mariastefania Antica

15:45 **Dubravko Forčić / Beata Halassy, University of Zagreb**
Preoperative shrinkage of a localized breast cancer recurrence by intratumoral injections of measles and vesicular stomatitis viruses – a case study

16:00 **Carmen Rozmanić**, University of Rijeka
Early life cytomegalovirus infection extensively reshapes the transcriptional profile and functionality of NK cells

16:15 **Alenka Gagro**, University of Zagreb
Call for collaboration in the field of monogenic autoinflammatory disease: trained immunity and deficiency of mevalonate kinase

16:30 **Maria Mazor**, University of Rijeka
Innate immunity responses to MCMV infection in the ovaries and adrenal glands

16:45-17:15 INVITED LECTURE:

Chairs: Dora Višnjić & Mariastefania Antica

Anže Smole

National institute of Biology, Ljubljana, Slovenia

`*Next generation chimeric antigen receptor (CAR) T cells*`

17:15-18:15 DRINKS

19:00-20:30 DINNER

FRIDAY October 7th 2022

08:15-09:00 GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY

09:00-09:30 INVITED LECTURE:

Chairs: Danka Grčević & Ilija Brizić

Liborija Lugović Mihić

University of Zagreb, Zagreb, Croatia

'Assessment of atopic dermatitis patients' quality of life and related factors: disease severity, patient perception of the disease and personality features'

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

Chairs: Danka Grčević & Ilija Brizić

09:30 **Jelena Železnjak**, University of Rijeka
Cytomegalovirus and host interplay via Ly49 receptors – the story's not over yet!

09:50 **Magdalena Medved**, University of Rijeka
Modulation of CD8+ T cell response mediated by mouse cytomegalovirus immunoevasins MATp1 and m04 /gp34

10:10 **Dora Gašparini**, University of Rijeka
Type 2 diabetes is associated with increased cytokine production by cytotoxic blood lymphocytes: a human flow cytometry study

10:30-11:00 COFFEE BREAK

Sponsored by Gorea Plus d.o.o.

11:00-11:30 INVITED LECTURE:

Chairs: Stipan Jonjić & Ivana Munitić

James di Santo

Institut Pasteur, Paris, France

`*Innate lymphoid cells: Development, Differentiation and Dynamics'*

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

Chairs: Stipan Jonjić & Ivana Munitić

11:30 **Paola Kučan Brlić**, University of Rijeka
Translational Opportunities for Antibodies targeting PVR (CD155)

11:45 **Jelena Korać Prlić, University of Split**
The antitumor effect of shikonin in mouse urinary bladder cancer model

12:00 **Marko Šestan**, Champalimaud Centre for the Unknown, Lisbon, Portugal
Ménage à trois: Neuro-endocrino-immune regulation of metabolic homeostasis

12:15 **Karlo Mladenić**, University of Rijeka
NKG2D-mediated immunosensing of metabolically stressed hepatocytes by innate-like T cells is essential for initiation of NASH and fibrosis

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12:30-15:30 LUNCH & LEASURE TIME

15:30-16:30 EFIS-IL LETURE AWARD CEREMONY:

Chairs: Felix Wensveen & Ineš Drenjančević

Klaas P.J.M. Van Gisbergen

Sanquin Research and Landsteiner Laboratory, University of Amsterdam, Amsterdam, The Netherlands.

`I*mmunological memory of the tissues*`

16:30-18:30 POSTER SESSION & DRINKS

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19:30-23:00 GALA DINNER

SATURDAY October 8th 2022

09:00-09:30 INVITED LECTURE:

Chairs: Bojan Polić & Marina Babić Čač

Daniel Konrad,

University of Zürich, Zürich, Switzerland

'A role of ASK1 in obesity and associated co-morbidities?'

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

Chairs: Bojan Polić & Marina Babić Čač

09:30 **Nikolina Kolobarić**, J. J. Strossmayer University of Osijek
Dietary intake of n-3 PUFAs enriched hen eggs changes Treg population frequencies in PBMCs of young, healthy individuals –randomized controlled study

09:45 **Andrea Mihalić**, University of Rijeka
Glial cell adaptation to latent virus infection in the CNS

10:00 **Barbara Tomić**, University of Zagreb
The role of p21 and WEE1 kinase in monocytic differentiation

10:15 **Mia Krapić**, University of Rijeka
NK cell derived IFN-γ causes free fatty acid release by adipocytes to promote B cell responses during viral infection

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10:30-11:00 COFFEE BREAK

11:00-11:30 INVITED LECTURE:

Chairs: Alenka Gagro & Ines Mrakovčić-Šutić

Ilija Brizić

H University of Rijeka, Rijeka, Croatia

`*Congenital Cytomegalovirus Infection: Neuroinflammation and Pathology'*

11:30-12:00 INVITED LECTURE:

Chairs: Alenka Gagro & Ines Mrakovčić-Šutić

Bojan Polić

University of Rijeka, Faculty of medicine

`V*irus-induced changes in blood glucose levels: friends or foes of viral immunosurveillance?*`

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

INVITED LECTURES

NOTCH SIGNAL MODULATES PHENOTYPE AND FUNCTION OF OSTEOCLASTS DIFFERENTIATED FROM COMMON TRILINEAGE MYELOID PROGENITOR UNDER INFLAMMATORY CONDITIONS

Alan Šučur

School of Medicine, University of Zagreb, Zagreb, Croatia

Osteoclasts (OC), macrophages (MA) and dendritic cells (DC) may be derived from common trilineage myeloid progenitor of hematopoietic origin. Progenitor commitment is susceptible to regulation through Notch signaling. Moreover, Notch dysregulation has been implicated in a number of inflammatory diseases. We aimed to determine the effects of Notch signaling modulation on common trilineage myeloid progenitor commitment and functional properties of differentiated cells under inflammatory conditions.

As a source of common bone marrow progenitor (CD45+CD3-B220-NK1.1-CD11bloCD115+), we used CX3CR1CreERT2NICD1 (Cre+NICD1) mice to overexpress Notch 1 signal and CX3CR1^{Cre}ERT2RBPJκ (Cre+RBPJκ) mice to inhibit Notch signal. Cre-recombinase, under the control of CX3CR1 promoter, expressed in monocyte/macrophage lineage, was induced *in vitro* by 4-hydroxytamoxifen. Differentiation of OCs was induced by M-CSF/RANKL; MAs by M-CSF; DCs by IL-4/GM-CSF; inflammation by E. coli lipopolysaccharide. Functionally, DCs were tested for the ability to process and present antigen; MAs to phagocytose E. coli particles, and OCs to resorb bone and express TRAP.

Among tested lineages, Notch 1 overexpression suppressed OC formation, whereas Notch deletion enhanced osteoclastogenesis, resulting in a greater number and larger osteoclasts. In accordance, RANK protein expression was upregulated in osteoclastogenic cultures from Cre+RBPJκ mice, as well as the gene expression of cFos and CatK. Notch modulation did not seem to affect the number of MAs or DCs. Functional assays under inflammatory conditions confirmed that Notch silencing amplifies antigen presentation by DCs and TRAP expression by OCs, whereas the enhanced phagocytosis by MAs was less prominent.

Although Notch signaling modulation affected functional properties of all three lineages, the major effect was observed in OCs, with enhanced differentiation and function by Notch signal silencing. Our results indicate that Notch signaling participates as the negative regulator of OC activity during inflammation, which may be relevant in immune and bone diseases.

NEXT GENERATION CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS

Anže Smole

1. Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA**.**
2. Parker Institute for Cancer Immunotherapy, USA.
3. National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Immunology and Cellular Immunotherapy (ICI) Group, Ljubljana, Slovenia.

Immunosuppressive tumor microenvironment (TME) and T cell intrinsic dysfunctions influence the clinical efficacy of CAR T cells. We have developed a genetic approach that combines autonomous antigen-triggered production of an accessory molecule, along with constitutive CAR expression in a single lentiviral vector called **Uni-Vect**. Here we present two distinct therapeutic applications of Uni-Vect. In a first therapeutic approach, we introduced inducible expression of IL-12 (**iIL-12-CAR T**) to augment efficacy in a safe manner. We demonstrated that only iIL-12-secreting, and not conventional CAR T cells, were capable of eradicating solid tumors *in vivo* without detectable toxicities. In a second therapeutic approach, we demonstrated modulation of CAR T cell intrinsic properties by transient activation-inducible transcription factor expression (**iTF-CAR T**). iTF-CAR T cells demonstrated enhanced antigen-dependent proliferation and a less differentiated phenotype after repeated stimulation *in vitro*. CyTOF analysis of iTF-CAR T cells showed that antigen-inducible expression of a single TF favorably affected T cell markers of efficacy. Finally, we tested activity *in vivo* in tumor xenograft model in which iTF-CAR T cells demonstrated increased expansion in blood compared with control CAR T cells. Importantly, the expansion of T cells was transient and ultimately contracted to normal levels after the tumor was cleared.

Together, we demonstrated that iIL-12 remarkably enhanced anti-tumor responses in established solid tumors *in vivo* and that iTF expression endowed CAR T cells with improved therapeutically relevant T cell states and *in vivo* expansion. Overall, our work provides a foundation for clinically actionable next-generation cellular immunotherapies.

Declaration of interests: AS is co-inventor on a PCT International Patent Applications by The Trustees of the University of Pennsylvania, which incorporate discoveries and inventions described here.

ASSESSMENT OF ATOPIC DERMATITIS PATIENTS' QUALITY OF LIFE AND RELATED FACTORS: DISEASE SEVERITY, PATIENT PERCEPTION OF THE DISEASE AND PERSONALITY FEATURES

Liborija Lugović Mihić

Department of Dermatovenereology, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia.

Quality of life concerns are often a basis for decisions on patient treatment, including decisions on therapy for patients with atopic dermatitis (AD). AD is a significant burden on patients and their families and society and its presence can severely limit both specific and broad dimensions of daily functioning, significantly lowering a person's quality of life. Our study included 84 adult AD patients: 42 with clinical AD manifestations and 42 in remission. To analyze different aspects of AD patients' quality of life, we used the SCORAD index (AD severity), the Dermatology Life Quality Index (DLQI), the World Health Organization Quality of Life Brief Version (WHOQOL-BREF), the Brief Illness Perception Questionnaire (Brief IPQ), and the Crown–Crisp Experiential Index (CCEI) to analyze personality traits. According to our results, SCORAD correlated positively and linearly with DLQI ($p<0.001$) and with disease impact on life, disease control, and disease symptoms ($p\leq0.023$). DLQI was also related to certain personality characteristics (free-floating anxiety disorder, obsession, somatization, and depression ($p\leq0.032$)). Symptomatic AD patients had a significantly more impaired DLQI than asymptomatic patients ($p<0.001$) and the two groups differed in some IPQ dimensions, but they did not differ significantly concerning the WHOQOL-BREF dimensions and personality traits (CCEI). So, AD patient quality of life is dependent not only on disease severity but is also influenced by patient personality characteristics and their concomitant psychological disturbances (anxiety disorder, obsession, somatization, depression). Thus, improving AD patients' quality of life requires clinicians to consider a multidisciplinary treatment approach with psychological support strategies.

INNATE LYMPHOID CELLS: DEVELOPMENT, DIFFERENTIATION AND DYNAMICS

James P di Santo

Institut Pasteur, Université Paris Cité, Innate Immunity Unit, Paris, France.

The intestinal barrier comprises a layered defense system involving 'non-specific' (epithelial, innate immunity) as well as 'specific' (adaptive immunity) components that work together to maintain tissue tolerance and protection the organisms against invasion by potential pathogens. While antigen-specific T and B cells are recognized for their essential roles in barrier defense and mucosal immune responsiveness, knowledge on how the innate immune system play regulatory and/or protective roles within the intestine is less defined. Emerging evidence highlights the importance of tissue-resident group 3 innate lymphoid cells (ILC3) in intestinal homeostasis and immunity. Through their ability to produce copious amounts of cytokines and their strategic location, ILC3 appear positioned to promote rapid, early protection in the context of stress and infection. Still, the spatio-temporal regulation of intestinal ILC3 responses as well as the impact of commensal and pathogen exposure on their long-term attributes remains poorly understood. New results concerning these two facets of ILC3 biology will be discussed.

IMMUNOLOGICAL MEMORY OF THE TISSUES

Klaas P.J.M. Van Gisbergen

Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

An important component of acquired T-cell driven immunity derives from tissue-resident memory CD8 T cells (Trm). These Trm form local populations in and directly underneath the epithelial layer of skin, gut, lungs and other relevant entry sites of pathogens. This strategic position enables them to directly respond upon reinfection with the production of pro-inflammatory cytokines and cytotoxic molecules to counter the invading pathogens. Trm have not only been found essential in protection against reinfection, but they also appear to substantially improve disease outcome in cancer patients. We have developed Hobit reporter/deleter mice to take advantage of the Trm-restricted expression of Hobit. Hobit was upregulated in a subset of LCMV-specific T cells located within peripheral tissues during the effector phase of the immune response, which were identified as Trm precursors. Transcriptional profiling of Hobit+ effector T cells underlined the early establishment of Trm properties including downregulation of tissue exit receptors and upregulation of Trm-associated molecules. Importantly, we identified Eomes as a key factor instructing the early bifurcation of circulating and resident lineages.

We also found that Trm are able to expand after rechallenge and can generate substantial effector responses in the local tissues. Assessment of the contribution of Trm and circulating memory T cells to secondary effector and memory T cells indicated that a large proportion originated from Trm rather than circulating memory T cells. Potentially, the size of the primary Trm population compared to that of circulating memory T cells and their location in barrier tissues at pathogen entry sites thereby permitting early activation contribute to the dominant contribution of Trm to secondary T cell responses. However, the ex-Trm were distinct from other circulating memory T cells and appeared to retain an imprint of their Trm history, which may enable these cells to preferentially home to their tissue of origin.

Thus, our findings suggest that Trm are not terminally differentiated, but that these memory T cells have an important contribution in the shaping of secondary effector and memory T cell responses. Moreover, our findings also suggest that Trm can be expanded in in vitro cultures, suggesting the possibility to exploit these memory T cells for therapeutic purposes.

A ROLE OF ASK1 IN OBESITY AND ASSOCIATED CO-MORBIDITIES

Daniel Konrad

Division of Pediatric Endocrinology and Diabetology, University Children's Hospital Zurich, University Zurich, Switzerland

Activation of stress pathways in adipose tissue and the liver are involved in the pathogenesis of obesity and associated co-morbidities such metabolic dysfunction-associated fatty liver disease (MAFLD). In particular, obesity-induced inflammation and/or suppression of "browning" impairs adipose tissue function, resulting in a deteriorated fat-liver cross talk that induces hepatic insulin resistance and steatosis. On the other hand, hepatic steatosis and insulin resistance may be a direct consequence of obesity-mediated liver dysfunction and/or hepatic inflammation. The apoptosis signal-regulating kinase-1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, acting as a signaling node in which different stressors such as endoplasmic reticulum, oxidative and inflammatory stresses converge. I will present recent and ongoing work from our laboratory demonstrating a role for ASK1 in the development of obesity as well as of associated co-morbidities. Our data provide evidence for the ASK1 pathway as potential novel therapeutic target to relieve obesity, obesity-induced insulin resistance and MAFLD..

CONGENITAL CYTOMEGALOVIRUS INFECTION: NEUROINFLAMMATION AND PATHOLOGY

Ilija Brizić,

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

Human cytomegalovirus (HCMV) is a highly prevalent herpesvirus that can cause severe disease in immunocompromised individuals and immunologically immature fetuses and newborns. Congenital human cytomegalovirus (cHCMV) infection of the brain is associated with a wide range of neurodevelopmental and cognitive sequelae. We are using infection of newborn mice with mouse cytomegalovirus (MCMV) as a reliable model that recapitulates many aspects of cHCMV infection, including virus dissemination to the central nervous system (CNS), altered neurodevelopment, and sensorineural hearing loss. The inflammatory response in the brain is required to control the infection, however, inflammation causes alterations in cerebellar development, suggesting that host inflammatory factors are key drivers of neurodevelopmental defects (Kosmac et al PLoS Pathog 2013, Kveštak et al J Exp Med 2021). Furthermore, MCMV establishes latency in the brain causing lifelong adaptation of glial cells to this persistent infection and retention of MCMV-specific T cells in the brain tissue. Here, our recent studies on cytomegalovirus infection in the brain, local immune response to infection, and mechanisms leading to CNS sequelae will be discussed.

VIRUS-INDUCED CHANGES IN BLOOD GLUCOSE LEVELS: FRIENDS OR FOES OF VIRAL IMMUNOSURVEILLANCE?

Bojan Polić

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

Activation of the immune system by a viral infection has a major impact on systemic metabolism, but much is unknown about underlying mechanisms and whether it has any benefit for the resolution of infection. In our previous work we have shown that a mild viral infection induces insulin resistance in skeletal muscle that results in transient compensatory hyperinsulinemia to keep normal blood glucose levels. This effect is mediated by IFNγ which downregulates the insulin receptor on myocytes. Hyperinsulinemia in turn enhances specific CD8-mediated anti-viral response. However, these mechanisms are derailed in obesity-induced hyperglycemic mice.

More recently, we examined how strong viral infection impacts systemic blood glucose levels and whether this influences viral immunosurveillance. We could show that strong, non-lethal viral infection causes a transient reduction of blood glucose levels. This is induced by IFNγ, produced by γδ T cells, in concert with IL-1β. These cytokines directly stimulate insulin secretion by pancreatic β-cells in the islets of Langerhans. High fasting blood insulin levels reduce hepatic glucose output by inhibiting glycogenolysis in hepatocytes. Relative hypoglycemia enhances the IFN type I production of non-immune cells by reducing lactate-mediate inhibition of IRF3 and NF-kB signaling. Correction of relative hypoglycemia by glucose feeding or viral infection of obesity-induced hyperglycemic mice results in impaired viral immunosurveillance and higher viral titers than in hypoglycemic controls.

Thus, virus-induced immuno-endocrine interactions transiently regulate blood glucose set points to fine-tune immune defense mechanisms. The level of adaptation corresponds to the strength of infection, and ultimately functions to benefit the host. These mechanisms are defective in diabetes, leading to increased susceptibility to viral infections.

ORAL PRESENTATIONS
Session 1

PREOPERATIVE SHRINKAGE OF A LOCALIZED BREAST CANCER RECURRENCE BY INTRATUMORAL INJECTIONS OF MEASLES AND VESICULAR STOMATITIS VIRUSES – A CASE STUDY

Dubravko Forčić[1,6,#], Karmen Mršić[2,#], Melita Perić-Balja[3,#], Tihana Kurtović[1,6], Snježana Ramić[3], Tajana Silovski[4], Ivo Pedišić[5], Ivan Milas[3], Beata Halassy[1,6]

1. University of Zagreb, Centre for Research and Knowledge Transfer in Biotechnology, Zagreb, Croatia
2. University Hospital Centre "Sestre Milosrdnice", Clinical Department of Diagnostic and Interventional Radiology, Zagreb, Croatia
3. University Hospital Centre "Sestre Milosrdnice", University Hospital for Tumours, Zagreb, Croatia
4. University Hospital Centre Zagreb, Department of Oncology, Zagreb, Croatia
5. Radiochirurgia Zagreb, Sveta Nedelja, Zagreb, Croatia
6. Center of Excellence for Virus Immunology and Vaccines, Zagreb, Croatia

Intratumoral oncolytic virotherapy may have promise as a means to debulk and downstage inoperable tumours in preparation for successful surgery. Here we describe the pioneering case of a 50-year-old virologist with locally recurrent muscle-invasive breast cancer who was able to proceed to tumour resection after receiving multiple intratumoral injections of viruses, first an Edmonston Zagreb measles vaccine strain (MeV) then vesicular stomatitis virus Indiana strain (VSV). The intratumoral virus therapy was well tolerated and led to transient pseudoprogression followed by partial tumour remission. Frequent imaging studies and regular clinical observations documenting size, consistency and mobility of the injected tumour demonstrate that both MeV and VSV contributed to the overall favourable response. Two months after the start of virus injections, the shrunken tumour was no longer invading the skin or underlying muscle and was surgically excised. The excised tumour showed strong lymphocytic infiltration, with increase in both CD20-positive B cells and CD8-positive T cells, as well as macrophages. PD-L1 expression was detected in contrast to the PD-L1 negative phenotype before the treatment. Although an isolated case, the study provides a strong rationale for the formal testing of oncolytic virotherapy also in patients with early stage cancer. vaccinees' sera of comparable SARS-CoV-2 neutralisation power indicate that convalescents are armed with antibodies of additional specificities and/or classes, that contribute to SARS-CoV-2 neutralisation.

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

Carmen Rožmanić[1], Berislav Lisnić[1], Lea Hiršl[1], Marina Pribanić Matešić[1], Eugene Park[2], Ana Lesac Brizić[1], Vanda Juranić Lisnić[1], Kristina Gotovac[3], Fran Borovečki[3], Wayne M. Yokoyama[2], Astrid Krmpotić[1], Stipan Jonjić[1], Ilija Brizić[1]

1. Center for proteomics and Department for histology and embryology, Faculty of Medicine, University of Rijeka, Croatia
2. Division of Rheumatology, Department of Medicine, Washington University School of Medicine, St. Louis, USA
3. Department for functional genomics, Center for translational and clinical research, School of medicine, University of Zagreb, Croatia

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

CALL FOR COLLABORATION IN THE FIELD OF MONOGENIC AUTOINFLAMMATORY DISEASE: TRAINED IMMUNITY AND DEFICIENCY OF MEVALONATE KINASE

Alenka Gagro[1], Ksenija Fumić[2,3]

1. Department of Pediatrics, Children's Hospital Zagreb, University of Zagreb School of Medicine, Zagreb
2. Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia

Abnormalities of the innate immunity machinery make up a group of rare diseases, named 'autoinflammatory', which are caused by mutations in genes involved in different immune pathways. Self-limited and recurrent inflammatory attacks involving skin, serosal membranes, joints, gut and other compartments of the body are hallmarks of these autoinflammatory diseases (ADs). Dysregulated inflammasome activity, overproduction of interleukin (IL)-1 beta or other IL-1-related cytokines, delayed cessation of inflammation and risk for secondary amyloidosis are pivotal keys in the majority of ADs. Mevalonic aciduria (MVA) and hyperimmunoglobulinemia D syndrome (HIDS) represent the two ends of a clinical spectrum of a rare AD caused by deficiency of mevalonate kinase (MVK), the first committed enzyme of cholesterol biosynthesis, leading to the accumulation of mevalonate. It is inherited in an autosomal recessive manner. More than 300 individuals worldwide are known to have the disorder, but the true number is likely greater. Most people with the disorder are individuals of western European heritage with approximately 60% occurring in Dutch or French individuals. Monocytes of patients with HIDS, have a constitutive trained immunity phenotype at both immunological and epigenetic levels, which could explain the attacks of sterile inflammation that these patients experience. Although there were attempts to use statins for therapy, based on the most recent recommendations, IL-1 blocking therapy is needed in patients with MVK/HIDS. We aim to present three children with genetically confirmed HIDS diagnosis using next-generation sequencing gene panel. The median age at onset was 0.7 years. Patients had on average 9 episodes per year. All patients had gastrointestinal symptoms, lymphadenopathy and myalgia. Increased IgD was found in one patient only. None of the patients had findings of altered parameters of acquired immunity or findings associated with autoimmunity. Based on the lack of knowledge of genotype-phenotype correlation in these patients as well as proposed importance of immunometabolism in trained immunity of myeloid cells, investigations that require collaboration between basic and clinical immunologists are highly required in order to improve therapy in these patients.

INNATE IMMUNITY RESPONSES TO MCMV INFECTION IN THE OVARIES AND ADRENAL GLANDS

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

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

Cytomegalovirus (CMV) is not only an excellent model for studying antiviral immune responses, but also a highly relevant and widespread human pathogen. Owing to its wide tropism, it infects nearly every tissue, yet our understanding of immune responses is limited to only few organs. Among those, both ovaries and adrenal glands have been equally neglected. Using mouse CMV (MCMV), we have recently shown that MCMV readily and strongly infects ovaries and adrenal glands; with peak of infection at day 4-6 and virus clearance by day 8. Surprisingly, extremely tissue-specific MCMV infection in the ovaries has been observed, with strong infection of corpora lutea (CL) and no signs of infection in the follicles. High infection densities indicated complete failure of immune control in CL cells, resulting in progesterone insufficiency and pregnancy loss. While corpora lutea infection might lead to miscarriage, infection of follicles may promote sterility. Our results revealed that innate immune responses, and among those NK cells, play crucial role in protection of ovarian follicles and fertile potential. In this work we performed detailed high throughput analysis of phenotype and functional profile of NK cell subsets in uninfected and MCMV infected ovaries and adrenal glands. The MCMV infection in the ovaries appeared to be mainly controlled by tissue resident ILC1 – like cells, while cNK cells play a predominant role in control of viral infection in the adrenal glands.

ORAL PRESENTATIONS
Session 2 – Bright Sparks

CYTOMEGALOVIRUS AND HOST INTERPLAY VIA LY49 RECEPTORS – THE STORY'S NOT OVER YET!

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Cytomegaloviruses' (CMVs) ability to manipulate immune-cell recognition is well established, especially fine-tuning of MHC I expression to avoid both antigen presentation to CD8 T cells and NK cell-mediated missing-self recognition. Mouse CMV (MCMV) encodes two proteins – m04 and MATp1, which rescue selected MHC I alleles from retention or degradation during infection and allow them to reach the cell surface. We have shown that these MATp1/m04-modified altered-self MHC I molecules engage inhibitory Ly49 NK cell receptors more strongly than MHC I molecules alone in uninfected cells. The result is an efficient avoidance of NK cell missing-self lysis of infected cells even when MHC I surface levels are severely diminished. This finding provided an explanation for the longstanding question why licensed NK cells cannot efficiently control MCMV infection despite marked down-regulation of MHC I. Furthermore, we have also shown how this viral strategy could have prompted the evolution of activating NK cell receptors since at least 3 activating Ly49 receptors (Ly49D2PWK/Pas, Ly49LBALB/c, Ly49PMA/My) specifically recognize MCMV-infected cells via MATp1/m04/MHC I complex. We have now turned our attention to C57BL/6 NK cell responses and observed attenuation of MCMV strains lacking MATp1 followed by increase in cells expressing activating Ly49H and inhibitory Ly49I which might indicate that MATp1 modulates m157 as well, in addition to MHC I. We are also investigating the role of MATp1/m04-modified MHC I molecules in the formation and maintenance of memory-like NK cells, especially in mice in which activating Ly49 receptors have been shown to play a role.

MODULATION OF CD8+ T CELL RESPONSE MEDIATED BY MOUSE CYTOMEGALOVIRUS IMMUNOEVASINS MATP1 AND M04 /GP34

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Cytomegaloviruses possess numerous immunoevasive genes that modulate immune system. To avoid immune recognition of infected cells by CD8 T cells, CMVs prevent the surface expression of MHC I molecules. However, a complete lack of MHC I on the surface activates NK cells. MCMV encodes two immunoevasive proteins - m04 and MATp1 that bind to certain alleles of MHC I molecules and ensure their expression on the cell surface. In that way these two immunoevasins inhibit NK cells. This study will examine the effect of modulation of MHC I molecules on the short and long-term response mediated CD8 T lymphocytes.

In vitro and in vivo experiments on BALC/c as well as on C57BL/6 mice showed a better response of CD8+ T lymphocyte when immunoevasin MATp1 was not present. Preliminary analyzes have shown that the MATp1 protein could cause qualitative and quantitative alterations the presented peptides and thus impair the recognition of infected cells via cytotoxic T lymphocytes. We have now constructed stable transfectants of MEF cells and antigen presenting cells producing H-2D^{d} MHC I molecule without the transmembrane domain which is then secreted into the supernatant – soluble MHC I (sMHC I). We now optimizing production, purification and quantitation of sMHC I necessary to proceed to mass-spec analysis.

In addition to expanding our understanding of the mechanisms by which CMV evade the immune response, this research has the potential to identify new CD8+ T cell epitopes that can be used as tool in further research into the immune response to CMV.

PE 2 DIABETES IS ASSOCIATED WITH INCREASED CYTOKINE PRODUCTION BY CYTOTOXIC BLOOD LYMPHOCYTES: A HUMAN FLOW CYTOMETRY STUDY

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Background: Chronic systemic low-grade inflammation has been recognized as a prominent feature of type 2 diabetes. Research has shown that elevated levels of proinflammatory cytokines in patients with diabetes are contributing to an increased risk of morbidity and mortality after viral infection. However, which immune cells are involved in the underlying mechanism is mostly unknown. The aim of this study is to characterize peripheral blood lymphocytes and determine the nature of diabetes-induced changes in the antiviral arm of the immune system. Methods: Study participants underwent initial clinical and laboratory evaluation to exclude subjects with any immunomodulating factors. After signed informed consents were obtained, peripheral blood mononuclear cells were isolated from the blood of patients with diabetes and age- and gender-matched control subjects. Multiparametric flow cytometry was used to analyze the phenotype and cytokine production by CD8+ T, NK and gamma delta T cells after in vitro stimulation with Phorbol 12-myristate 13-acetate and Ionomycin. Results: Phenotypic analysis of peripheral blood mononuclear cells showed only minor differences in the percentage of lymphocyte subpopulations between study groups. However, significantly increased production of tumor necrosis factor by CD8+ T cells and Granzyme B by NK cells and gamma delta T cells was observed in patients with diabetes in comparison to the control group. Conclusion: Our findings suggest that cytotoxic immune cells permanently change their functional profile in the context of diabetes and may therefore play an important role in diabetes-induced immune dysfunction.

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ORAL PRESENTATIONS
Session 3

TRANSLATIONAL OPPORTUNITIES FOR ANTIBODIES TARGETING PVR (CD155)

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Poliovirus receptor (PVR/CD155), expressed by many types of cancer cells, 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 immune cell receptors that leads to inhibition of their anti-cancer activities. 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 NTX1088, 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. Moreover, it uniquely blocks the interaction between PVR and the activating receptor DNAM1, preventing PVR-induced internalization of DNAM1, leading to robust antitumor activation. Efficacy of NTX1088 was validated using human immune cells, and tumor cell lines both *in vitro* and in humanized murine models *in vivo*. *In vitro* data showed that NTX1088 increased activation of CD8 T- and NK-cells. *In vivo*, NTX1088 induced a robust tumor growth inhibition, accompanied by higher prevalence of DNAM1+ CD8+ tumor infiltrating cells. Altogether, NTX1088 shows novel triple mechanism of action, not shared by other drugs. Excelent drug manufacturing and safety properties allowed FDA clearance for NTX1088 as an investigational new drug (IND). NTX1088 is currently evaluated in a Phase 1, First-in-Human Study in patients with advanced solid malignancies (NCT05378425).

THE ANTITUMOR EFFECT OF SHIKONIN IN MOUSE URINARY BLADDER CANCER MODEL

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Bladder cancer is one of the most common malignancies in man. Although great progress has been achieved in terms of bladder cancer management, survival rates for muscle-invasive bladder cancer are still poor, which warrants the search for new therapeutic approaches. Shikonin, a natural compound isolated from the roots of the plant Lithospermum erythrorhizon, has long been used in traditional Chinese medicine to treat dermatitis, wounds, measles and burns, and today is known for its anti-inflammatory, antioxidant and antitumor effects. In this study, the antitumor effect of shikonin was studied on murine bladder cancer induced by carcinogen N-butyl-N-(4- hydroxybutyl)-nitrosamine (BBN). Our results demonstrate that shikonin slows down tumor growth by induction of necroptosis and apoptosis and by changing immune cell composition in the tumor microenvironment. Altogether, shikonin is a potential new therapeutic approach in bladder cancer management.

MÉNAGE À TROIS: NEURO-ENDOCRINO-IMMUNE REGULATION OF METABOLIC HOMEOSTASIS

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It has now been acknowledged for decades that the immune system is implicated in the control of metabolism, a function not linked to the classical immune response of the defence. While inflammation has long been considered as a significant pathogenic feature of diabetes development, recent studies have shown that immune cells are also important for maintenance of metabolic homeostasis in a steady state. Nevertheless, how immune cells integrate local and systemic cues to regulate metabolic processes remains elusive. Herein, we hypothesise that neuro-endocrine cues can regulate innate lymphocytes, forming an organismal neuro-endocrine-immune circuit that ensures metabolic homeostasis and prevention of obesity. By using combined cutting-edge immunology, neuroscience and endocrinology approaches we were able to decipher how this unconventional multi-organ, neuro-endocrine-immune circuit is controlled to regulate the host euglycemia in the health and how it can derail in obesity.

NKG2D-MEDIATED IMMUNOSENSING 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. What triggers inflammation in MAFLD is unknown. We find that lipid accumulation in hepatocytes induces expression of ligands for the activating immune receptor NKG2D. Tissue-resident innate-like T cells are activated through NKG2D and secrete IL-17A. IL-17A licenses hepatocytes to produce chemokines that recruit pro-inflammatory cells into the liver, causing NASH and fibrosis. NKG2D-deficient mice did not develop fibrosis in a dietary model for NASH and had a marked decrease in the incidence of hepatic tumors. Importantly, the frequency of IL-17A+ γδ T cells in the blood of MAFLD patients correlated with liver pathology. Our findings identify a key molecular mechanism through which stressed hepatocytes trigger inflammation in context of MAFLD.

ORAL PRESENTATIONS
Session 4

GLIAL CELL ADAPTATION

TO LATENT VIRUS INFECTION IN THE CNS

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

DIETARY INTAKE OF N-3 PUFAS ENRICHED HEN EGGS CHANGES TREG POPULATION FREQUENCIES IN PBMCS OF YOUNG, HEALTHY INDIVIDUALS –RANDOMIZED CONTROLLED STUDY

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Objectives: To determine the effects of n-3 polyunsaturated fatty acid (PUFA) supplementation, i.e., α-linolenic (~230 mg), eicosapentaenoic (~15 mg), and docosahexaenoic acid (~105 mg), on the prevalence of T regulatory (Treg) lymphocytes and their subsets in healthy individuals.

Participants and Methods: 40 participants were randomized and divided into: 1) Control group (N=21) that consumed regular hen eggs (n-3 PUFAs: ~249 mg/per day), 2) n-3 PUFAs group (N=19) that consumed enriched hen eggs (n-3 PUFAs: ~1053 mg/per day). The study protocol included two appointments and participants were instructed to eat three hard-boiled hen eggs/day for three weeks. Venous blood samples were taken before and after the dietary protocol for serum and peripheral blood mononuclear cells isolation. Frequency of Treg lymphocytes were determined by flow cytometry. Measurements were carried out by BD FACSCanto II cytometer equipped with blue Argon 488 nm and Red HeNe 633 nm laser lines.

Results: Both study groups showed reduced frequencies of peripheral Treg lymphocytes. There was a significant reduction of CD4+CD25+Foxp3high subpopulation corresponding to 'real' regulatory T cells (nTreg) in both groups. Further, rates of recently activated T cells were positively associated, while the % of nTreg was inversely related to serum IL-22 levels in the n-3 PUFAs group.

Conclusion: Both dietary protocols resulted in reduced frequencies of peripheral nTreg lymphocytes. Although, regarding the same study groups, we previously reported that functional capacity of T cells for cytokine secretion was significantly altered only in the n-3 PUFAs group through increased anti-inflammatory and reduced pro-inflammatory cytokine secretion.

THE ROLE OF P21 AND WEE1 KINASE IN MONOCYTIC DIFFERENTIATION

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Ataxia telangiectasia and RAD3-related (ATR)/checkpoint kinase (Chk) DNA damage response has been described to promote macrophage differentiation in granuloma. Our previous studies revealed that two potent pyrimidine synthesis inhibitors, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) and brequinar, induce monocytic differentiation of leukemia cells through activation of checkpoint kinase 1 (Chk1). A similar mechanism was observed in response to low dose cytarabine. Furthermore, *CDKN1A/p21(CIP1)* was upregulated in both U937 monocytic leukemia cell line and primary acute myeloid leukemia (AML) blasts differentiated with AICAr. In addition, all differentiation agents increased inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1). WEE1 kinase phosphorylates and inhibits CDK1, but its role in leukemia cell differentiation has not been investigated. The aim of this study was to test for the role of p21 and WEE1 kinase in differentiation of AML cells. Gene set enrichment analysis revealed upregulation of the hematopoietic cell lineage genes and downregulation of cell cycle pathway genes in samples treated with pyrimidine synthesis inhibitors and cytarabine. Downregulation of p21 diminished differentiation in cytarabine-treated U937 cells, but had no effects on AICAr- and brequinar-mediated differentiation. MK-1775, a pharmacological inhibitor of WEE1 kinase, in a dose that effectively decreases inhibitory CDK1 phosphorylation, significantly reduced differentiation effects of all agents tested. siRNA transfection targeting WEE1 decreased the expression of differentiation marker CD11b in cells treated with pyrimidine synthesis inhibitors. Therefore, our results suggest that cytarabine-mediated differentiation of AML cells depends on p21, while differentiation in response to pyrimidine synthesis inhibitors relies on WEE1 kinase.

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NK CELL DERIVED IFN-γ CAUSES FREE FATTY ACID RELEASE BY ADIPOCYTES TO PROMOTE B CELL RESPONSES DURING VIRAL INFECTION.

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Adipose tissue (AT) is a major lipid storage organ which releases and distributes lipids to maintain energy homeostasis. In 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. In an *in vitro* model of adipocyte differentiation, we could show that the pro-inflammatory cytokine IFN-γ significantly reduces cellular lipid content. High-throughput transcriptome analysis of these cells demonstrated that IFN-γ mediates down-regulation of PPAR-γ, a master regulator of AT metabolism, causing a net efflux of lipids. Infection of mice with cytomegalovirus induced a striking reduction of adipocyte cell size and induced a change in the transcriptional profile of these cells corresponding with an IFN-γ imprint. This corresponded with a systemic increase of adipose tissue derived metabolites 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 free fatty acids promote the acute B cell 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.

POSTER PRESENTATIONS

PITFALLS IN UNDERSTANDING AND HANDLING OF COVID-19 VACCINES

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The short incubation period of COVID-19 requires a steady protective antibody titer to avert infection, achieve herd immunity, and terminate the pandemic spread. COVID-19 vaccines attained 95% effectiveness within the clinical trials with follow-up of 4-6 months. The hurry reflected in the choice of a 2-dose schedule with doses 3-4 weeks apart, and immunity waning in 4-6 months, were not compensated by the roll-out fast enough to avoid the appearance of breakthrough infections and new variants. The protective neutralization titer against against any infection was found to be 20% of the mean convalescent titer (corresponding to neutralization titer of 1:10-1:30). From the present perspective, it was naive to believe that 2 vaccine doses given at the 3-4 weeks interval could reliably counter COVID-19. Moreover, it has not been appreciated that more generous spacing between the doses increased immunogenicity, and that a single vaccine dose at the appropriate post-recovery interval induced powerful hybrid immunity. Indeed, as of 24 June 2022, in the USA, both mRNA vaccines are offered as a series of 3 doses at longer intervals: dose 1 to 2 at least 3-8 weeks (BNT162b2) or 4-8 weeks (mRNA-1273), dose 2 to 3 at least 5 months. Dose 4 is indicated for ages ≥50 years at least 4 months after dose 3. The perplexing point about the above facts is that they had to be rediscovered in the present pandemic, without recognizing their time-honored scientific and empirical validation in the past.

QUALITATIVE AND QUANTITATIVE DIFFERENCES IN SARS-COV-2 - NEUTRALIZING ANTIBODY RESPONSE INDUCED BY FOUR COVID-19 VACCINES OR DEVELOPED IN COVID-19 CONVALESCENTS

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In the COVID-19 pandemic, we witnessed an impressive speed in the development of effective vaccines that have been approved for use in humans on the basis of the clinical studies comparing their efficacy to placebo. Head-to-head comparisons of several approved vaccines are rare, as well as comparisons of immune responses generated by vaccination to the ones created by the disease. Due to lack of appropriate standardization of assays that evaluate immune response, it is difficult to compare results from different studies. However, such analyses are inevitable in order to properly assess the correctness of epidemic measures applied in an effort to combat the pandemic. Here we report on a head-to head comparison of the first four vaccines authorized for emergency use in Europe (BNT162b2, mRNA-1273, AZD1222 or Ad26.COV2.S) in their ability to induce neutralizing antibody response (NT). All four deliver genetic information for the full-length spike protein into the host cells and induce both humoral and cellular branch of spike protein-specific immune response. Thus, NT response could serve as an indicator of the overall immune response induced by these vaccines. Well standardized wild-type neutralization assay1 enabled reliable comparison of antibody response induced by vaccines to the response induced in convalescents. By analysing the response in 113 vaccinees (≥18 per vaccine) we observed that the highest NTs were induced by mRNA vaccines, followed by AZD1222 and finally Ad26.COV2.S. In comparison to convalescents, these NTs were significantly higher, comparable and significantly lower, respectively. NT decline was significantly slower in convalescents. Better complement-activating properties of antibodies developed in natural infection as well as lower anti-spike IgG content in convalescents' in comparison to vaccinees' sera of comparable SARS-CoV-2 neutralisation power indicate that convalescents are armed with antibodies of additional specificities and/or classes, that contribute to SARS-CoV-2 neutralisation.

MODULATION OF NOTCH SIGNALLING PATHWAY IN ACTIVATED HEPATIC STELLATE CELLS HAS NO INFLUENCE ON DEVELOPMENT OR RESOLUTION OF HEPATIC FIBROSIS

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We used transgenic mice to investigate the effect selective Notch inhibition or forced activation in activated hepatic stellate cells (aHSCs) has on liver fibrosis. We used two models, carbon tetrachloride (CCl4) treatment and DDC-supplemented diet. The effect of Notch inhibition was investigated in SmaCre+ΔRbpjκΔ mice, while Notch activation was studied using SmaCre+NICD1 mice. Mice were treated with 75μg/g of tamoxifen i.p. twice weekly. Identically treated Cre- littermates served as controls. CCl4 and DDC caused a similar degree of fibrosis (Sirius red stained area, hydroxyproline content, aminotransferase activities, gene expression of Col1a1 and Acta2) in experimental and control groups in Notch-inhibition as well as Notch-activation experiments. We further tested whether forced Notch activation in aHSCs delays resolution of fibrosis. SmaCreNICD1 mice were treated with either CCl4 or DDC, and tamoxifen for 10 days, and then recovered for one month. Recovery was similar in both SmaCre+NICD1 and SmaCre-NICD1 mice, suggesting that forced Notch activation doesn't impair recovery. We also found that tamoxifen per se protects against DDC-induced fibrosis. This was evidenced by a lower Sirius red-stained area, lower hydroxyproline content, and further confirmed by qPCR showing a lower expression of genes for Col1a1, Acta2, Sox9, Pdgf, and Krt19, indicating the inhibitory effect on HSCs, collagen production, and biliary duct proliferation. This effect highlights the importance of a proper Cre- littermate control in this investigation. We conclude that modulation of Notch activity in aHSCs changes neither the degree of liver fibrosis nor does it impair recovery once the noxious stimulus is withdrawn.

TRANSCRIPTOME PROFILING OF CCR2^{HI} AND CCR2^{LO} OSTEOCLAST PROGENITOR SUBPOPULATIONS ASSOCIATED WITH ARTHRITIS

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As the existence of different osteoclast progenitor (OCP) subsets has been confirmed, their detailed characterization is required to understand the pathophysiology of increased osteoclast activity causing periarticular and systemic bone resorption in arthritis. We previously defined OCP subsets based on the level of CCR2 expression, as circulatory-like committed CCR2hi OCPs, substantially expanded in arthritis, and marrow-resident CCR2lo OCPs of immature phenotype and behavior. We sorted CCR2hi and CCR2lo OCPs of mice with collagen-induced arthritis (CIA) and control mice (n=4 for each group), and performed next-generation RNA sequencing. We confirmed a disparity between the transcriptomes of CCR2hi and CCR2lo OCP subsets, and identified pathway enrichment for osteoclast differentiation, chemokine and NOD-like receptor signaling in the CCR2hi OCP subset and ribosome biogenesis in eukaryotes and pyrimidine metabolism in the CCR2lo OCP subset. Genes associated with the osteoclastogenic pathway (Fcgr1, Socs3), and genes involved in cell adhesion and migration (F11r, CD38, Lrg1) were used to both identify the CCR2hi subset and distinguish CIA from control group, as validated by qPCR (n=6 for control, n=9 for CIA). The latter set positively correlated with arthritis clinical score and percentage of CCR2hi OCPs, indicating potential disease markers. Moreover, osteoclast pathway-identifying genes remained upregulated in committed preosteoclasts cultured for two days, suggesting their importance for enhanced osteoclastogenesis of CCR2hi OCPs in arthritis. Our approach identified differentially expressed genes that allow detection of distinct OCP subsets associated with arthritis and well as indicate possible therapeutic targets aimed to modulate progenitor migration, proliferation, differentiation or activity.

DIFFERENTIAL ROLE OF NEURONS AND GLIAL CELLS IN CYTOMEGALOVIRUS INFECTION AND IMMUNE CONTROL

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Human cytomegalovirus (HCMV) infection is the leading cause of congenital viral infections, which can cause a wide range of neurological sequalae. After the acute infection has been resolved, the virus remains in the central nervous system (CNS) in a state of latency. The pathogenesis of congenital CMV infection (cCMV) remains insufficiently understood. To elucidate the mechanisms of brain infection and pathogenesis during cCMV infection, we are using a murine model. As reported for HCMV, murine cytomegalovirus (MCMV) efficiently infects neurons, astrocytes and microglia. To study viral entry and dissemination in the developing murine brain, we have utilized a cell-type-specific virus labeling system. Here we show that MCMV can infect the brain independently of infection of endothelial cells, the main component of the blood-brain barrier (BBB). Following CMV entry into the CNS, infectious virus was produced by astrocytes, microglia and neurons. Even though astrocytes are initial cellular source of infectious virus in the brain, a significant proportion of the virus bypasses astrocytes and replicate in other cells in the brain. Microglia is strongly infected during the peak of MCMV infection in brain, however, the microglia-derived virus does not spread to other cells, indicating efficient microglial mechanism of virus containment. At later time points of acute infection, when immune control is established, neurons are the main source of infectious virus, suggesting impaired immune control of virus in neurons. Furthermore, we provide evidence that neurons are potential site of MCMV latency and reactivation.

IMMUNIZATION AGAINST SARS-COV-2 USING DIFFERENT VIRAL VECTORS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current worldwide COVID-19 pandemic, with over 600 million infected cases and over 6 million related deaths so far. Although efficient in preventing severe forms of the disease, vaccines developed to fight COVID-19 cannot warrant complete protection against breakthrough infections. One of the main reasons for this could be suboptimal mucosal immunity at the site of virus entry. To tackle this problem, we compared humoral and cellular responses in experimental mice after intranasal and intramuscular immunization with adenoviral vector ChAdOx1-S expressing the full-length Spike protein of SARS-CoV-2. We showed that both routes of vaccination elicited a potent IgG antibody response and a robust neutralizing capacity, whereas intranasal vaccination elicited superior IgA antibody titers in the sera and the respiratory mucosa. Furthermore, substantially higher frequency of epitope-specific CD8 T cells exhibiting a tissue-resident phenotype was found in the lungs of intranasally immunized animals.

Additionally, we have constructed and tested recombinant cytomegalovirus vectors expressing SARS-CoV-2 S protein. Immunization with this vector gave rise to outstanding CD8 T cell response and the generation of neutralizing antiviral antibodies. Importantly, not only immunization via systemic route but also via intranasal application of our recombinant vector, resulted in the induction of protective immune response.

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

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

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Research during recent years identified cytomegalovirus (CMV) as an attractive vaccine vector against infectious diseases and tumors. CMV encodes many non-essential immunomodulatory genes that can be easily manipulated to modulate its virulence and the immune response. Additionally, CMV infection in human and mice is lifelong and induces an atypical CD8 T cell response characterized by expansion and maintenance of effector memory T cells in peripheral tissues, a process termed memory inflation. As inflationary T cells are highly functional, CMV-based vaccines have gained substantial interest for vaccination purposes. The exact mechanisms underlying inflation of these CMV-specific CD8 T cell populations are still poorly understood. Therefore, understanding how this effector memory pool is generated and maintained is of great importance to optimize the efficacy of CMV-based vaccines.

In this study we investigated the contribution of costimulatory molecules in CD8 T cell response upon mouse CMV (MCMV) infection and their role in CD8 T cell inflation. We infected mice with WT MCMV or recombinant MCMV viruses lacking viral proteins that negatively regulate expression of different CD8 T cell costimulatory molecules and recombinant viruses expressing cellular ligands for CD8 T cells costimulatory receptor NKG2D (RAE-1γ, MULT-1 and H60) and followed CD8 T cell response over time. Our results show that upon infection with recombinant MCMV expressing NKG2D ligands, as well as with most of the MCMV mutants lacking genes that regulate expression of the costimulatory molecules, higher frequency of MCMV-specific memory precursor effector cells ($KLRG1^{-/}CD127^{+}$) is established early during infection and we will investigate whether this effector memory pool serves as a source of inflationary cells in peripheral tissues

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

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Human cytomegalovirus (HCMV) infection is the most common congenital viral infection and the leading cause of lasting perinatal brain damage, with the inflammatory response being the primary cause of pathogenic manifestations. Accordingly, it has been shown that anti-inflammatory drugs can reduce abnormalities in new-born mice infected with mouse cytomegalovirus (MCMV). Since contemporary antiviral drugs have inadequate efficiency, finding new therapeutic targets that can reduce brain damage is of utmost importance. There are growing indications that cellular prion protein (PrPC) dampens the immune response in various organs and prevents collateral, immune response-mediated pathologies. In addition to being expressed on immune cells, PrPC can also bind to immune cells, suggesting its interaction with immune receptors. Our primary goal is to characterize the role and impact of PrPC on the course and severity of congenital cytomegalovirus infection and associated brain pathology.

We show that CMV infection in different cell lines and primary cells affects the amount of PrPC. After initial strong induction of PrPC expression at early time points of infection, cell-associated PrPC levels are largely reduced, partially by triggering its ADAM10-mediated cell surface shedding. Intriguingly, PrP-/- mice have significantly lower virus titres in multiple organs, including the brain. Immune cell subsets and mechanisms responsible for more efficient virus clearance in the absence of PrPC protein are still being investigated.

Obtained data indicate that PrPC is involved in the immune response to CMV infection in new-born mice. Absence of PrPC improves control of the virus due to enhanced immune system activation.

THE ROLE OF MMP-2 AND 9, THEIR TISSUE INHIBITORS TIMP-1 AND 2 AND INNATE IMMUNITY IN PATIENTS WITH COLORECTAL CANCER

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Background: Colorectal cancer (CRC) is one of the primary causes of cancer-related mortality worldwide with approximately one million new cases every year and is accompanied with high mortality rate. The human response to malignancies includes an alteration in immune response, observed as decreases in natural killer (NK) cell cytotoxicity, lower antibody titers and decreased lymphocyte proliferation. The family of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play important role in colon carcinogenesis. AIM: The purpose was to examine the changes in percentage of innate immune cells: NKT and regulatory T cells (Tregs) in peripheral blood and the correlation with the levels of MMP-2 and 9 and TIMP 1 and 2 in urine of patients with colorectal cancer.

Subjects and methods: A total of 145 patients with CRC and 50 healthy control subjects were included in this study. Human peripheral blood mononuclear cells (PBMNC) from patients with CRC, classified as Dukes' B or Dukes' C, were taken before the operation and analyzed by flow cytometer. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of MMP-2 and 9, TIMP 1 and 2.

Results: our results showed a large increase in the concentration of both enzymes in the urine of patients with CRC and their correlation with the stage of tumor. The percentage of NKT cells were increased, while Tregs were diminished.

Conclusion: We verified the activity of MMPs in the urine of patients with diagnosed colorectal cancer in different stages of disease.

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HIGH NACL DIET INDUCES LOW GRADE INFLAMMATION BY TH17/TREG AXIS DYSREGULATION AND VASCULAR WALL REMODELING, AND CHANGES LEUKOCYTE EXPRESSION OF VLA-4, LFA-1 AND MAC-1 INTEGRINS IN SPRAGUE-DAWLEY RATS

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Background and aim: High salt (NaCl; HS) intake induces oxidative stress and inflammation leading to endothelial dysfunction and atherosclerosis. However, data on the salt-induced immunomodulatory effects in the earliest phase of salt loading, are scarce. This study aimed to assess the effects of short-term HS diet on the peripheral blood leukocytes (PBL) activation status, Th17/Treg axis and vascular structure in healthy rats. Materials and Methods: Distribution of PBL subpopulations and surface expression of integrins were determined using FACS in Sprague-Dawley rats on 0.4% NaCl diet (LS group) or 4% NaCl diet (HS group) for 7 days. Vascular tissue samples were processed for quantitative rtPCR, and total proteome analysis, while blood samples were analysed with multiplex immunoassay. Results: Frequency of granulocytes was increased, while the frequency of lymphocytes was reduced in HS group. Expression of total CD11b/c in granulocytes and CD3 lymphocytes and expression of CD11a was significantly increased in HS group. CD49d expression on all PBL subsets was significantly decreased. Tnf and Il1a gene had a central role in regulating cytokine, chemokine and cell adhesion molecule gene expression, as determined by total transcriptome network analysis. HS diet had distinct, lymphoid compartment-specific effects on leukocyte subpopulations, which could be attributed to the increased expression of salt-sensitive SGK-1 kinase Vascular upregulation of Il-6 gene was accompanied by increased TNFalpha serum concentration HS diet. HS diet induced tissue remodelling. Conclusions: Present results suggest that short-term HS diet alters leukocytes' activation status involving Th17/Treg axis dysregulation and promotes vascular low-grade inflammation and vascular wall remodelling.

NKG2D REGULATES THE EFFECTOR FUNCTIONS OF CD4+ T CELLS AND SHAPES THE INFLAMMATORY PROGRAMS OF MICROGLIA AND MONOCYTE-DERIVED CELLS DURING NEUROINFLAMMATION

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The role of danger signals in driving aberrant immune responses during multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), a mouse model thereof, remains largely unclear. We performed single cell transcriptome analysis of splenic versus CNS CD4$^+$ T cells at disease peak revealing a transcriptional continuum within CNS CD4$^+$ T cells with distribution skewed by the expression of key effector cytokines and activation markers. One prominent feature associated to CNS as compared to splenic CD4$^+$ T cells was the expression of innate receptors, particularly *Klrk1*, coding for Natural Killer Group 2, Member D (NKG2D), a key innate sensor of cellular danger signals. Moreover, expression of NKG2D ligands was detected in CNS-derived activated microglia and monocyte subsets. CNS derived antigen-specific CD4$^+$ T cells from mice with *Klrk1*-deficiency in the T cell compartment (*Klrk1*$^{\Delta CD4}$) were impaired in the production of inflammatory cytokines, particularly IFN-g and GM-CSF, as well as in the recruitment of inflammatory myeloid cells, which further displayed transcriptional changes associated with cellular activation and metabolism as compared to littermate controls (*Klrk1*^{FLOX}). Importantly, we could demonstrate that *Klrk1*$^{\Delta CD4}$ mice show significant resistance to EAE when compared to *Klrk1*^{FLOX}. Altogether, our findings suggest the role for the stress-sensing innate receptor NKG2D in the modulation of Th cell-mediated neuroinflammation.

THE ROLE OF CD16, NKG2D AND NCR1 ACTIVATING RECEPTORS IN NK CELL ACTIVITY

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Human NK cells express a large number of germline encoded activating and inhibitory receptors to sense their target cells. Rather than one dominant activating signal, it is believed that the balance of signals received CD16, NKG2D and NCR1 controls whether or not an NK cell gets activated.

Both NKG2D and NCR1 recognize stress-induced ligands and play an important role in the fight against different types of infections and tumors, whereas CD16 recognizes Fc tails of cell-bound antibodies and is responsible for induction of antibody-dependent cellular cytotoxicity (ADCC).

In this study, we investigated whether triple knockout mice can efficiently control tumors the killing of which is mediated by NK cells. As a model we used, B16 melanoma cell line which generates lung metastases upon intravenous injection, and RMA-S lymphoma cell line. Intravenous B16 melanoma inoculation resulted in prolonged survival of CD16-/-NKG2D-/-NCR1gfp/gfp mice compared to the wild type controls. This better control of the tumor is NK cell dependent and mediated via NCR1 receptor, as previously shown by our group. In case of intravenous RMA-S administration there were no survival differences between the groups.

In vivo mcmv infection of mixed bone marrow chimeras showed no differences in NK cell maturation and activation, while NK cells derived from CD16-/-NKG2D-/-NCR1gfp/gfp mice showed lower expression of CD107a, a degranulation marker, and higher granzyme B levels. These findings indicate that whereas CD16, NCR1 and NKG2D are important activating receptors, their deficiency causes only moderate defects in the NK cell compartment

ACTIVATION OF ARF GTPASES DURING MURINE CYTOMEGALOVIRUS INFECTION

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Arf proteins are small GTP-binding proteins (GTPases) that are one of the main regulators of endocytosis and intracellular trafficking. They function as molecular switches, meaning that they continuously cycle between active (GTP) and inactive (GDP) form. When activated they bind to the membranes of endosomal compartments where they recruit their effector molecules. We have previously shown that Arf GTPases have important role in pathogenesis of murine cytomegalovirus (MCMV) infection. While Arf1 and Arf6 are important for the establishment of MCMV infection, Arf3 and Arf4 have important role in biogenesis of virion assembly compartment. The aim of this study was to determine the degree of Arf GTPases activation during MCMV infection. For that purpose, Balb 3T3 cells were infected with Δm138 MCMV and at 0, 6, 16 and 30 hours post infection degree of Arf GTPases activation was determined by 3 different methods: subcellular fractionation, precipitation of Arf proteins with GGA3 PBD agarose beads and by analysing intracellular localization of Arf effector molecules. Our results have shown that the main effector molecules of Arf proteins are accumulated in the juxtanuclear area of MCMV infected cells with a pattern like that of the Arf proteins. Furthermore, the proportion of Arf proteins that are precipitated with GGA3 PBD agarose beads increases with progression of MCMV infection, suggesting that Arf proteins are overactivated during MCMV infection, although we did not observe an increase in membrane fraction of Arf proteins.

MORE MALIGNANT BREAST CANCER SUBTYPES INFLUENCE M2 POLARISATION OF MACROPHAGES AND NK CELL DYSFUNCTION

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INTRODUCTION: Breast cancer is the greatest threat to women's health and highly heterogeneous disease. Tumour cells induce the infiltration of various immune cells distributed in different sites and perform a variety of functions that have recently been proposed to predict clinical outcome. Numerous studies have confirmed that tumour-associated macrophages (TAMs) are important for increasing angiogenesis, tumour invasion, and suppressing immunity. TAMs could affect the impaired cytotoxicity of natural killer (NK) cells and induce a tolerogenic phenotype. The aim of this study was to investigate the phenotype and activation status of TAMs and NK cells and to understand their cell plasticity within the TME in different breast cancer subtypes. MATERIAL AND METHODS: Immunohistology was used to detect the presence and localization of CD68, CD163, and CD56 in paraffin-embedded normal and tumorous breast tissue sections. The abundance of NKG2A, NKG2C, and NKp46 in the population of NK cells and of CD80, CD86, and CD206 in suspensions of peripheral blood mononuclear cells was assessed by flow cytometry. The mRNA of cytolitic mediators in all subtypes of breast cancer was detected by quantitative RT -qPCR. RESULTS: The percentage of CD68+, IL163+, and CD56+ cells was significantly higher in triple-negative breast cancer. The frequency of NK cell activating receptors was decreased in all breast cancer subtypes, whereas the inhibitory receptor NKG2A was increased. The decreased frequency of CD80+ and CD83+ monocytes indicated an M2 polarisation status. Gene expression of cytolitic mediators at the local level was downregulated in luminal A and triple-negative breast cancer. CONCLUSION: Modulation of peripheral blood NK cell activity and tumour-infiltrating NK cells by M2 macrophages at local and systemic levels may be involved in the pathogenesis of more malignant breast cancer. Understanding the complex interaction complexity of TAMs and NK cell plasticity within the TME could improve new strategies to enhance NK-based immunotherapeutic approaches.

HUMAN THYMUS CONTAINS EPITHELIAL CELLS WITH STEM CELL FEATURES LONG AFTER BIRTH

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Thymus function regarding T lymphocyte development and selection is mainly considered to end at early age in human. Therefore when life saving cardiac surgery in children born with a complex heart disorders is needed, it is part of the procedure to perform thymectomy. Several groups have shown that thymus is active during the whole life, although it involutes at puberty and its function to keep the organism healthy decreases with age. Therefore the reactivation of the thymus either *in vivo* or by reassembling it *in vitro* from thymic epithelial stem cells would bring considerable benefits to patients with damaged thymus function either by age, injury or iatrogenically induced.

By means of immunohistochemistry and confocal microscopy methods we attempted to find cells from the human thymus with stem cell characteristics. Also we analysed thymi of children of different age, from newborn to 11 years old. We describe here a marked difference in histology sections of thymus from children of different ages and our results indicate that although there are specific non hematopoietic $CD45^-$ progenitor populations in postanatal human thymus, the aging is an important factor for cell recovery and possibly functionality. When cultured *in vitro* their ability to support lymphocyte differentiation depends strongly on the age of the patient.

Our work shows that in the human thymus there are rare populations of epithelial stem cells and although very rare they can be the initial population that can be isolated and used for the thymus regeneration.

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IN VIVO GROWTH INHIBITION AND ANTI-ANGIOGENIC EFFECTS OF RESVERATROL AND ITS NANOCRYSTALS ON SOLID TUMOR IN MICE

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Due to low toxicity and multiple mechanisms, natural compounds such as resveratrol could play a significant role in the treatment of cancers. Nevertheless, therapeutic use of resveratrol is limited by its physico-chemical and pharmacokinetic properties. To overcome its traits, we produced rasveratrol as nanocrystals suspension, as several formulations of flavonoid nanocrystals have shown enhanced pharmacological effects. The aim of this study was to investigate *in vivo* effects resveratrol and its nanocrystals on angiogenesis-dependent, fast-growing Ehrlich ascites tumor (EAT) in solid form, as well as potential toxicity caused by the interacion of nanocrystals with biological tissue through biochemical and histological changes of kidney and liver. Tumor was induced by subcutaneous injection of 1×10^6 EAT cells into the muscle tissue of the right hind leg of Swiss albino mice. Treatment of animals with EAT started on day 1 of the experiment by intratumoral injection of resveratrol or resveratrol nanocrystals at dose of 50 mg/kg every other day for 14 days. The results indicate that resveratrol and resveratrol nanocrystals have an inhibitory effect on tumor growth. Reduced microvessel density in the tumor, liver and kidney tissue confirms the anti-angiogenic potential of resveratrol *in vivo*. Antioxdant and anti-inflammatory effect is seen through reduced NO levels and elevated Arg1 levels in kidney tissue, as well as biochemical parameters in serum.

Based on the obtained results, we can conclude that resveratrol and its nanocrystals successfully inhibit the growth and angiogenesis of solid form of EAT and have protective effect on the liver and kidney.

ADVANCED CHARACTERIZATION OF IGG FORMULATIONS BY MULTI-DETECTION SIZE EXCLUSION CHROMATOGRAPHY

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Size Exclusion Chromatography (SEC) coupled with multi-detector consisting of refractive index (RI), UV/Vis absorbance, light scattering (LS) and intrinsic viscosity (IV) detectors has already been recognized as a valuable analytical technique for the characterization of macromolecules as well as monitoring of the process control, manufacturing and formulation of biotechnology products. It provides deep insight into sample's absolute molecular weight, molecular size (hydrodynamic size and radius of gyration), intrinsic viscosity, but also informations concerning structure, concentration, recovery, and potentially, absorption profile and compositional analysis.

The aim of this work was to investigate the possibilities of the multi-detection size exclusion chromatography in advanced characterization of IgG-based antivenoms obtained by different purification principles. The absolute molecular weight (Mw) of the sample peaks, their hydrodynamic radius (Rh) and polydispersity (Mw/Mn) were determined. Trace amounts of aggregates and the differences in molecular nature of the polymeric structures (dimer, trimer, etc.) were detected. The obtained absolute Mw values were in line with the expected ones for dimeric and monomeric IgG species. Polydispersity value of ~1 confirmed molecular homogeneity and symmetrical size distribution for monomers and dimers. On the contrary, the peaks that correspond to soluble aggregates showed higher polydispersity values, indicating their heterogenous composition. This methodology proved as promising and powerful technology for the quality control of therapeutic IgGs during the process development.

ESTABLISHING OF PREREQUISITES FOR THE COVID-19 CONVALESCENT PLASMA USAGE IN CROATIA

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In 2020, a worldwide spread of SARS-CoV-2 caused the global COVID-19 pandemic. Experience from previous outbreaks with other coronaviruses showed that convalescent sera contained neutralizing antibodies (NAbs) against the virus and that their use was beneficial to the treated patients. Although convalescent plasma therapy was considered generally beneficial, the scientific medical community lacks definitive proof of its efficacy coming from carefully designed clinical trials. The reasons for this can be found in its short-term usage only during epidemics caused by a new and insufficiently know pathogen, in a period when pathogen specific therapy and vaccines are lacking. During this period, methods for plasma neutralization potency determination are usually lacking or if they exist, they are neither standardized nor validated. All this results in the variability within different individual trials. Here we describe an approach of establishing antibody characterisation in emergent times which would, if followed, enable comparison of results from different studies. We established an assay of wild-type SARS-CoV-2 neutralisation, as the most relevant for antibody protectivity. To ensure the maximal reproducibility, we used a banking system of its biological components - a challenge virus, cells and an anti-SARS-CoV-2 antibody in-house standard, calibrated to the First WHO International Standard immediately upon its availability. Consequently, all collected serological data were retrospectively expressed in an internationally comparable way. The neutralising antibodies (NAbs) among convalescents ranged from 4 to 2869 IUmL-1, being in significant positive correlation to the disease severity. NAbs decline in convalescents was on average 1.4-fold in a one-month period. Heat-inactivation of sera samples resulted in a 2.3-fold decrease of NAb titres in comparison to the native sera, implying significant complement activating properties of SARS-CoV-2 specific antibodies. The monitoring of NAb titres in the sera of immunocompromised COVID-19 patients that lacked their own antibodies evidenced the successful transfusion of antibodies by the COVID-19 convalescent plasma units with NAb titres of 35 IUmL-1 or higher.

VENOM/ANTIVENOM DISTRIBUTION IN EXPERIMENTAL SHEEP MODEL

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In envenomation distribution of s.c. or i.m. injected venom into the interstitial space occurs. An absorption process, by blood or lymphatic capillaries, is necessary before it reaches the bloodstream. Viper venoms are mostly absorbed through the lymphatic system prior their delivery into the systemic circulation. Antivenoms constitute the mainstay in the snakebite envenoming therapy. There is a prevailing opinion that i.v. administration of antivenoms is more effective than i.m. application, based on the studies on venom/antivenom pharmacokinetics in the bloodstream. Recently, it was hypothesized that neutralization in the lymphatic system might be of great importance for clinical outcome. Therefore, need to reconsider (dis)advantages of each therapeutic principle has emerged by monitoring the antivenom's effect on venom levels in both body compartments. We established large animal model, using ovalbumin as a model protein, and developed procedures and surgical techniques for blood and lymph sampling as well as assays for ovalbumin quantification in these two body fluids. We aimed to perform a pharmacokinetic study of venom and antivenom in these two compartments using experimentally envenomed and treated sheep. Each animal received s.c. injected venom in the dose corresponding to the amount of venom injected in a typical envenoming. Antivenom was delivered i.m. as a bolus or i.v. as an infusion. Blood samples were collected from the jugular vein, and lymph by continuous drainage from ductus thoracicus. Venom and antivenom quantification has been performed by respective in-house ELISA assays. The preliminary results on venom/antivenom pharmacokinetic distribution will be presented.

LONGITUDINAL FOLLOWUP OF NEUTRALIZING CAPACITY AGAINST SARS-COV-2 IN NAIVE AND COVID-19 RECOVERED HEALTH CARE WORKERS FOLLOWING BNT162B2 VACCINATION

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The emergence of SARS-CoV-2 on the pandemic level needed prompt reaction of preventive measures. Therefore, first mRNA vaccine Comirnaty-BNT162b2 (BioNTech/Pfizer) was approved by the US FDA already in December 2020. Healthcare professionals, as SARS-CoV-2 front-line workers, were the first being vaccine administered in Croatia. Early studies on vaccinated individuals have largely focused on measuring total IgG antibodies without insight in their real protective potential. In our study we focused on measuring neutralizing capacity against SARS-CoV-2 induced by the vaccine. We also studied longevity of SARS-CoV-2 specific antibody response after vaccination in SARS-CoV-2 naïve subjects and one that previously experienced COVID-19 infection. Vaccinees achieved neutralizing titers comparable to neutralizing titers achieved during natural SARS-CoV-2 infection. <1% of SARS-CoV-2 naïve individuals did not responded to the two-dosage protocol vaccination by activation of humoral immunity. Neutralization titer gradually decreased over time but high neutralization activity was present still six months after in all vaccinees. Previous natural SARS-CoV-2 infection is associated with a more rapid and robust neutralizing response than individuals who were not infected. Maximal response was induced already with the single dose of vaccine and was not associated with the time elapsed from the COVID-19 recovery. Longevity of SARS-CoV-2 specific antibody response after vaccination was associated with better individual humoral response, regardless of prior exposure to SARS-CoV-2. Measuring short-term antibody titers alone may predict long-term immunity elicited by the vaccine.

GENETIC STABILITY OF RECOMBINANT VACCINE CANDIDATE F-RSV-MRV2 UNDER SELECTIVE PRESSURE

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Respiratory syncytial virus (RSV) usually causes mild cold-like symptoms, but in infants it can lead to serious diseases like bronchiolitis and pneumonia. Despite great efforts, not a single vaccine is available yet. One of the approaches in the development of RSV vaccines is the preparation of chimeric recombinant viruses expressing RSV glycoprotein (G) or fusion protein (F). Albeit, it has been shown that such constructs can be genetically unstable, resulting in mutation of inserted gene which can reduce or disable expression.

We prepared a recombinant virus, F-RSV-MRV2, which contains mumps virus as a vector with inserted F-RSV gene. The aim of this research was to investigate genetic stability of F-RSV-MRV2 under selective pressure of two cell lines (Vero and A549) and high and low multiplicity of infection (MOI). Analysis of variability of viral populations was studied by passaging virus stock for 10 passages in triplicate and subsequent sequencing by next-generation sequencing.

Our results show that viruses passaged in Vero accumulated mutations in greater numbers and at higher frequencies than viruses passaged in A549. However, all samples had similar number of mutations in the insert. Surprisingly, in all high MOI Vero samples deleterious mutations that would prevent the expression of the F-RSV appeared in the insert.

These results show that cell lines have different effects on genetic stability of viruses, while the influence of MOI was not prominent. Also, regular monitoring of the viral genome composition is required during the preparation of recombinant vaccine candidates that are produced in Vero cells.

IFN-γ IS A MAJOR MEDIATOR OF NEUROINFLAMMATION, BRAIN PATHOLOGY AND NEUROFUNCTIONAL DEFICITS FOLLOWING CONGENITAL CMV INFECTION

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Congenital human cytomegalovirus (cHCMV) infection of the brain is associated with a wide range of neurodevelopmental and cognitive sequelae. We are using infection of newborn mice with mouse cytomegalovirus (MCMV) as a reliable model that recapitulates many aspects of cHCMV infection, including virus dissemination to CNS, altered neurodevelopment, and sensorineural hearing loss. We have shown that mitigation of inflammation prevented alterations in cerebellar development, suggesting that host inflammatory factors are key drivers of neurodevelopmental defects. Here, we show that MCMV infection causes a dramatic increase in the expression of the microglia-derived chemokines CXCL9/CXCL10, which recruit NK and ILC1 cells into the brain in a CXCR3-dependent manner. By using archived human brain tissues of legally terminated cHCMV cases we confirmed inflammatory response in infected tissue. Surprisingly, brain-infiltrating innate immune cells not only were unable to control virus infection in the brain but also orchestrated pathological inflammatory responses, which lead to delays in cerebellar morphogenesis resulting in impaired motor coordination. Our results identify NK and ILC1 cells as the major mediators of immunopathology in response to virus infection in the developing CNS, which can be prevented by anti–IFN-γ antibodies. Furthermore, upon establishment of MCMV latency in the brain we observed lifelong adaptation of glial cells at the single-cell level to persistent infection. This was most pronounced in microglia which exhibited a prominent proinflammatory state and emergence of a new population of cells associated with latency. Finally, we show that IFN-γ contributes to persistent neuroinflammation in the brain of cCMV infected mice.

BONE MARROW STROMAL CELLS INHIBIT MONOCYTIC DIFFERENTIATION INDUCED BY LOW-DOSE CYTARABINE

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Bone marrow stromal (BMS) cells form niche that regulates both normal and malignant myeloid differentiation. Our previous studies showed that low-dose cytarabine (LDAC) and pyrimidine synthesis inhibitors, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) and brequinar, induce differentiation in acute myeloid leukemia (AML) cells by activating checkpoint kinase 1 (Chk1). The murine stromal cell line MS-5 in known to attenuate cytarabine-induced killing of AML cells, but the effects on cytarabine-induced differentiation are unknown. Present study investigates the effects of differentiation agents in a co-culture of BMS MS-5 and AML U937 cell lines. Results show that the presence of MS-5 cells reduces toxicity and S-phase arrest caused by differentiation agents in U937 cells. However, the expression of differentiation marker CD11b decreases only in U937 cells treated with LDAC and preliminary data from transwell experiments suggest that these effects do not depend on cell-to-cell contact. Both AICAr and high-dose cytarabine inhibit the growth of MS-5 cells, and AICAr induced their fibrocyte-like morphology. The addition of nucleosides completely prevents the effects of AICAr and brequinar on cell cycle and differentiation of AML cells, but has no effects on AICAr-induced changes in BMS cells. Preliminary data suggest that AICAr activates AMP-activated kinase (AMPK) and inhibits mammalian target of rapamycin (mTOR) in MS-5 cells. In conclusion, BMS decreases differentiation of AML cells in response to LDAC. AICAr induces fibrocyte-like changes of stroma independent of pyrimidine synthesis inhibition. These results suggest that mechanisms responsible for phenotypic changes induced by AICAr differ in AML and stromal cells.

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INNATE AND MUCOSAL IMMUNE RESPONSE IN COVID-19 PATIENTS

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The first line in the battle with respiratory viral infections is innate and mucosal immunity, which aims to prevent virus invasion and replication before adaptive immunity is developed. The contribution of NK cells, monocytes/macrophages, and mucosal immunity in COVID-19 immunopathogenesis is poorly understood. To better understand their role in COVID-19 pulmonary infection and inflammation, we analyzed the changes in the main elements of nasopharyngeal mucosal immunity and the dynamic changes in NK cells and monocytes in PBMCs of COVID-19 patients. Patients were hospitalized in our Clinique and classified as moderate to severe according to clinical parameters. Based on disease onset, all patients were stratified into early and late acute stages of the disease.

Data analysis showed increased expression of the M2 marker CD206 in monocytes of early and late acute patients. CD206 promotes the resolution of inflammation, healing, and homeostasis after infection. Expression of the activating receptor NKG2D was increased at a later stage of the disease. NKG2D positive NK cells are highly potent effector cells primed with stress response ligands, but prolonged activation of NK cells can lead to reduced responsiveness and anergy.

In nasopharyngeal swabs of COVID-19 patients, the concentrations of IP-10 and proinflammatory cytokine IL-6 were significantly higher in hospitalized compared to outpatients. The concentration of specific IgA antibodies, a marker of active mucosal immunity and the first line of defense against infection was detected in serum, nasopharyngeal swabs, and saliva, suggesting humoral response of the mucosal barrier in the oral and nasal cavity.

COLLECTION OF HIGHLY SPECIFIC SARS-COV-2 MONOCLONAL ANTIBODIES

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With the outbreak of COVID-19 in the early 2020, research, infection prevention and control has become of utmost importance. Here we present our collection of monoclonal antibodies (mAbs) directed against different SARS-CoV-2 proteins, including some of the most characterized structural and many non-structural proteins. Briefly, following recombinant SARS-CoV-2 protein production and purification, mice were immunized and used to develop monoclonal antibody producing hybridoma cell lines. We generated mAbs against all structural proteins, Spike, Nucleocapsid, Envelope and Membrane protein, various non-structural proteins (nsp1, nsp7, nsp8, nsp9, nsp10, nsp12 nsp16, including mAbs against nsp7-8 complex) and accessory factors (ORF3a, ORF7b, ORF8, ORF9b, ORF9c). All our antibodies are applicable in at least one of the following methods: ELISA, Western blot, flow cytometry and immunofluorescence. Continuous development of SARS-CoV-2 research tools will have a great impact on research directed towards understanding of SARS CoV-2 biology and pathogenesis which will finally contribute to the better control of the pandemic. This work has been fully supported by Croatian Science Foundation under the project IP-CORONA-04-2073.

THERAPEUTIC POTENTIAL OF RESVERATROL AND CISPLATIN ON MOUSE TUMOR GROWTH IN HYPERTHERMAL CONDITIONS

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The aim of this study was to investigate the therapeutic potential of resveratrol in combination with cisplatin on mouse tumor growth in hyperthermal conditions, i.e. the effect of this combination on inhibition of angiogenesis and tumor development, and on macrophage polarization. In addition, it was investigated whether a multimodal approach with hyperthermia and resveratrol could abolish cisplatin resistance in tumor cells through modulation of HDAC activity and heat shock proteins, contributing to direct toxicity on tumor cells. The tumor was induced by injecting 1×10^6 EAT cells subcutaneously (*sc*) into the thighs of Swiss albino mice. Mice were treated with resveratrol per os for five consecutive days beginning on day 2 after tumor injection and/or by injecting cisplatin intraperitoneally (*ip*) at a dose of 2.5 mg/kg on days 10 and 12 and a dose of 5 mg/kg on day 15. Immediately thereafter, mice were exposed to systematic hyperthermia for 15 min at a temperature of 41 °C. Results show that the administration of resveratrol and cisplatin with hyperthermia is an effective approach in tumor therapy. It successfully inhibits angiogenesis and tumor growth and modulates macrophage polarization to the M1 phenotype. Also, it abolishes tumor cells' cisplatin resistance through modulation of HDAC activity and concentration of HSP-70 and HSP-90, contributing to the increased lifespan of mice.

MUMPS VIRUS INFECTION OF GUINEA PIGS

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Mumps is an acute respiratory infection transmitted by aerosol and respiratory droplets, which can be prevented with a vaccine. It is caused by mumps virus (MuV), a non-segmented enveloped RNA virus belonging to the *Paramyxoviridae* family. In the last decade, we have encountered repeated outbreaks of mumps even in highly vaccinated populations, which calls into question the effectiveness of available vaccines. Animal models are needed to investigate the causes of outbreaks and to understand MuV pathogenesis. The search for an animal model for mumps has been going on for decades. Experimentally, only primates show symptoms of the disease after MuV infection. The lack of suitable small-animal model for studying pathogenesis and development of vaccines is one of the most serious obstacles to research progress. The aim of this research was to examine mumps-guinea pig interaction after intranasal infection (the natural route of MuV transmission) and evaluate the humoral and cellular immune responses in infected animals. We observed a significant viral replication in tissues of infected guinea pigs for up to 4 days post-infection and seroconversion in infected animals, without clinical signs of disease. Activation of cellular immunity was also recorded in the first 7 days after infection, which was more pronounced in bronchoalveolar lavage fluid than in whole blood. The obtained results clearly show that animals intranasally infected with MuV develop detectable antibody titers during 28 days. We also show for the first time that MuV-infected guinea pigs develop moderate to severe bronchopneumonia on day 28 post-infection. Our study demonstrates that guinea pigs are highly susceptible to MuV infection.

EFFICIENT AND SUSTAINABLE PLATFORM FOR PREPARATION OF A HIGH-QUALITY IMMUNOGLOBULIN G AS AN URGENT TREATMENT OPTION DURING EMERGING VIRUS OUTBREAKS

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During the pre-vaccine era of the COVID-19 pandemic convalescent plasma (CP) has once again emerged as a potential therapeutic form. But, there is a growing concern that variable concentration of neutralizing antibodies, present in CP which originates from different donors, affects its effectiveness. The drawback can be overcome through the downstream process of immunoglobulin fraction purification into a standardized product of improved safety and efficacy. All modern procedures are lengthy processes. They are also based on fractionation of large plasma quantities whose collection is not attainable during an epidemic. When outbreaks of infectious diseases are occurring more frequently, there is a need for a more sustainable production approach that would be goal-oriented towards assuring easily and readily available immunoglobulin of therapeutic relevance.

We proposed a simple strategy for the IgG preparation. It was designed as a small but scalable process to offer an immediately available treatment option that would simultaneously be harmonized with an increased CP availability over the viral outbreak time-course. The proof of concept was demonstrated on anti-SARS-CoV-2 plasma but is likely applicable to any other type depending on the current needs. Our procedure provided a high-quality IgG product of above the average recovery. It was proved free from IgA and IgM as mediators of adverse transfusion reactions, as well as of any other residual impurities. Undisturbed IgG subclass composition was accomplished. However, the fractionation principle affected the final product's capacity to neutralize SARS-CoV-2 infectivity. Decrease in neutralization potency significantly correlated with the IgM amount in the CP.

ACTIVATION OF GRANULOCYTES IN RESPONSE TO A HIGH PROTEIN DIET LEADS TO THE FORMATION OF NECROTIC LESIONS IN THE LIVER

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People are more and more known to follow extreme diets in their pursuit of healthy appearance and modern lifestyle. However, the acute impact of these diets on overall body homeostasis and organs regulating systemic metabolism is not well characterized. Here, we investigated the acute impact of several extreme diets on the liver in mice including diets rich in fats, cholesterol, protein, carbohydrates, etc. Most diets did not result with overt liver pathology after short-term (2 weeks) feeding. However, two weeks of feeding with a high protein diet (HPD) resulted in an acute increase of liver enzymes in the blood, indicative of liver damage. Histology revealed the formation of necrotic lesions in the liver which persisted for several weeks. Flow cytometric analysis of hepatic immune cell populations showed that HPD feeding induced activation of both macrophages and neutrophils. Neutralization of the pro-inflammatory cytokine IL-1β or depletion of macrophages with clodronate loaded liposomes or with genetic models did not fully abrogate liver necrosis. In contrast, depletion of neutrophils reduced HPD-induced hepatic inflammation. HPD-induced liver damage was associated with a strong increase of the cytokines IL-10 and IL-27, suggesting that anti-inflammatory mediators are activated in order to prevent nutrient-overload induced damage to the liver. In summary, our findings indicate that extreme diets with high protein content may lead to severe, acute damage to the liver as a consequence of metabolic stress and should therefore be implemented with caution.

RELATIVE HYPOGLYCEMIA INDUCED BY STRONG INFECTION ENHANCES INNATE ANTI-VIRAL IMMUNE CONTROL AND IS DEFECTIVE IN DIABETES

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Viral infection has a major impact on systemic metabolism, but much is unknown about how this benefits the host. Here we show that strong, non-lethal infection causes a transient lowering of blood glucose levels that benefits the innate anti-viral immune response. IFNγ produced by γδ T cells in concert with IL-1β directly stimulate pancreatic β-cells and enhance glucose-induced release of insulin. Infection-mediated hyperinsulinemia lessens hepatic glucose output by reducing glycogenolysis of hepatocytes during fasting. Glucose restriction enhances type-I interferon production by non-immune cells by curtailing lactate-mediated inhibition of IRF3 and NF-κB signaling. Glucose feeding or diabetes-induced hyperglycemia resulted in lower IFN-I production and higher viral titers upon strong infection. Our findings indicate that the reduction of blood sugar levels during strong infection is part of a physiological anti-viral response. This mechanism is defective in diabetes, leading to increased susceptibility to viral infection.

THE ROUTE OF ANTIGEN EXPOSURE IMPACTS THE IN VIVO RECALL CAPACITY OF SARS-COV-2 SPECIFIC MEMORY CD8 T CELLS

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Memory CD8 T cells play an important role in the protection against breakthrough infections with SARS-CoV-2. Whether the route of antigen exposure impacts these cells at a functional level is incompletely characterized. Here we compared the memory CD8 T cell response against a common SARS-CoV-2 epitope after vaccination, infection, or both. CD8 T cells demonstrated comparable functional capacity when restimulated directly ex vivo independent of the route of immunization. However, clonal analysis showed that vaccination resulted in a narrower scope than infection alone or in combination with vaccination, especially at the TCR-Vβ level. Importantly, in a unique in vivo recall model, memory CD8 T cells isolated from infected individuals showed equal proliferative capacity but secreted less cytokines compared to those from vaccinated people. This defect was reverted when infected individuals had also been vaccinated. Our findings shed more light on the differences in susceptibility to reinfection after infection and vaccination and may explain why a combination provides the best protection.

ROLE OF NUTRITION IN ALLERGY PREVENTION IN URBAN AND RURAL AREAS OF CROATIA

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Introduction: Nutritional habits have been associated with differing allergy risk. The aim of this study was to explore the role of the frequency of consuming different food in the urban (city of Zagreb) and rural (Natural park Lonjsko polje) in development of allergic symptoms. Methods: Schoolchildren aged 6-13 years old, from the city of Zagreb and Lonjsko polje, participated in the study. The prevalence of allergic symptoms, as well as the frequency of consuming different food (fresh green vegetables, raw green vegetables, fruits, fruit juice, meat, fish, sparking drinks and hamburgers), were assessed using the International Study of Allergy and Asthma in Children (ISAAC) questionnaire. The association of allergic symptoms (asthma, allergic rhinitis (AR) and atopic dermatitis (AD)) and food was assessed using logistic regression, based on the odds ratio (OR) and 95% confidence interval (CI). Results: 1741 children participated in the study, 885 girls and 856 boys, 648 from Lonjsko polje and 1093 from the city of Zagreb. In the city of Zagreb, consumption of raw green vegetables was associated with the lower risk of wheezing during last 12 months (OR 0.751, 95% 0.567-0.994, p=0.044), while in the area of Lonjsko polje meat consumption was associated with the lower risk of wheezing during last 12 months (OR 0.025, 95% CI 0.001-0.025, p=0.012). Consumtion of sparkling drinks was a risk factor for wheezing ever-in-a-lifetime in Lonjsko polje (OR 0.767, 95% CI 0.631-0.934, p=0.008). In the city of Zagreb, consumption of raw (OR 0.759, 95% CI 0.596-0.965 p=0.024) and cooked green vegetables (OR 0.751, 95% CI 0.569-0.991, p=0.043) were associated with the lower risk of AR symptoms during last 12 months. In Lonjsko polje, meat consumption was associated with the lower risk of diagnosed AR (OR 0.594, 95% CI 0.424-0.833, p=0.002). Consumption of hamburgers in Lonjsko polje was associated with the increased risk of AR symptoms ever-in-a-lifetime OR 1.226, 95% CI 1.039-1.446, p=0.015, during last 12 months (OR 1.226, 95% CI 1.039-1.446, p=0.015) and diagnosed AR (OR 1.351, 95% CI 1.003-1.819, p=0.048). In the city of Zagreb, consumption of cooked green vegetables was associated with lower risk of AD symptoms during last 12 months (OR 0.833, 95% CI 0.697-0.995, p=0.043). Conclusion: Our study showed raw and cooked green vegetables to be associated with lower risk of the symptoms of asthma, AR and AD (current activity of the disease) in the urban area. Fast food consumption was a risk factor for AR symptoms and asthma in the rural area. Green vegetables consumption should be encouraged in allergic patients in order to control the symptoms. Fast food consumption should be avoided in order to prevent the diseases.

NEW GENERATION VACCINE ADJUVANTS BASED ON PEPTIDE HYDROGEL WITH BUILT-IN DESMURAMYLPEPTIDE IMMUNOPOTENTIATORS: PREPARATION AND IN VIVO EVALUATION

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Adjuvants are essential for enhancing vaccine potency by improvement of the humoral and cell-mediated immune response to vaccine antigens. Adjuvants of new generation are adjuvant systems composed of various combinations of classical adjuvants designed to adjust the adaptive immune responses against pathogens. The challenge for this strategy is to define an effective and safe formulation in which individual components can synergize with one another to elicit a more robust immune response. Self-assembling peptides have a great potential for applications in bionanotechnology and it has been shown that certain peptides possess immunostimulatory activity. Muramyl dipeptide (MDP) is a synthetic immunoactive peptide consisting of N-acetyl muramic acid attached to a dipeptide L-Ala-D-isoGln. It was first identified in bacterial cell wall peptidoglycan as the smallest fragment possessing adjuvant activity. Desmuramylpeptides have been extensively studied in an attempt to increase adjuvant activity and boost the immune response effectively for clinical use in the treatment of cancer and other diseases. We report here the preparation and characterization of adjuvant system based on supramolecular hydrogel of a self-assembling tripeptide Ac-L-Phe-L-Phe-L-Ala-NH2 with built-in desmuramylpeptide immunopotentiators. Recently, a series of novel acyl tripeptides mimicking MDP were identified as potent nanomolar NOD2 agonists. The most potent derivatives were incorporated into hydrogel and tested in the mouse model of adjuvancy. The obtained results show that adjuvant system based on a hydrogel of a self-assembling tripeptide Ac-L-Phe-L-Phe-L-Ala-NH2 with built-in desmuramylpeptides elicits stronger specific immune response in comparison with antigen and adjuvant alone.

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