Baltic-Polish School of Immunology 2025

BPSI 2025
VILNIUS 12-13 May
Baltic-Polish School of Immunology

Lietuvos imunologijos draugija • Lithuanian Society for Immunology

ABSTRACT BOOK

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ABSTRACT BOOK

Baltic-Polish School of Immunology (BPSI 2025)

Vilnius University Life Sciences Center,

May 12-13, 2025

Cover photo was kindly donated by MB R. Davidonio studija

Foreword

The Baltic-Polish School of Immunology (BPSI 2025) is organized by the Lithuanian Society of Immunology in cooperation with Baltic and Polish Immunological Societies and kindly supported by the European Federation of Immunological Societies (EFIS) and European Journal of Immunology (EJI). Over 100 participants are registered to the BPSI 2025. Thanks to EFIS-EJI support, 26 travel grants for early-career researchers are provided.
The BPSI 2025 aims to promote regional collaboration of immunologists with a particular focus to a dialogue between researchers and medical doctors, between young and experienced immunologists.
The regional Schools of Immunology are expected to become traditional events organized every year in a different neighboring country. This trend started in 2023 with the ABC7 Summer School in Tallinn (Estonia) followed by the School of Immunology "50 Shades of Immunology" in 2024 in Bialystok (Poland).
The scientific program of BPSI 2025 covers 4 broad thematic areas: *Host-pathogen interaction, Inborn errors of immunity, Sterile inflammation and autoimmunity, Tumor immunology*. The lectures are given by experts from different European countries, while early career researchers are presenting posters and short talks. In this electronic issue, the abstracts of 21 lecture and 66 poster presentations are published.

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Lithuanian Society for Immunology
Co-organizers:
Vilnius University (VU) Life Sciences Center
Estonian Society for Immunology and Allergology
Latvian Association of Immunologists
Polish Society for Fundamental and Clinical Immunology
National Cancer Institute

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We stand with Ukraine

SCIENTIFIC PROGRAM

Monday, May 12

9:00-9:50 Registration, welcome coffee
9:50-10:00 Opening
10:00-10:40 **Keynote lecture**
The causative role of Epstein-Barr virus in multiple sclerosis.
Christian Münz (University of Zurich, Switzerland)

10:40-13:40 **Session 1: Host-pathogen interaction**
Moderators: Aurelija Žvirblienė and Maciej Kurpisz

10:40-13:00 Plenary lectures
10:40-11:10 To fragment or fuse? Mitochondrial architecture decides the niche for pathogenic survival.
Bhupesh K Prusty (Rīga Stradiņš University, Latvia)
11:10-11:40 Why we get sick; On the immunology of sickness metabolism.
Felix M. Wensveen (University of Rijeka, Croatia)
11:40-12:10 Coffee break and poster viewing
12:10-12:40 Post-pandemic increase in *S. pyogenes* infections: a role of herd trained immunity.
Janusz Marcinkiewicz (University of Agriculture, Krakow, Poland)
12:40-13:00 Clinical features and outcomes of multisystem inflammatory syndrome in children.
Inga Ivaškevičienė (Vilnius University, Lithuania)
13:00-13:10 Presentation of *Ateities Biomedicinos Fondas (Future Biomedicine Foundation)*
13:10-13:40 Short talks
13:40-14:30 Lunch and poster viewing

14:30-15:30 **Round-table discussion** "Interdisciplinarity and hyperspecialization in science: how to keep the balance?"
Moderator: science journalist Elizabet Beržanskytė (Lithuania)

15:30-18:40 **Session 2: Inborn errors of immunity**
Moderators: Kai Kisand and Martynas Simanavičius

15:30-18:10 Plenary lectures
15:30-16:00 Hipogammaglobulinemia without limits: SID and PID crossroad.
Ewa Więsik-Szewczyk (Military Institute of Medicine, Warsaw, Poland)
16:00-16:30 Many faces of primary immunodeficiencies: guess what? (an interactive lecture).
Brigita Gradauskienė (Lithuanian University of Health Sciences, Kaunas, Lithuania)
16:30-17:00 Coffee break and poster viewing
17:00-17:30 Special considerations for HSCT in patients with inborn errors of immunity.
Manfred Hönig (University Hospital Ulm. Germany)
17:30-17:50 CARD11 gene: Function and alterations.
Anastasia Bondarenko (International European University, Kyiv, Ukraine)
17:50-18:10 Newborn screening for SCID in Latvia: first results.
Natalja Kurjāne (Rīga Stradiņš University, Latvia)
18:10-18:40 Short talks
18:50 Bus to the city center
19:30-22:00 Welcome reception at the "Arkangelo Konferencijų ir Meno Centras (AKMC)", Maironio str. 11. Concert of Future Cello *(Justas Kulikauskas)*

Tuesday, May 13

9:00-12:50 **Session 3: Sterile inflammation and autoimmunity**
Moderators: Agata Mlynska and Felix Wensveen

9:00-12:10 Plenary lectures

9:00-9:30 The persistence and plasticity of (regulatory) T cells: lessons from autoimmune inflammation and tumor microenvironments.
Femke van Wijk (Utrecht University, the Netherlands)

9:30-10:00 Cross-talks between the immune and DNA repair systems.
Nelson Gekara (University of Freiburg, Germany)

10:00-10:30 Long COVID and autoimmunity: transfer of IgG from long COVID patients induces symptomology in mice
Hung-Jen Chen (Amsterdam University Medical Centers, the Netherlands)

10:30-11:00 Coffee break and poster viewing

11:00-11:30 Inflammation and reproductive health.
Maciej Kurpisz (Institute of Human Genetics, Poznan, Poland)

11:30-11:50 Cytokine autoantibodies: friends or foes?
Kai Kisand (University of Tartu, Estonia)

11:50-12:10 Baby-led weaning complementary foods and food allergy development among Ukrainian mothers and babies after six months of age.
Olena Sharikadze (Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine)

12:10-12:20 Single cell immune profiling
Aistė Vitkūnaitė (Linea Libera)

12:20-12:50 Short talks

12:50-14:00 Lunch and poster viewing

14:00-16:00 **Session 4: Tumor immunology**
Moderators: Natalja Kurjane and Jan Krasko

14:00-15:30 Plenary lectures

14:00-14:30 Neutrophils in cancer: state of the art.
Sven Brandau (University Hospital Essen, Germany)

14:30-15:00 Tumor microenvironment - key to successful management of cancer-induced immunosuppression
Agata Mlynska (National Cancer Institute, Vilnius, Lithuania)

15:00-15:30 The role of microbiota in anti-tumor immune response
Julia Shvets (Taras Shevchenko National University of Kyiv, Ukraine)

15:30-16:00 Short talks

16:00-16:30 Coffee break

16:30-17:15 Awards for the best posters/short talks, interactive feedback from the participants, closing remarks

17:30 Bus to the city center

18:00-19:30 Guided tour to Vilnius old-town

ABSTRACT OF A KEY LECTURE

THE CAUSATIVE ROLE OF THE EPSTEIN-BARR VIRUS IN MULTIPLE SCLEROSIS

Christian Münz

Institute of Experimental Immunology, University of Zürich, Switzerland

The Epstein Barr virus (EBV) establishes persistent infection in nearly all human adults. In a small subset of these, EBV is associated with lymphomas and carcinomas, as well as some autoimmune diseases. Primarily, EBV has been suggested to initiate the prodromal phase of multiple sclerosis (MS), a demyelinating autoimmune disease of the central nervous system (CNS). Our studies could show that EBV specific immune control that safeguards the healthy carrier status relies on early differentiated CD27+CD8+ T cells. In tissues, like mucosal secondary lymphoid tissues, in which T cells are further differentiated to tissue resident memory T cells (Trm), EBV specific immune control is less potent. This probably also applies to the CNS into which EBV infection drives virus differentiated T-bet+ memory B cells to recruit and restimulate inflammatory lymphocyte infiltrates. Thus, EBV infection spreads in memory B cells to tissues in which CD8+ T cell mediated immune control is less efficient due to terminal Trm differentiation. This might also apply to the CNS, causing immune pathology that results in MS.

***Prof. Christian Münz** is a Professor and Co-director of the Institute of Experimental Immunology at the University of Zürich, Switzerland. He is known for his pioneering work in viral immunobiology and tumor immunology, especially in relation to Epstein-Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV). Prof. Münz's team works on developing the immunotherapies and vaccination strategies to restore immune function in patients with virus-associated malignancies. His research team has characterized protective antigens of EBV, which are now widely used in current vaccine candidates. He extensively investigates immune mechanisms related to dendritic cell, NK cell, and T cell response, exploring antigen processing via autophagy and developing immunotherapies for virus-associated cancers, and providing insights how a comprehensive immune control could be re-established in cancer patients through vaccination. Prof. Münz has notable awards, including the Burroughs Welcome Fund Investigators in Pathogenesis of Infectious Disease Award and Sobek Award, recognizing his contributions to infectious disease pathogenesis and tumor immunology.*

ABSTRACTS OF PLENARY LECTURES

1. HOST-PATHOGEN INTERACTION

TO FRAGMENT OR FUSE? MITOCHONDRIAL ARCHITECTURE DECIDES THE NICHE FOR PATHOGENIC SURVIVAL

Bhupesh Kumar Prusty

Institute of Microbiology and Virology, Rīga Stradiņš University, Riga, Latvia

We have learned to understand the pathomechanism of infectious diseases by linking them to specific pathogens (the one pathogen-one disease model). However, in reality, we exist in a nearly symbiotic relationship. It is entirely possible for multiple pathogens, some of which reside within our cells, to interact and influence each other, ultimately shaping the fate of the affected cell. This talk will explore the intriguing world of the intracellular obligate bacterium Chlamydia and the common DNA virus from the human herpesvirus family, HHV-6, along with the essential role of mitochondrial architecture in the life cycle of these distinct pathogens.

Prof. Bhupesh K. Prusty *is a Professor of Science at Rīga Stradiņš University, Riga, Latvia, where he leads a laboratory dedicated to studying the role of viral infections in post-viral chronic illnesses and autoimmune diseases. Prof. Prusty began his academic journey in rural India and earned a prestigious doctoral fellowship from the Council of Scientific and Industrial Research (CSIR), India. His Ph.D. research at the Institute of Cytology and Preventive Oncology has been focused on the role of human papillomaviruses (HPVs) in cervical cancer, earning him multiple awards, including the Young Scientist Award from the Indian Science Congress and the Shakuntala Amir Chand Prize from the Indian Council of Medical Research. He conducted postdoctoral research at the German Cancer Research Center (DKFZ) in the laboratory of Nobel Laureate Prof. Harald zur Hausen. He later established his independent research group at Julius-Maximilians-University of Würzburg, where he earned recognition for his groundbreaking studies on human herpesvirus 6 (HHV-6) pathophysiology. His work has been supported by numerous international grants and awards, such as the prestigious Experiment! research grant from Volkswagen Stiftung and the Ramsay Research Award from Solve ME/CFS Initiative. After receiving his habilitation in Virology from the Julius-Maximilians-University of Würzburg, Prof. Prusty joined Rīga Stradiņš University and opened here a new laboratory to study the pathophysiology of latent viral infections.*

WHY WE GET SICK: ON THE IMMUNOLOGY OF SICKNESS METABOLISM

Felix M. Wensveen

University of Rijeka, Faculty of Medicine, Croatia

When we get sick, we feel miserable. We get a temperature, we feel weak and we just want to lay in bed. We experience this as a pathology. After all, how can feeling bad be good? But in fact, the alterations to our physiological state in response to infection are the result of a carefully regulated set of metabolic changes mediated by the immune system. Its purpose is to generate a metabolic environment in which the body is optimally able to fight infection, while denying pathogens vital nutrients for their replication. Infection-induced metabolic changes, also known as sickness metabolism, depend on tissue-specific interactions between the immune system and organs involved in regulation of systemic homeostasis. Alterations to homeostatic set points leads to altered production and uptake of nutrients in circulation, which modifies the metabolic rate of key organs. This is what we experience as being sick. Surprisingly, whereas we are all familiar with being sick, the underlying mechanisms are only now starting to be understood. In this presentation I will provide some new insights into how the immune system modulates systemic physiology following viral infection and how this benefits the anti-pathogenic response. In addition, I will explain how these mechanisms are chronically activated in obesity and thus contribute to the development of metabolic disease.

Prof. Felix M. Wensveen *is a Professor at the Department for Histology and Embryology at the Faculty of Medicine of the University of Rijeka, Croatia. He is a member of EFIS Vaccine Task Force and the Past-President of the Croatian Immunological Society. He received his PhD in 2010 from the University of Amsterdam. Following his doctoral studies, he completed his postdoctoral training at the University of Rijeka and then established his research group in 2015, expanding his expertise initially in NK cell biology and later transitioning to systemic immunometabolism (sickness metabolism). Currently, Prof. Wensveen investigates sickness metabolism of the liver and the endocrine systems. His work has provided important insights with therapeutic potential for both metabolic and infectious diseases. In recognition of his research, Prof. Wensveen received the prestigious EFIS Eastern Star Award in 2022.*

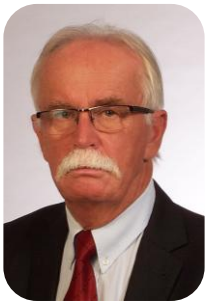
POST-PANDEMIC INCREASE IN S. PYOGENES INFECTIONS: A ROLE OF HERD TRAINED IMMUNITY

Janusz Marcinkiewicz

Faculty of Veterinary Medicine, University of Agriculture, Kraków, Poland

Herd Trained Immunity - the missing phenomenon of human immunity. In this presentation the data and opinions which support this idea will be discussed.

To wit: there is an unexpected global increase in the incidence of some infectious diseases observed since the ending of the COVID-19 pandemic. Various factors might contribute to this phenomenon (e.g. new more virulent pathogens). However, it could be hypothesized that primarily this increase results from the long term isolation of the majority of people during the global pandemic lockdown resulting in an extreme reduction of contact with environmental human microbiota. This, in turn, led to a silencing state of the body's defense systems, including a decline of the pre-pandemic trained immunity (innate memory) that persists only for weeks-to-months after exposure. This decrease in trained immunity may be especially important for morbidity of infectious diseases without currently available vaccines, such as scarlet fever and invasive Group A *Streptococcus pyogenes* (GAS) infections, primarily streptococcal toxic shock syndrome (STSS). Moreover, it has been resulted in a surge of the human reservoir of *S. pyogenes*. Therefore, it might be speculated that trained innate immunity within an entire population can lead to the development of Herd Trained Immunity (HTI), the novel coined medical term. HTI can supplement classical antigen specific herd immunity (B and T cells memory) and plays a key role in preventing various infectious diseases, including invasive GAS infections. Much as the global HTI has been overthrown during the COVID-19 pandemic, but it will be restored shortly.



Prof. Janusz Marcinkiewicz *worked at the Department of Immunology at Jagiellonian University Medical College (UJCM) from 1975 until 2024, leading this department from 1999 to 2022. Since 2024, he is affiliated with the Faculty of Veterinary Medicine at the University of Agriculture in Kraków. Prof. Marcinkiewicz is recognized on the 2024 Stanford/Elsevier prestigious list of the World's Top 2% Scientists for his outstanding contributions to immunology. His research is focussed on innate immunity and inflammation. His pioneering work includes the discovery of selective suppression of Th1-type cytokine synthesis by prostaglandins and contribution to the identification of nitric oxide (NO) as a key regulator of immune responses. His research also revealed the hyperinflammatory response of biofilm-stimulated macrophages, which has implications for understanding chronic infections. He was the first to show the role of herd trained immunity in defending against bacterial infections, that are not protected by antigen-specific adaptive immunity. As former President of the Polish Society of Experimental and Clinical Immunology (2008–2014), Prof. Marcinkiewicz actively promoted immunology research in Poland. His extensive career has established him as a respected mentor and leader in studying immune mechanisms in health and disease.*

CLINICAL FEATURES AND OUTCOMES OF MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C)

Inga Ivaškevičienė

Clinic of Children's Diseases, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Lithuania

Multisystem Inflammatory Syndrome in Children (MIS-C) is a rare but potentially severe hyperinflammatory condition associated with SARS-CoV-2 infection, typically occurring after the acute phase of COVID-19.
While the pathophysiology of MIS-C is not well understood, it is hypothesized that the syndrome arises from a dysregulated immune response to SARS-CoV-2.
MIS-C primarily affects children and adolescents, with the majority of cases occurring in those aged 5 to 11 years. Clinically, MIS-C is characterized by systemic inflammation that involves multiple organ systems, including the cardiovascular, gastrointestinal, mucocutaneous, renal, and neurological systems. Common presenting symptoms include persistent fever, abdominal pain, diarrhea, rash, conjunctivitis, mucous membrane involvement, respiratory symptoms, and lymphadenopathy. Laboratory findings often reveal markedly elevated inflammatory markers, such as C-reactive protein (CRP), interleukin-6 and procalcitonin. Additionally, other findings may include elevated cardiac biomarkers, indicative of myocardial involvement, and coagulopathy.
While the majority of affected children respond well to treatment and recover fully, MIS-C can lead to severe, life-threatening complications. These include myocarditis, shock, acute respiratory distress, and multi-organ failure, all of which require urgent medical intervention. Immunomodulatory therapies, such as intravenous immunoglobulin (IVIG) and corticosteroids, have shown efficacy in mitigating the inflammatory response and improving clinical outcomes. However, delayed or inadequate treatment can result in long-term sequelae, particularly related to cardiac dysfunction, and in some cases, can be fatal. Early recognition and prompt intervention are critical for improving patient outcomes.

Dr. Inga Ivaškevičienė *is the Head of the Department of Paediatric Infectious Diseases at Vilnius University Hospital Santaros Klinikos since 2008. She is an expert in paediatric infectious diseases and immunology. She graduated at the Faculty of Medicine and completed her residency in the Department of Paediatric Diseases at Vilnius University. She further advanced her training with a two-year postgraduate course in paediatric infectious diseases at the University of Oxford (2009-2010). Since 2007, Dr. Ivaškevičienė has built her academic career at the Faculty of Medicine, Vilnius University, starting as a junior researcher and advancing to her current role as an Associate Professor. She is actively involved in numerous national and international scientific societies, contributing to research, monitoring and policy of paediatric infectious disease. She is a member of the Lithuanian Paediatric Society (LPD), the European Society for Paediatric Infectious Diseases (ESPID), and a member of the Group of Independent Experts of the National Immunoprophylaxis Programme at the Ministry of Health of Lithuania. She is also involved in the Paediatric Tuberculosis Network (pTBNet), the Central European Vaccination Awareness Group (CEVAG), and the Awareness of Influenza Strategies in Europe (RAISE) group.*

2. INBORN ERRORS OF IMMUNITY

HIPOGAMMAGLOBULINEMIA WITHOUT LIMITS: SID AND PID CROSSROAD

Ewa Więsik-Szewczyk

Military Institute of Medicine, Warsaw, Poland

A 50-year-old female presents with enteropathy refractory to treatment. She had mild hypogammaglobulinemia and was referred to an immunologist by a gastroenterologist as SID for immunoglobulin replacement.
Her disease started at the age of 18 with severe diarrhea, abdominal pain and significant weight loss. Over life she has never achieved remission. During repeated examinations no evidence was found for: autoimmune colitis, Crohn's disease, lymphoma, Whipple's disease, giardiasis, acute or chronic infection and food allergy. The main diagnoses were: celiac disease resistant to gluten-free diet (1999), lymphocytic and collagenous colitis (2014), atrophic gastritis (2014).
She received systemic corticosteroids, several lines of immunosuppression (mercaptopurine, azathioprine, thioguanine) and anti-TNFi (infliximab, adalimumab). The treatment was ineffective or complicated by severe side effects, autoimmune and inflammatory phenomena including severe ILD.
Other comorbidities included: central chorioretinopathy of the right eye, renal failure, severe dyselectrolitemia, osteoporosis, parotiditis. Her infections history before immunosuppressive therapy was unremarkable. During immunosuppression she suffered from pneumonia, *Clostridium diff.* infection and Salmonellosis.
Immunology work-up revealed, chronic lymphopenia, low IgG 245 mg/dl (700-1600)) high IgA 615 mg/dl (70-400), T CD4+ 134 c/ul (500-1300), T CD8+ 149 c/ul (280-900), NK 34 c/ul (90-630) and B (CD19+) 193 c/ul (120-400 k/ul). We performed genetic testing which identified a heterozygous pathogenic variant in STAT3 gene (p. Arg152Trp), present HLA-DQ2.5 haplotype and two rare variants in NOD2 gene (VUS).
After diagnosis she started tofacitinib as a targeted therapy.
To conclude if histological features of the enteropathy are atypical or in the occurrence of an unsatisfactory response to conventional therapy PIRDS should be considered.
Our case was unique due to relatively late onset of the disease, unusual constellation of symptoms without cytopenia, lymphadenopathy, coexisting celiac disease.

Dr. Ewa Więsik-Szewczyk *is the Head of Out-Patient Unit of Clinical Immunology, Military Institute of Medicine, National Research Institute, Poland. She is a leading clinical immunologist and rheumatologist. With over two decades of experience, her research and clinical expertise focus on the management of primary and secondary immunodeficiencies, autoimmune and autoinflammatory diseases, particularly in complex cases involving connective tissue diseases and pregnancy. Dr. Więsik-Szewczyk also participated in clinical trials aimed at optimizing treatments for various conditions, including rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus. Her academic career includes the Associate Professorship and teaching roles at Warsaw Medical University. She actively contributes to the field of clinical immunology as a member of the European Society of Primary Immunodeficiencies, the Clinical Immunology Society, Polish Society of Clinical and Experimental Immunology, advisory committees on ultra-rare diseases and systemic vasculitis. Dr. Więsik-Szewczyk co-organizes national conferences on immunology and rare diseases, promoting the translation of clinical insights into novel therapeutic approaches in immunology.*

MANY FACES OF PRIMARY IMMUNODEFICIENCIES: GUESS WHAT?

Brigita Gradauskienė

Department of Immunology and Allergology, Faculty of Medicine, Lithuanian University of Health Sciences

Primary immunodeficiencies, also known as inborn errors of immunity (IEI), are a group of more than 400 rare disorders that vary in severity and clinical picture. A properly functioning immune system protects the host from foreign substances (e.g. infection agents, abnormal cells, etc.) that are responsible for diseases. That is why IEI may cause increased susceptibility to various infections and certain conditions or disorders – autoinflammatory diseases, allergy, autoimmunity, bone marrow failure, and/or malignancy. The discovery of novel IEI demonstrates that distinct types of variants in the same gene may cause disparate clinical conditions. Undiagnosed cases of IEI, delayed diagnosis and/or delayed treatment contribute to the remarkable increase of morbidity and mortality in this group of patients. During an interactive case-based lecture, knowledge about primary immunodeficiencies will be enhanced and encourage participants to think outside the box.

Prof. Brigita Gradauskienė *is the Head of the Department of Immunology and Allergology at the Lithuanian University of Health Sciences in Kaunas, Lithuania. Since 1999, she has gained extensive experience as an allergologist and clinical immunologist at Kaunas Medical University Hospital. Prof. Gradauskienė has led and contributed to over 50 international multicenter clinical trials since 1996. Her research interests include autoimmune diseases, immunodeficiencies, allergic airway diseases, and oncology. An influential figure in her field, she is an active member of prestigious organizations such as the U.E.M.S. Section and Board of Allergology, the European Academy of Allergy and Clinical Immunology, the European Respiratory Society, and the Lithuanian Society of Pulmonology and Allergy. Prof. Gradauskienė's career reflects her commitment to integrating clinical practice with research, fostering national and international collaborations, and training the next generation of specialists in immunology and allergology.*

SPECIAL CONSIDERATIONS FOR HSCT IN PATIENTS WITH INBORN ERRORS OF IMMUNITY

Manfred Hönig

University Medical Center Ulm, Department of Pediatrics, Ulm, Germany

Hematopoietic stem cell transplantation (HSCT) in patients with inborn errors of immunity (IEI) looks back on a long and successful history pioneering many aspects of this procedure, which nowadays are state of the art in HSCT and have been transferred to other patient groups e.g. with malignant disease or hemoglobinopathies.
With a still increasing number of currently about 500 different IEIs, the understanding of the primary disease has become increasingly important for an individualized and patient centered therapeutic approach. This includes pretransplant considerations as the timing and the general indication for HSCT, diagnostic strategies to identify infectious agents or coping with inflammation caused by the primary disease. Donor choice and the intensity of conditioning needs to be adapted to individual constellations and center experience. Last but not least the interpretation of posttransplant outcome includes parameters as lineage specific chimerism and decisions need to be made on the necessity and continuation for immunomodulatory or -supportive therapies. The “ideal” strategy for each individual patient can therefore only be achieved in a multidisciplinary dialogue between physicians and scientists. Clinical examples will be given to illustrate this crosstalk.

Prof. Manfred Hönig *is a Senior Physician at the Clinics for Pediatrics and Adolescent Medicine at University Hospital Ulm, Germany. He is a pediatrician and immunologist specializing in pediatric hematology, oncology, and immunodeficiencies. He began his career as a Pediatric Hemato-Oncologist in 1999 and currently holds the position of Vice-Director of the Pediatric Department at Ulm Medical Center. Prof. Hönig is actively engaged in scientific research with a strong focus on severe combined immunodeficiency (SCID) and complex immune disorders, where he has significantly advanced therapeutic approaches, particularly through hematopoietic stem cell transplantation. He efficiently integrates these research-driven insights into clinical practice.*

CARD11 GENE: FUNCTION AND ALTERATIONS

Anastasiia Bondarenko[1,2], Olena Sharikadze[2], Ida Rumiantseva[3], Baerbel Keller[4,5], Svetlana Sharapova[6], Ganna Brudna[7], Andrii Budzyn[7], Oleksandr Lysytsia[7], Klaus Warnatz[4,5,8]

[1]International European University, Kyiv, Ukraine, [2]Shupyk National Healthcare University, Kyiv, Ukraine, [3]Municipal Non-Profit Enterprise City Children's Hospital No. 5 of the Zaporizhia City Council, Zaporizhia, Ukraine, [4]University of Freiburg, Department of Rheumatology and Clinical immunology, University Medical Center, Freiburg, Germany, [5]University of Freiburg, Center for Chronic Immunodeficiency, Medical Center, Freiburg Im Breisgau, Germany, [6]Belarussian Center for Pediatric Oncology, Hematology and Immunology, Reseach, Minsk, Belarus, [7]National Specialized Children's Hospital OHMATDYT, Kyiv, Ukraine, [8]University Hospital Zurich, Department of Immunology, Zurich, Switzerland

CARD11 is a protein which plays a crucial role in connecting antigen recognition with downstream activation of the NF-κβ pathway in lymphocytes. The mutations in CARD11 gene are associated with autosomal recessive combined immunodeficiency, autosomal dominant B-cell expansion with NFKB and T-cell anergy (BENTA) and autosomal dominant immunodeficiency with atopic disease.
Methods: Clinical observations, immunological investigation including serum Ig and lymphocytes subsets, PID Panel Genetic sequencing (Invitae), IkBa degradation, p65 phosphorylation in B and T cells on frozen PBMCs.
Results: We report 3 clinical cases of Ukrainian children with CARD11 deficiency all of them presented with severe recurrent erythroderma since 2-5 month of age, infectious syndrome from mild respiratory and skin infections to pneumonia and severe hypohammaglobulinaemia with minor abnormalities in lymphocyte subsets. In first girl PID Panel Genetic sequencing identified two variants in CARD11 gene, further IkBa degradation assay showed severely impaired CARD11 function upon PMA stimulation suggesting c.1145C>A (p.Ala382Asp) and c.922G>A (p.Asp308Asn) as LOF mutations. At 2,5 y.o. hematopoietic SCT was successfully performed with 300 days uneventful. In two other children, pathogenic variant c.88C>T (p.Arg30Trp) in CARD11 gene was identified in a boy and VUS c.155T>C (p.Ile52Thr) in a girl. Children receive immunoglobulin replacement therapy, antimicrobial prophylaxis, nutritional support and local skin care.
Conclusion. Genetic testing is crucial in confirming the diagnosis and determining the patient's management, since clinical manifestations are similar with different genetic variants in the same gene. More clinical observations are needed to understand the phenotypes of CARD11 deficiency and the effectiveness of different treatment approaches.

Prof. Anastasia Bondarenko *is the Head of the Department of Paediatrics, Immunology, Infectious and Rare Diseases at the European Medical School of the International European University in Kyiv, Ukraine. She is also a Professor in Dermatovenerology, Allergology, Clinical and Laboratory Immunology at Shupyk National Healthcare University of Ukraine. Prof. Bondarenko began her academic journey at the National Medical University in Kyiv, receiving her medical degree in 2000. She got her PhD in 2006 at Shupyk National Medical Academy, where she studied age-dependent aspects of meningitis in children. She later completed her Doctor of Medical Sciences degree, investigating primary immunodeficiency diagnostics and social support in children. With over two decades of experience, she had key teaching and research roles in paediatric infectious diseases and immunology fields, including the Assistant and Associate Professorships at Shupyk National Medical Academy. Prof. Bondarenko is a consultant in paediatrics, infectious and immunology-related diseases at Kyiv Children's Hospital #1. Her research interests include primary immunodeficiencies and the immunoprophylaxis of infectious diseases, solidifying her role as an expert in both academic and clinical paediatric immunology in Ukraine.*

NEWBORN SCREENING FOR SCID IN LATVIA: FIRST RESULTS

Natalja Kurjāne[1,3,4], Marija Rozevska[1,3], Svetlana Vorslova[2], Maija Konika[2], Agnese Vētra[1], Madara Auzenbaha[1,3]

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Objectives

Neonatal screening for severe combined immunodeficiency (SCID) started in Latvia on 1 April 2002. The aim of this study was to analyse the screening results during the first two years.

Materials and methods

Data on screening results were collected from the only newborn screening centre in Latvia, the Children's Clinical University Hospital. TREC (quantitative measurement of T cell receptor excision circles) and KREC (kappa-deleting recombination excision circles) were used for detection of T and B cell immunity deficiencies. Patients with a positive result after three tests were referred to an immunologist for in-depth immunological investigation and then, if necessary, to a geneticist for whole genome sequencing.

Results

During the two years of screening for SCID, 25,920 newborns were screened. TREC positive were 97, KREC positive were 128, inconclusive results were in 61 cases and both TREC and KREC positive were 6 infants. After further immunological investigation, two were diagnosed with DiGeorge syndrome and one with Jacobsen syndrome. One child died (without diagnosis).

Conclusions

The implementation of newborn screening using the TREC and KREC assays allowed early diagnosis of inborn errors of immunity, not only SCID, with the aim of early observation and treatment if necessary.

Prof. Natalja Kurjāne *is the Head of the Center of Clinical Immunology and Allergology established in 2023 at Pauls Stradiņš Clinical University Hospital in Riga, Latvia. She is also a Professor at the Department of Biology and Microbiology at Riga Stradiņš University and serves as a lead researcher at the Scientific Laboratory of Molecular Genetics. As the President of the Latvian Immunological Society, Prof. Kurjāne plays a pivotal role in advancing the field of immunology in Latvia. Prof. Kurjāne's research addresses a range of immunological disorders, with a particular focus on primary immunodeficiencies, predominantly antibody deficiencies, as well as autoimmune diseases, hereditary angioedema, and chronic spontaneous urticaria. Her work also encompasses autoinflammatory syndromes, contributing significant insights into both the fundamental mechanisms and clinical management of these conditions. Through her research, Prof. Kurjāne has established herself as a leading expert in Latvia's medical and scientific communities, advancing both academic knowledge and clinical practice in immunology.*

3. STERILE INFLAMMATION AND AUTOIMMUNITY

SPATIAL AND FUNCTIONAL REGULATION OF DENDRITIC CELLS DURING HEALTH AND DISEASE

Andreas Schlitzer

Biology of Inflammation, Life & Medical Sciences Institute, University of Bonn, Germany

Dendritic cells (DCs) form coordinated networks orchestrating inflammatory responses across tissues. Conventional DCs undergo transcriptomic, phenotypic, and functional changes upon activation, entering the mregDC (mature DCs enriched in immunoregulatory molecules) cell state. mregDCs have been described in a range of cancers. However, how mregDCs contribute to the induction and maintenance of human chronic inflammation have yet to be explored. To address this, we aimed to investigate mregDCs across a range of chronically inflamed human tissues, focusing on cervical lymph nodes (LNs). We performed scRNAseq and flow cytometry on healthy, acutely, chronically inflamed and cancer-associated human cervical (LNs) and identified a novel inflammatory mregDC (imregDC) cell state, found only within chronically inflamed LNs. imregDCs were defined by a unique cytokine expression profile (CXCL9, CXCL10, IL1B) and could be phenotypically distinguished from other mregDC by their elevated expression of CD1C, CD206, CD319, and CD274. Functionally, we found that IFNg signaling induces DC2s to enter the imregDC cell state and that this process can be abrogated by inhibition of JAK-STAT signaling. Using spatial transcriptomics, we observed LN imregDC residing within a specific chronic inflammatory niche, which was also enriched for inflammatory monocytes, NK cells and CD8+ T cells. Extrapolating our findings to other chronic inflammation-associated diseases, we similarly observed the emergence of imregDCs within the intestines of treatment-resistant Crohn's Disease patients. Similarly to the lymph node, imregDCs were found to reside in conserved chronic inflammation-associated spatial niches in the colon with equivalent transcriptional characteristics and cell type compositions. Collectively, these data highlight a previously unexplored role of mregDCs in human chronic inflammatory diseases and propose a conserved imregDC-populated inflammatory niche associated with resistance to therapy. These findings draw attention to future possibilities of shaping inflammatory trajectories via targeting of imregDCs and their niches, for example, via inhibition of JAK-STAT signaling.

Prof. Andreas Schlitzer *is the Head of the Department of Quantitative Systems Biology at the Life & Medical Sciences Institute of the University of Bonn, Germany. He studied Molecular Biology at the University of Marburg, then Immunology & Immunogenetics at the University of Manchester (UK) and completed his PhD in myeloid cell biology in 2008 at the University of Marburg. He received notable awards, including EFIS Bright Sparks Award in 2014 and the Postdoctoral Award from Robert Koch Foundation in 2016. His lab investigates the development, functional specialization and spatial organization of mononuclear phagocytes and their role in inflammation and immunity. His research contributes to understanding how local molecular cues govern the differentiation and function of these cells in both health and disease, with a particular focus on the transition from acute inflammatory conditions to chronic diseases, including idiopathic pulmonary fibrosis, inflammatory bowel disease, and psoriatic arthritis. Prof. Schlitzer is an Editorial board member of Frontiers in Immunology and a member of EFIS Study Group on "Innate immunity in sterile inflammation, autoimmunity, and their resolution".*

THE PERSISTENCE AND PLASTICITY OF (REGULATORY) T CELLS: LESSONS FROM AUTOIMMUNE INFLAMMATION AND TUMOR MICROENVIRONMENTS

Gerdien Mijnheer, Lisanne Lutter, Nila Hendrika Servaas, Jing Yao Leong, Alessandra Petrelli, Jorg van Loosdregt, Sebastiaan Vastert, Rob de Boer, Salvatore Albani, Jose Borghans, Aridaman Pandit, **Femke van Wijk**

University Medical Center Utrecht, Utrecht University, The Netherlands

Regulatory T cells (Tregs) play a crucial role in immune homeostasis and tissue adaptation. In autoimmune diseases like juvenile idiopathic arthritis (JIA), persistent antigen-driven T cell clones infiltrate affected tissues, yet the extent to which these clones persist across distinct sites remains unclear. Here, we performed CyTOF analysis and T cell receptor (TCR) sequencing to characterize the immune cell composition and clonal expansion of circulating and joint-derived Tregs and non-Tregs in JIA. We found striking similarities in immune infiltrates between affected joints within individual patients and observed a strong overlap in dominant T cell clones, particularly Tregs. These clones persisted over the course of relapsing-remitting disease, with some also detectable in circulation. Further analysis revealed high sequence similarity among TCRs, suggesting antigen-driven expansion and the presence of TCR clusters responsive to shared antigens. To further elucidate mechanisms of Treg adaptation, we re-analyzed publicly available bulk RNA-sequencing datasets, comparing Tregs from peripheral blood, homeostatic tissue, and inflammatory or tumor microenvironments. We identified shared and distinct transcriptional programs, with HOX genes playing a regulatory role in tissue adaptation. Both inflammatory and tumor-infiltrating Tregs displayed increased oxidative phosphorylation and expressed an effector gene signature. Key transcription factors, including BATF and VDR, were implicated in tissue adaptation, while FOXP3, HDGF, and NR3C2 were shared regulators in both inflamed and tumor tissues. These findings highlight the persistence and adaptability of Tregs in autoimmune and tumor settings, providing insights for therapeutic strategies targeting Treg function in inflammatory diseases and cancer

Prof. Femke van Wijk *is a Professor in Tissue Immunology at the Center for Translational Immunology at the University Medical Centre Utrecht (UMCU) in the Netherlands. Her team aims to elucidate peripheral and local T cell responses in health and inflammation and to translate these insights into tools for (pre-clinical) disease monitoring and therapeutic targeting in chronic inflammatory diseases. She takes a disease- and age-overarching approach to decipher common and specific pathogenic processes underlying different inflammatory conditions and one of her specific interests is early imprinting of disease. Since 2022, Prof. van Wijk has been the manager of research for the division of pediatrics at the Wilhelmina Children's Hospital. In this role, Prof. van Wijk is responsible for research policy and talent management within the division and is committed to empowering the next generation of clinical scientists. Since 2020, Prof. van Wijk has also served as the scientific director of the Federation of Clinical Immunology Societies (FOCIS) Center of Excellence (FCE). Prof. van Wijk is an advocate for a transition in science towards less ego, more inclusivity, greater diversity, and more impact, as reflected in her inaugural lecture of 2023, titled "The Great Reset." In 2023 she received the Athena award for outstanding female researchers from the Dutch Research Council.*

CROSS-TALKS BETWEEN THE IMMUNE AND DNA REPAIR SYSTEMS

Nelson Gekara

Universitätsklinikum Freiburg, Germany

The innate immune system, which orchestrates rapid responses to infections, and the DNA repair system, which safeguards genomic integrity and ensures the accurate transmission of genetic information, serve as primary defense mechanisms against both endogenous and exogenous threats. Dysregulation in either of these systems is central to many health disorders. It is increasingly becoming evident that these defense systems are highly interdependent and regulated by overlapping cellular components. In this talk, I will discuss some of our discoveries in this emerging field at the intersection of innate immunity and DNA repair. In the process, I will highlight how these defense system could be targeted simultaneously in the management of complex health conditions.

Prof. Nelson Gekara *is a Full Professor of Microbiology and Molecular Infection Immunology at the Medical Center of the University of Freiburg, Germany, and Stockholm University, Sweden. His research focus on understanding the regulation of the innate immune and DNA repair systems and exploring how these mechanisms can be manipulated to enhance health outcomes, particularly in infectious diseases, inflammation, and cancer. Prof. Gekara investigates the regulation of pattern recognition receptor signalling pathways and the implications of their dysregulation, which can lead to inflammation or weakened anti-microbial host defenses. His recent findings have elucidated how DNA damage and the ubiquitin system influence inflammatory responses, revealing complex interactions between immune and DNA repair systems. Prof. Gekara's career includes prominent positions at leading institutions, including the Helmholtz Center for Infection Research and Umea University, advancing the understanding of immune regulation and host-microbe interactions. His research bridges fundamental cell biology and medical science with a translational focus.*

LONG COVID AND AUTOIMMUNITY: TRANSFER OF IgG FROM LONG COVID PATIENTS INDUCES SYMPTOMOLOGY IN MICE

HJ Chen, B Appelman, HLDM Willemen, N Eijkelkamp, J den Dunnen

Center for Infection and Molecular Medicine, Amsterdam UMC. Center for Translational Immunology, University Medical Center Utrecht, the Netherlands

SARS-CoV-2 infections worldwide led to a surge in Long COVID cases, a post-infectious syndrome. It has been hypothesized that autoantibodies play a crucial role in the development of Long COVID and related syndromes, such as fibromyalgia and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). In this study, we tested this hypothesis by transferring IgG from Long COVID patients to mice. Using Glial Fibrillary Acidic Protein (GFAP) and type-I interferon (IFN) expression, we stratified patients into three Long COVID subgroups, each with unique plasma proteome and autoantibody targets. Two subgroups were characterized by increased plasma levels of GFAP and Nueorfilement Light chain and antibodies against constituents of the epidermis, or elevated leukocyte activation markers and antibodies against Transforming Growth Factor-β signaling. Remarkably, IgG transfer from the two subgroups induced pronounced and persistent sensory hypersensitivity with distinct kinetics. Conversely, IgG transfer from the third subgroup, which was characterized by enriched type-I IFN, striated muscle proteins, and anti-IFN antibodies, reduced locomotor activity in mice without affecting their motor coordination. These findings demonstrate that transfer of IgG from Long COVID patients to mice replicates disease symptoms, underscoring IgG's causative role in Long COVID pathogenesis. This work proposes a murine model that mirrors Long COVID's pathophysiological mechanisms, which may be used as a tool for screening and developing targeted therapeutics.

Dr. Hung-Jen Chen, *is a Postdoctoral researcher at the Center for Experimental and Molecular Medicine of Amsterdam University Medical Centers (AUMC's), the Netherlands. He completed his MD at National Taiwan University and received his PhD from AUMC under the supervision of Prof. Menno de Winther. With expertise in macrophage biology, immunometabolism, epigenetics, and Fc receptor signaling, Dr. Chen leads a project within the Dutch Long COVID Foundation, investigating immune dysregulation in Long COVID. His research seeks to clarify immune mechanisms in chronic post-viral syndromes, providing insights for potential therapeutic approaches.*

INFLAMMATION AND REPRODUCTIVE HEALTH

Maciej Kurpisz

Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

Basic elements of inflammatory reactions within reproductive tract/organs are: infectious agents (bacteria, viruses), leukocytes producing biological mediators (mainly cytokines), associated metabolic reactions including augmented secretion of reactive oxygen species (ROS) which in concerted action may transfer innate immunity reactions into adaptive ones creating antisperm antibodies (AsA) both in autoimmune as well as allogeneic scenario. Critical parameters for all listed elements (present in male reproductive tract) are available for: bacteria, leukocytes and antisperm antibodies while reference values for cytokines and pro/antioxidant balance have been still a matter of dispute. Vicious circle can be created through participation of infectious agents attacking biological membranes and triggering ROS release (NADP(H) membrane pump and mitochondrial superoxide anion) that through signal transduction may activate pro- and antioxidant compounds which may prevail in induction of transcription factors either stimulating antioxidants to quench initiated inflammatory reaction or in the contrary activating DNA sequences responsible for further transcription of cytokines closing the vicious circle". Cytokines may augment leukocyte chemotaxis and their activation status which may then downgrade the semen quality measured by conventional (sperm concentration, motility and morphology) and unconventional parameters (architecture of sperm biological membranes, mitochondrial potential and redox status, malonylodialdehyde concentration and DNA fragmentation). We have divided inflammatory reaction into initial phase (bacteria), developed phase (bacteria + leukocytes) and resolution phase (leukocytes alone) analyzing the mentioned parameters. Possible ways of treatment including targeted antibiotic therapy (based upon sensitivity assay), probiotics and antioxidants will be discussed including also selective anti-cytokine trapping."

Prof. Maciej Kurpisz *is the Head of the Department of Reproductive Biology and Stem Cells at the Institute of Human Genetics, Polish Academy of Sciences, in Poznań, Poland. Prof. Kurpisz and his team focus on the genetic and immunological underpinnings of male and couple infertility, particularly on developing diagnostic and therapeutic strategies for these conditions. His research advances the knowledge in reproductive immunology, aiming to unravel the genetic factors and immune processes that impact male infertility, including sperm function, redox balance, and inflammatory responses. Additionally, he explores new therapeutic approaches, such as probiotic treatments and innovative therapies for azoospermia. Since 1987, Prof. Kurpisz has been a licensed clinical andrologist at the Medical Center for Postgraduate Education in Warsaw. Over 35 years of clinical practice, he has introduced reproductive immunology in Poland, chairing the National Reproductive Immunology Group, and serving as President of the European Society for Reproductive Immunology (2002–2004). He also chaired the Polish Society for Fundamental and Clinical Immunology from 2021 to 2023. His work has earned him prestigious awards from the Polish Academy of Sciences and the Ministry of Health. He was awarded the title of Doctor Honoris Causa by Lviv Medical University and received the prestigious prize Polonia Restituta Officer's Cross for his contributions to Polish science.*

CYTOKINE AUTOANTIBODIES: FRIENDS OR FOES?

Kai Kisand

University of Tartu, Estonia

Cytokines are small proteins that play a critical role in immune system regulation, inflammation, and various physiological processes. Acting as signaling molecules, they mediate communication between cells to coordinate immune responses, including pro-inflammatory and anti-inflammatory actions. This balance is essential for maintaining health and combating infections, inflammation, trauma, and diseases such as cancer and autoimmune disorders.

Cytokine autoantibodies (AAbs), add another layer of complexity to immune regulation. While they may help modulate excessive cytokine activity in some contexts, their inappropriate production can lead to immune dysregulation and increased susceptibility to infections or autoimmune conditions. For example, AAbs against interferons or interleukins have been implicated in diseases like mycobacterial infections, COVID-19 and autoimmune pulmonary alveolar proteinosis. Thus, understanding the interplay between cytokines and their autoantibodies is crucial for advancing treatments for immune-mediated disorders and infections.

Prof. Kai Kisand *is a Research Professor in Cellular Immunology at the Institute of Biomedicine and Translational Medicine at the University of Tartu, Estonia. After receiving her MD and PhD from the University of Tartu and completing postdoctoral training at Uppsala University, Prof. Kisand has focused her research on molecular mechanisms of autoimmune diseases. She investigates monogenic autoimmunity and immune responses to SARS-CoV-2 and COVID-19 vaccines. Her work has been pivotal in demonstrating the role of Th17 cytokines in protecting epithelial surfaces from Candida infection and the importance of type I interferons in preventing severe COVID-19. Alongside her research, Prof. Kisand is deeply committed to teaching immunology to postgraduate students, supervising numerous MSc and PhD projects. She was the President of the Estonian Society of Immunology and Allergology (2001–2004 and 2015–2018). In 2022, her contributions to immunology were recognized with the Estonian Natural Science Award in Medicine.*

THE BLW COMPLEMENTARY FOODS AND FOOD ALLERGY DEVELOPMENT AMONG UKRAINIAN BABIES AFTER 6 MONTHS OF AGE

Olena Sharikadze

Shupyk National Healthcare University of Ukraine

The BLW method is new variant of complementary food introduction. This is method over the past two decades and is gaining popularity in Ukraine. At the same time, the prevalence of food allergy (FA) has been increasing, primarily among the child population. Infant nutrition, especially during the period of introduction of complementary foods after six months of age, is key for preventing the development of FA and the manifestation of atopic pathology.

The aim of this study was to evaluate the level of sensitization to food the main food allergens in the children on traditional and BLW variants feeding

Results. For the period 2023-2024 were examined 120 children who, along with breastfeeding, began to be given BLW complementary foods (60 people) and traditional complementary foods (60 people) from 6 months of age. We used enzyme immunoassay Immulite 2000 for evaluation the total IgE and specific IgE to 22 food allergens, twice before and after 12 months of introduction of complementary foods.

The results of the analysis showed that the highest level of sensitization to food allergens was found in infants of groups 1 and 3 with atopic dermatitis (AD), regardless of the type of complementary feeding. Among children of group 2 who received complementary feeding according to the BLW method without AD, a significantly higher level of sensitization to food allergens was observed compared to children of group 4 who received traditional complementary feeding without a history of AD ($p=0.015$).

Conclusion: 1. children with manifestations of AD are more often sensitized to food allergens, which can be clinically reflected by various manifestations of food hypersensitivity; 2. infants on BLW complementary feeding, regardless of the presence of AD, are more prone to sensitization to food allergens.

Prof. Olena Sharikadze *is the Director of the Institute of Postgraduate Education at Shupyk National Healthcare University of Ukraine. She is an expert of clinical immunology, paediatric respiratory diseases, and molecular allergy diagnostics. She also serves as a scientific consultant at the Paediatric and Endocrinology Department of National Children's Hospital 'Okhmatdyt', and works as an allergologist at Divero Medical Center, Ukraine. With over 20 years of teaching experience, Prof. Sharikadze has trained numerous students in the fields of clinical immunology and allergology. Her research is focussed on the accuracy and safety of food allergy diagnosis, the mechanisms of food allergy and oral tolerance, the efficacy of allergen immunotherapy in children, and the management of asthma, allergic rhinitis, and chronic urticarial. She is an active member of the European Academy of Allergy and Clinical Immunology.*

3. TUMOR IMMUNOLOGY

NEUTROPHILS IN CANCER: STATE OF THE ART

Sven Brandau

University Hospital Essen, Germany

In human oncology a high infiltrate of the cancer with so called tumor-associated neutrophils (TAN) is associated with poor prognosis in the majority of cancer types. Certain therapeutic interventions can revert this scenario. In my presentation I will provide an overview on basic concepts of TAN and circulating neutrophils with a focus on human immuno-oncology. I will also highlight major immunosuppressive and pro-tumorigenic mechanisms that our lab discovered over the last couple of years. These pro-tumorigenic mechanisms include the execution of T cell suppression (classically known as MDSC activity) and the induction of metastasis. I will also provide some ideas on a new concept that defines the pro-tumor and anti-tumor activity not at the level of the neutrophil itself, but rather at the level of the tumor target cells.

***Prof. Sven Brandau** is the Head of Experimental & Translational Research, University of Duisburg-Essen, Germany. He is an expert in tumor immunology, specializing in the immune response to cancer. His research focus on the role of myeloid cells in inflammation and cancer, particularly in head and neck tumors. Prof. Brandau has led international initiatives such as Mye-EUNITER and Mye-InfoBank, which aim to advance the understanding of myeloid regulatory cells and translate their molecular profiles into biomarkers for cancer and inflammation. His team conducts clinical studies, analyzing patient' tissue and blood using digital pathology to assess biomarkers and immune function in cancer. These investigations contributed to biomarker identification and the development of standardized analytical methods for studying immune cells, including myeloid-derived suppressor cells.*

NOVEL DEVELOPMENTS IN CANCER IMMUNOTHERAPY

Jon Amund Kyte

Oslo University Hospital, Norway

Immunotherapy has over the last decade given important progress in cancer treatment. The immune checkpoint inhibitors (ICI) are effective in many cancer forms, but work through removing breaks on existing immune responses and rarely show efficacy in "immune-cold" tumors. Metastatic breast cancer (mBC) is among the most common causes of cancer-related death. In mBC, only a minority respond to ICI. We have conducted two randomized trials, called ALICE and ICON, evaluating the addition of ICI to selected chemotherapy with immumomodulating properties. The studies were the first to evaluate ICI combined with anthracyclines (stimulates immunogenic cell death) and metronomic cyclophosphamide (counters immunosuppressive regulatory T cells) in mBC. ALICE and ICON targeted triple negative and hormone receptor positive (HR+) mBC, respectively. The ALICE trial (Nat Med Dec 2022; doi 10.1038/s41591-022-02126-) is the first to show benefit from adding ICI to chemotherapy in PD-L1negative mBC. This finding supports the use of selected chemotherapy to make "immune-cold" tumors responsive to ICI. In ICON, we found no benefit from concomitant addition of ICI to chemotherapy, but obtained responses from ICI after chemotherapy (J Immunother Cancer 2024; doi 10.1136/jitc-2023-007990). In both trials, we found that the chemotherapy reduced regulatory T cells, as hypothesized. Looking forward, it is important to investigate biomarkers and mechanisms of effect. We are currently conducting translational projects that will provide insight into immune-tumor co-evolution and inform new clinical trials. We are also developing CAR T cell therapy for solid cancers, exploring new targets and strategies for overcoming tumor heterogeneity and immune suppression.

Prof. Jon Amund Kyte *is leading the Section for Experimental Cancer Treatment, Department of Oncology at Oslo University Hospital (OUH) and serving as a professor at Oslo Metropolitan University and the University of Oslo. His work focuses on developing innovative approaches for cancer immunotherapy, including adoptive cell therapies and chimeric antigen receptor (CAR) T cells, targeting solid tumors and advancing personalized oncology. Prof. Kyte has played a pivotal role in translating basic research into clinical applications through his leadership in national and international multi-center clinical trials, such as ICON, ALICE, and REPORT. He has authored over 50 peer-reviewed articles, holds numerous patents related to cancer therapies, and has received extensive research funding, including grants from the Norwegian Cancer Society and the European Research Council. In 2023, Prof. Kyte co-founded the biotech company ImmunoQuest, furthering his commitment to bridging academic innovation with real-world applications. For his dedication to cutting-edge cancer research and therapy development, he was awarded the Research Prize of the Norwegian Society of Immunology in 2023.*

TUMOR MICROENVIRONMENT – KEY TO SUCCESSFUL MANAGEMENT OF CANCER-INDUCED IMMUNOSUPPRESSION

Agata Mlynska[1,2], Eglė Žymantaitė[1,3], Austėja Butkutė[1,3], Neringa Dobrovolskienė (1), Jan Aleksander Krasko[1,2], Olha Karaman[1], Karolina Suveizdė[1], Beatričė Gudaitė[1,3], Margarita Žvirblė[1,3], Emilija Paberalė[1,3], Vita Pašukonienė[1]

[1]Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania; [2]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania;[3]Life Sciences Center, Vilnius University, Vilnius, Lithuania

Different patterns of immune and stromal interactions within the tumor microenvironment significantly impact tumor behavior and response to treatment. Recent research has identified distinct immune subtypes—immune desert, excluded, and inflamed—each associated with specific mechanisms that impede anti-tumor immune responses. In the era of precision medicine, accurately stratifying patients into these subtypes is essential for optimizing cancer management and personalizing immunotherapy. The research team at National Cancer Institute in Lithuania addresses critical gaps in this field by focusing on several key areas. Our first focus is on developing reliable and accessible tools for determining patient immune subtypes. We are integrating transcriptomics and histology data to create a robust subtyping pipeline, which we are validating with real-world data and streamlining for clinical use. Another aspect of our work involves creating translatable preclinical models for cancer immunotherapy through utilizing 3D cell cultures and biocompatible scaffolds. We are developing druggable syngeneic murine tumor models that accurately reflect the three immune subtypes, enhancing the relevance of preclinical studies. Using both pharmaceutical and non-pharmaceutical approaches, we are tailoring immunomodulatory interventions to address the unique characteristics of each immune subtype. Joint efforts in these research areas could generate translatable insights, contributing to more precise cancer therapies and overcoming resistance mechanisms.

Dr. Agata Mlynska *is a Senior Researcher at the Laboratory of Immunology at the National Cancer Institute, Lithuania. She received her PhD from Vilnius University in 2018, where she advanced the understanding of how the tumor microenvironment and chemokine signaling influence ovarian cancer and melanoma progression and response to therapy. During her PhD and postdoctoral studies, she also conducted research in translational oncology at the Swiss Federal Institute of Technology and Lausanne University Hospital. Her research focuses on the dynamic interactions between immune cells and cancer cells that shape the tumor microenvironment, with a strong emphasis on personalized cancer therapies. Dr. Mlynska is a Board member of the Lithuanian Society for Immunology and a member of the European Society for Medical Oncology (ESMO).*

THE ROLE OF MICROBIOTA IN ANTITUMOR IMMUNE RESPONSE

Yuliia Shvets

Taras Shevchenko National University of Kyiv, Ukraine

Microbiota influences the development of human malignant neoplasms. Certain representatives of the microbiota demonstrate the ability to both stimulate the development of neoplasms and demonstrate antitumor activity, due to numerous signaling mechanisms involving the immune system.

When considering the topic of the influence of the microbiota on the antitumor immune response, attention is drawn to the following points:

1. Microbiota as a component of the tumor microenvironment;

2. The intestinal microbiota, according to the principle of the axis, affects various organs and tissues, including the immune system and the tumor;

3. Microbiota influences the effectiveness of antitumor therapy, primarily immunotherapy;

4. Microbiota as a factor in the prognosis of tumor development and sensitivity to antitumor therapy.

Of great practical interest is the topic of the possibility of influencing the composition and metabolic activity of the microbiota in order to correct it with the use of probiotics, prebiotics, synbiotics, metabiotics, postbiotics, as well as intestinal microbiota transplantation and careful selection of an individual diet.

Dr. Yuliia Shvets *is an Associate Professor in the Department of Biomedicine at Taras Shevchenko National University of Kyiv. She is also a scientific consultant for the neo-insurance company "Lilo", where she provides expertise on immunological aspects. After graduating in 1995 from Taras Shevchenko National University with a specialization in Microbiology and Immunology, she earned her Ph.D. in Oncology in 2000. Over her career, she has contributed extensively to the academic community, authoring numerous scientific articles and textbooks in medical microbiology and immunology, which are widely used by students in biological and medical fields. Dr. Shvets's research focus on the critical intersection of human microbiome and tumor immunology, particularly investigating how the microbiome influences the effectiveness of anticancer immunotherapies. Recognizing the translational potential of this field, she is dedicated to advancing microbiome research in Ukraine, aiming to build a foundation for therapeutic innovations that can be applied in future treatment strategies. Dr. Shvets is strongly committed to fostering the growth of Ukrainian scientific research and actively works to inspire young scientists to pursue innovative biomedical research addressing both national and global health issues.*

Round table discussion: Interdisciplinarity and hyperspecialization in science: how to keep the balance?

Moderator:
Elizabet Beržanskytė,
Science journalist, *the Lithuanian National Radio and Television*

Participants:
Dr. Jonas Čiurlionis, Science philosopher, *Vilnius University Faculty of Philosophy*
Dr. Rolandas Maskoliūnas, Science communicator, *the Lithuanian Academy of Sciences*
Prof. Andrei Spiridonov, V*ilnius University, Lithuania*
Dr. Agata Mlynska, *the National Cancer Institute, Lithuania*
Prof. Aurelija Žvirblienė, *Vilnius University, Lithuania*

Elizabet Beržanskytė *is a science journalist and editor at the Lithuanian National Radio and Television news network. She holds a Bachelor's degree in molecular biology from Vilnius University Life Sciences Center, where she also conducted research on stem cells. Alongside her studies, she was the organizer of the international life sciences conference "The COINS" in 2020. She started her career in video journalism in 2020, creating science communication series such as "The Paradox of Life" and "Lithuania is Closer to Space Than It Looks". In collaboration with the European Commission representatives in Lithuania, she has also highlighted EU-funded research projects. After receiving her Master's degree in analytical journalism from Vilnius University in 2023, she began her role as a science editor at the National Radio and Television presenting scientific innovations and conducting interviews with Lithuanian and foreign scientists. Her mission is to bridge the gap between science and the general public.*

Dr. Jonas Čiurlionis *is an Associate professor of Vilnius University Faculty of Philosophy. He received his PhD from Vilnius University in 2006 upon defense of PhD Thesis "Physicalistic problem of Space and Time". Since then, he has been working at Vilnius University where he gives lectures on various topics. He also lectures at foreign universities, participates in conferences, boards of scientific journals and supervises international students.*
He has published research articles in Lithuania and abroad. His research interests are: Philosophy of Space and Time; History and Philosophy of Science; Relation of Science and Metaphysics; Ancient Science; Philosophy of Music; Aesthetics.

Dr. Rolandas Maskoliūnas *is a biochemist, science communicator, Chief specialist for international communication at the Lithuanian Academy of Sciences. After receiving his PhD in Biological sciences, he has been creating science popularization programs on Lithuanian television for the past three decades: "Negali būti" ("That is impossible"), "Mokslo ekspresas" ("Science Express"), "Smalsumo genas" ("Curiosity Gene"). He is an author of two books: "I Believe in Science Almighty", "1922: Between Cannibalism and Modernism". He is an organizer of the National Science Festival "Spaceship Earth".*

Prof. Andrei Spiridonov *is a Professor at the Institute of Geosciences of Vilnius University and a member of Young Academy of the Lithuanian Academy of Sciences. he works on development of a comprehensive hierarchical view of the evolutionary process, with special attention to macroevolution and interaction of biological evolution with the multi-scale dynamics of the Earth system. His work led to the recognition of the spatio-temporal structure of Silurian oceanic extinctions events. He developed approaches for understanding evolution in the light of the dynamics of geographical barriers at many spatial scales (e.g. Geo-Red Queen hypothesis, Bretskyan hierarchy of geobiomes). More recently he made an excursion into the domain of the cultural evolution, where he tries to connect the evolution of culture and biological organismal and population level traits.*

ABSTRACTS OF POSTER PRESENTATIONS

1. HOST-PATHOGEN INTERACTION

1.1. CHRONIC HEPATITIS B: IS STERILISING CURE POSSIBLE?

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Introduction. Chronic hepatitis B infection remains a major global health concern, carrying a substantial risk of liver cirrhosis and hepatocellular carcinoma. Despite notable advances in vaccination and antiviral therapies, a sterilising cure not yet been realised, largely due to the persistence of covalently closed circular DNA (cccDNA) and the virus's capacity to evade or suppress the host immune response. Current treatment strategies therefore target a "realistic functional cure", which is, unfortunately, achieved in only approximately 1% of patients each year.

Clinical case. We report the case of a patient who achieved a realistic functional cure of chronic hepatitis B. In 2002, routine screening revealed the following serological markers: HBsAg (+), anti-HBcor (+), HBeAg (-), anti-HBe (+), anti-HBs (-), HBV-DNA 2.83 x 107 copies/mL, consistent with a diagnosis of HBeAg negative chronic hepatitis B. The patient underwent prolonged clinical monitoring and received multiple antiviral treatments, including lamivudine, interferon and entecavir. After more than a decade of entecavir therapy, the patient exhibited HBsAg clearance and undetectable HBV-DNA levels, meeting the criteria for a realistic functional cure, which persisted following cessation of treatment.

Discussion and Conclusions. The immunopathogenesis of chronic hepatitis B, particularly T cell exhaustion, restricts the efficacy of current antiviral strategies. Although a sterilising cure remains unachievable, realistic functional cure is infrequently achieved. Evidence suggests that extended nucleos(t)ide analogue therapy, combination treatment modalities, low baseline HBV-DNA and HBsAg levels, and specific host factors can improve the likelihood of achieving this outcome. In the present case, long-term entecavir therapy likely facilitated immune reconstitution, contributing to sustained viral suppression. Given the limited success rates of existing treatments, continued investigation into innovative therapeutic approaches is imperative to enhance realistic functional cure rates in chronic hepatitis B.

1.2. INVESTIGATING NEUTROPHIL RESPONSES TO SURFACE-ASSOCIATED VIRULENCE FACTORS OF ACINETOBACTER BAUMANNII

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Acinetobacter baumannii is an opportunistic pathogen that causes nosocomial infections. According to WHO, A. baumannii is a priority target for developing new drugs due to its antibiotic resistance. Little is known about the virulence factors of A. baumannii. Therefore, understanding the molecular mechanisms of host-pathogen interaction is crucial for controlling antibiotic-resistant A. baumannii. The innate immunity, particularly neutrophils, plays an important role in fighting infection. Neutrophils inactivate pathogens through NETosis, releasing neutrophil extracellular traps (NETs). However, it is poorly understood how A. baumannii induces NET formation. The pathogenicity of A. baumannii is determined by cell surface structures, therefore it is necessary to investigate their influence on the host immune system. Neutrophils were purified from human blood by density gradient centrifugation. Afterwards, they were infected with A. baumannii clinical isolate and mutant strains, generated by markerless gene-deletion. Deleted A. baumannii genes were associated with surface virulence factors: galU encodes an enzyme required for capsule biosynthesis, pmrC – lipid A phosphoethanolamine transferase, ompA – outer membrane porin. Confocal fluorescence microscopy was used for imaging. Following image analysis, the level of NET formation, the number of phagocytosed bacteria and viable neutrophils were evaluated and compared.
The purification of neutrophils from human blood was optimized; from ~9 mL of blood, approximately 6-8 106 neutrophils were obtained. Results from ex vivo infections demonstrated A. baumannii ability to induce NET production in neutrophils. Furthermore, phagocytosis level and neutrophil survival rates after infection were analysed. Results demonstrated the importance of A. baumannii surface-associated virulence factors during interaction with neutrophils.

1.3. CHANGES IN THE NUMBER OF MAJOR T-LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH LONG COVID, IN THE CONTEXT OF REACTIVATION OF HUMAN HERPESVIRUS 6 INFECTION

Olena Ivanytska

Danylo Halytsky Lviv National Medical University, Ukraine

Long COVID is a prolonged condition following an acute form of SARS-CoV-2 infection that causes alterations in the levels of CD4+, CD4+CD25+CD127− and CD8+ subpopulations of T-lymphocytes among patients diagnosed with this disease. The research focuses on clinical cases where the infection has been accompanied by reactivation of Human Herpesvirus 6 (HHV-6). Thereby, changes in the levels of subpopulations of T-lymphocytes serve as a reliable indicator of immune dysregulation.

The results demonstrate that in patients with HHV-6 reactivation in mild or moderate cases of long COVID, the percentage of CD4+, CD4+CD25+CD127− and CD8+ T-cells did not exhibit significant differences from control groups, which consist of healthy people without long COVID and without HHV-6. However, a more pronounced decrease in the percentage of CD4+ (p=0.045), CD4+CD25+CD127− (p=0,008), and CD8+ (p=0.005) subpopulations of T-lymphocytes was observed after severe cases of the disease, suggesting that HHV-6 contributes to further immune suppression (according to statistical research). These findings indicate that HHV-6 reactivation exacerbates T-cell depletion in severe long-standing cases of the disease, potentially increasing the risk of immune dysfunction. The results emphasize the importance of monitoring immune responses in patients with long COVID, particularly in those with reactivation of HHV-6, to better understand disease progression and potential therapeutic approaches.

1.4. IMPACT OF SARS-CoV-2 VIRAL INFECTION ON CYTOKINE LEVELS IN CHILDREN WITH COMMUNITY-ACQUIRED PNEUMONIA

Kateryna Smiian

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Pneumonia remains one of the most prevalent infectious diseases in children. The COVID-19 pandemic has significantly influenced the course and clinical presentation of the disease. The severity of pneumonia is largely determined by the host immune response, a critical component of which is cytokine regulation, particularly interleukins.

To determine the levels of anti-inflammatory interleukin-4 (IL-4) and pro-inflammatory interleukin-6 (IL-6) in children with community-acquired pneumonia during the acute phase, considering the presence of SARS-CoV-2 infection.

A total of 49 children aged 6 to 18 years with diagnosed community-acquired pneumonia were examined. Of these, 31 patients had confirmed SARS-CoV-2 infection, while 18 had no evidence of coronavirus infection. The control group consisted of 21 somatically healthy children matched by age and sex.

During the acute phase of pneumonia, children included in the study exhibited a significant increase in IL-4 ((27.54±1.21) pg/mL) and IL-6 ((15.23±1.21) pg/mL) levels compared to the control group ((15.43±1.12) pg/mL) and ((4.72±0.87) pg/mL), respectively. Specifically, IL-4 levels were significantly elevated in pneumonia patients and were highest in those with confirmed SARS-CoV-2 infection ((31.15±0.9) pg/mL). IL-6 levels ((19.83±1.0) pg/mL) were also significantly increased compared to the control group but did not differ between pneumonia patients with and without SARS-CoV-2 infection.

The observed increase in both pro-inflammatory and anti-inflammatory cytokines during the acute phase of pneumonia indicates activation of the cellular immune response, which is essential for combating infectious pathogens. Higher cytokine levels in children with SARS-CoV-2 infection may reflect a more intense inflammatory response in the body.

1.5. IMMUNOLOGICAL PARAMETERS OF SARS-CoV-2 RESISTANT INDIVIDUALS

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The course of SARS-CoV-2 can vary a lot – it can be completely asymptomatic or in some cases even fatal. There are also individuals who don't get infected despite being in intense contact with patients suffering from SARS-CoV-2, and who are therefore considered resistant against the virus. There has been a lot of research looking into the causes of severe cases of SARS-CoV-2 infection, but there's a lot less information available about resistant people. To get more insight into viral resistance and understand the pathology of this virus, those individuals must also be investigated. To do this, T cell response to viral antigens was measured from whole blood supernatants, secretion of IFNα after stimulating the samples with TLR7/8 and cGAS-STING signalling pathway ligands was determined and the presence of autoantibodies against type I and type III interferons was examined. It was found that some of the non-infected patients had cross-reactive T cells and some were completely naive. There were no AABs against type I IFNs among these

naive people and some individuals had high induced IFNα levels. The genetic causes behind this should be further investigated.

1.6. IMMUNOMODULATORY EFFECTS OF BACTERIOPHAGE-DERIVED DSRNA (LARIFAN) IN SARS-CoV-2-INFECTED K18-HACE2 MICE

Kristine Vaivode[1,2], Anna Esiņa[1,2], Madara Kreismane[1,2], Irina Verhovcova[1,2], Eva E. Morozova[1,2], Ramona Petrovska[2] and **Dace Pjanova**[1,2]

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The COVID-19 pandemic has highlighted the need for novel or adapted therapies. Bacteriophage-derived double-stranded RNA (dsRNA), Larifan, has demonstrated antiviral properties in vitro and in animal models; however, its immunomodulatory effects during SARS-CoV-2 infection remain incompletely understood.

This study evaluates Larifan's impact on macrophage responses, pro-inflammatory cytokine production, and disease progression in transgenic K18-hACE2 mice infected with SARS-CoV-2.

K18-hACE2 mice were intranasally infected with SARS-CoV-2 (1×10^4 PFU) and treated with Larifan (5 µg/kg) intranasally or intraperitoneally, either before or shortly after infection. Survival, weight, lung macrophage populations and serum cytokine levels were analyzed. SARS-CoV-2 presence in lung and brain tissues was confirmed via ddPCR and immunostaining.

Intranasal Larifan administration before infection improved survival, delaying symptom onset and extending survival to day 16 ($p < 0.001$). This effect was not observed when Lariafn was given after infection. Intraperitoneal administration post-infection was most effective when given two hours after infection compared to six hours after infection. Lung macrophage analysis revealed that Larifan did not significantly alter alveolar or monocyte-derived macrophage proportions early in infection. However, by day 5, a significant reduction in monocyte-derived macrophages ($p = 0.0003$) and total macrophages within the leukocyte population ($p = 0.0108$) was observed. Pro-inflammatory cytokine analysis detected IFN-α, TNF-α, IL-6, IL-1β, CXCL1, CCL2, CCL5, and CXCL10, while GM-CSF, IL-10, IL-12, IFN-β, and IFN-γ remained undetectable, with no significant differences between treated and untreated groups.

These findings suggest that Larifan enhances antiviral responses without exacerbating inflammation, supporting its potential as an immunomodulatory treatment for respiratory viral infections.

1.7. MAPPING OF RECOGNITION SITES OF NOVEL MONOCLONAL ANTIBODIES SPECIFIC TO SARS-CoV-2 NUCLEOCAPSID PROTEIN

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The SARS-CoV-2 nucleocapsid protein (NP) is a multifunctional structural protein essential for the viral life cycle, pathogenesis, and interaction with the host immune system. NP plays an important role in immune modulation by suppressing innate immune responses and inducing the release of inflammatory cytokines. Although NP is predominantly localized in the cell cytoplasm, its significant amounts are found

in the extracellular space. The impact of the extracellular form of NP on the immune response remains unknown. The emergence of new viral variants emphasizes the importance of advancing molecular research on SARS-CoV-2 NP.

This study aimed to characterize the collection of recently developed murine monoclonal antibodies (MAbs) specific to SARS-CoV-2 NP for comprehensive investigations. Firstly, the MAbs demonstrated cross-reactivity with the recombinant NP of the Omicron variant but did not recognize NPs of other human coronaviruses. To localize the binding sites of the MAbs, overlapping SARS-CoV-2 NP fragments were produced. The analysis indicated that MAbs 16D9, 18A8, and partially 7F10 bind within the RNA binding domain of NP. Likewise, the binding sites of MAbs 1A6, 4B3, 4G6, 6G11, and 12B2 are located within the C-terminal domain of NP, which is involved in both RNA binding and protein dimerization. These findings suggest that MAbs recognize epitopes located in functionally active regions of NP molecule. In silico computational analysis of epitopes revealed that MAbs do not recognize epitopes that include altered amino acids in different SARS-CoV-2 variants. Thus, the generated MAbs could be utilized in the in-depth studies of SARS-CoV-2.

1.8. CHRONIC URTICARIA AND ITS ASSOCIATION WITH HELICOBACTER PYLORI: ANALYSIS OF RECENT STUDIES

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Globally, an increasing number of people are facing the symptoms of chronic urticaria (CU), which significantly impacts their quality of life. This condition, characterized by complex pathophysiology and often idiopathic etiology, presents diagnostic and therapeutic challenges for healthcare professionals, remaining a significant public health concern. Recent studies have drawn attention to a potential connection between chronic urticaria and Helicobacter pylori infection. This systematic review revealed substantial geographical variations in H. pylori prevalence among CU patients: 27% in Tanzania, 33.8% in Croatia, 36% in Iran, 59.8% in Pakistan, 65% in Egypt and even 100% in Cuba. Notably, diagnostic methods varied significantly across studies, potentially influencing prevalence rates: from single antigenic tests to combined approaches including serological testing, urea breath test, and gastric biopsy. The analyzed studies indicated that H. pylori eradication achieved complete remission in 72.7-80% of cases, with the best results obtained through a 14-day eradication protocol. While these findings suggest H. pylori testing and eradication should be considered in CU management, the limited data from European populations, particularly Baltic countries, indicates a need for further regional studies to establish population-specific guidelines.

1.9. COMPARISON OF ANTIGENIC PROPERTIES OF RECOMBINANT DERIVATIVES OF DIPHTHERIA TOXIN

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Diphtheria remains a public health concern due to its potential for outbreaks in regions with declining vaccination rates. The causative agent, Corynebacterium diphtheriae, produces diphtheria toxin (DT), a

potent exotoxin responsible for disease pathogenesis. Recombinant DT derivatives offer safer immunization strategies.

This study compared the antigenic properties of recombinant DT fragments, including the non-toxic toxoid CRM197, subunit A (SbA), subunit B (SbB), and the receptor-binding domain (Rd). Proteins were expressed in E. coli, purified via metal-affinity chromatography, and used to immunize BALB/c mice. IgG levels were assessed by ELISA to determine immunogenicity.

CRM197 induced the highest IgG response, confirming its strong immunogenicity. The SbA-SbB combination also elicited a significant immune response, indicating potential for vaccine formulations. The receptor-binding domain (Rd) showed moderate immunogenicity, highlighting its role in antigen recognition.

These findings support the development of improved diphtheria vaccines and diagnostic tools. Further studies are needed to optimize antigenic formulations and assess their efficacy in neutralizing diphtheria toxin.

1.10. SEMLIKI FOREST VIRUS MEDIATED EXPRESSION OF IL-12 AS A POTENT ADJUVANT TO IMPROVE VACCINE-INDUCED IMMUNITY

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Interleukin-12 (IL-12) is a key pro-inflammatory cytokine that plays a crucial role in shaping the immune response by promoting Th1-type immunity, enhancing cytotoxic T cell activity, and stimulating natural killer (NK) cells. It is known to boost interferon-gamma (IFN-γ) production, which contributes to a more robust and durable immune response. Due to its potent immunomodulatory properties, IL-12 has been explored as an adjuvant in vaccine formulations to enhance antigen-specific immunity. In this study, we evaluated the adjuvant potential of IL-12 delivered via Semliki Forest virus (SFV) vector in combination with a SARS-CoV-2 vaccine prototype based on hepatitis B core/G (HBc/G) virus-like particles (VLPs). Mice were immunized with HBc/G-Gly-RBM VLPs alone or in combination with IL-12-expressing Semliki Forest virus (SFV-IL12). The neutralization capacity of induced antibodies was assessed using a murine leukemia virus (MLV)-based SARS-CoV-2 pseudotyped particle assay. Our findings demonstrate that SFV-mediated IL-12 delivery significantly enhances the efficacy of the HBc/G VLP-based SARS-CoV-2 vaccine. Mice immunized with the SFV-IL12 and HBc/G-Gly-RBM combination exhibited the highest virus neutralization capacity, and generated a long and effective T cellular response as shown by IFN-γ ELISpot test of splenocytes from immunized mice. These results underscore the strong adjuvant potential of IL-12, highlighting its potential to amplify immune responses and optimize vaccine formulations for infectious diseases and cancer.

1.11. COMPARISON OF POST-VACCINATION PERIOD WHEN USING VACCINES FROM DIFFERENT MANUFACTURERS

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Vaccination has saved millions of lives by protecting the population from infectious diseases and is one of the cornerstones of public health worldwide. From time to time, the incidence of vaccine-controlled infections increases worldwide due to insufficient vaccination coverage of children and adults. One of the reasons for postponing or refusing vaccination is the fear of increasing of post-vaccinal reactions when using vaccines from different manufacturers.

Objective: to evaluate the postvaccination period when using 6-valent vaccines against pertussis, diphtheria, tetanus, polio, hepatitis B and Haemophilus influenzae type b infection from different manufacturers within the same vaccination cycle.

Study design. The objectives were children aged under 36 months received a full cycle of vaccinations according to the Ukrainian national schedule from January 2020 to October 2023 at a private health care facility in Kyiv. Data from 343 children vaccinated with combined vaccines from different manufacturers were selected. We analyzed 2 types of combination vaccines for the prevention of diphtheria, tetanus, pertussis (acellular component), hepatitis B, polio and Haemophilus influenzae type b infection with a 3- and 2-component pertussis component (Vaccine 1 and Vaccine 2, respectively). The post-vaccination period was assessed by telephone survey of parents whose children had been vaccinated the day before. According to the combination of vaccines, children were divided into 5 groups: roup 1: 3 doses of vaccine 1 + 1 dose of vaccine 2 (26 children); Group 2: 2 doses of vaccine 1 + 2 doses of vaccine 2 (104 children); Group 3: 1 dose of vaccine 1 + 3 doses of vaccine 2 (98 children); Group 4: 4 doses of vaccine 1 (24 children); Group 5: 4 doses of vaccine 2 (91 children). The condition of children was assessed after each vaccination.

Results: Data were evaluated in total from all 4 doses. In group 1, 25% reported a fever of up to 38.5C the next morning and about 10% complained of limb pain the day after vaccination. In group 2, 12% reported a fever of up to 38.5°C and 2% the pain in the site of injection. In group 4% had a fever of up to 38.5°C and 1% complained of pain in the limb. In group 4, 32% of parents reported a fever of up to 38.5°C and 4% a pain in the limb and 4% complained of a child's moodiness. In group 5, 16% repotrted only a fever of up to 38.5°C. When assessing the reliability of the differences, it was found that they were not significant ($p > 0.05$).

Conclusions:

1. The spectrum and percentage of postvaccination reactions is similar in heterologous immunization against pertussis, diphtheria, tetanus, poliomyelitis, hepatitis B and infections caused by Haemophilus influenzae type b, with vaccines from different manufacturers with a 2- and 3-component pertussis component.

2. Combining doses of hexavalent vaccines from different manufacturers during one vaccination cycle is not accompanied by an increase in postvaccination reactions, on the contrary, there is a tendency to decrease them.

3. The combination of different hexavalent vaccines during one vaccine cycle is justified and contributes to optimal dose intervals.

1.12. *S. aureus* CARRIAGE AND SENSITIZATION IN ALLERGIC RHINITIS: A CASE-CONTROL STUDY

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Staphylococcus aureus produces proteins that influence allergic diseases. Recent advances in detecting hypersensitivity to S. aureus enterotoxins (A, B, C, TSST) enable investigation of carriage prevalence and specific enterotoxin sensitization patterns in allergic rhinitis (AR) patients.
Methods: Clinical files of 35 patients were analyzed: 57.1% patients with moderate to severe allergic rhinitis and 42.9% control subjects without AR. Evaluation included skin prick tests, total/specific IgE determination, ARIA-based severity assessment, nasal bacterial culture, and specific IgE to S. aureus enterotoxins.
Results: Gender distribution in the allergic rhinitis (AR) group are 60% women and 40% men. Bacterial colonization profile: 60% had S. aureus carriage alone, 10% had combined S. aureus and Klebsiella pneumonia carriage and 30% maintained normal flora. Enterotoxin sensitization analyze showed 50% of patients had positive antibodies to at least one staphylococcal enterotoxin. In the control group: 33.3% showed S. aureus carriage, 13.3% of carriers demonstrated sensitization to one staphylococcal enterotoxin. In the allergic rhinitis (AR) group compared to controls: S. aureus carriage rate was significantly higher (70% vs 33.3%) and enterotoxin sensitization was more prevalent (50% vs 13.3%). Total IgE levels were significantly elevated in the allergic rhinitis (AR) group with enterotoxin sensitization.

Conclusion: Findings indicate significantly higher prevalence of S. aureus carriage and enterotoxin sensitization in AR patients compared to controls, suggesting S. aureus's role in modulating allergic responses. Further studies with larger cohorts are needed to validate these findings and explore therapeutic implications.

1.13. DYNAMICS OF HAPTOGLOBIN (HP) CONCENTRATION IN RABBITS EXPERIMENTALLY INFECTED WITH LAGOVIRUS EUROPAEUS GI.1A-RHDVA

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Acute phase proteins (APPs) play a key role in mammalian immunity, including rabbits. Haptoglobin (Hp) is one of the "strong" APPs, and its dynamics in Lagovirus europaeus GI.1 infection, including GI.1a-RHDVa, have not been previously analyzed.

Aim: Changes in Hp concentration in rabbits experimentally infected with Lagovirus europaeus GI.1a, strain NL-2 were determined. Clinical signs and animal mortality during the experiment were also reported.

Methods: 20 serum of Polish crossbred rabbits (10 infected, 10 control) from the serum bank (Department of Immunology US) were used. Hp concentration was determined by ELISA at 0h and 8h, 12h, 24h, 36h, 48h after infection. Clinical signs and animal mortality were also reported.

Results: Hp concentration in infected rabbits was 0.36-0.48 g/l (SD±0.05-0.08), while in controls it was 0.58-0.60 g/l (SD±0.02-0.05). Statistically significant changes were recorded as decreases from 8h to 48h after infection. Animal mortality was recorded from 24h to 48h and reached 80%.

Conclusions: The decreases in Hp between 8h and 48h are not analogous to the results of other rabbit "strong" APPs obtained by different authors, and do not correspond with the results of the dynamics of Hp concentration in other mammals in viral infections. This gives a view of a specific immune response to the NL-2 strain, associated with its different virulence and immunogenicity. They are an indication for the study of other APPs in rabbits and those infected with other strains, as well as a signal for the study of factors regulating their synthesis in hepatocytes.

1.14. INVESTIGATION OF ANTIBODY RESPONSES TO VARIOUS VIRUSES IN BALTIC SEA GREY SEALS

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Infectious diseases that are capable of infecting and propagating in multiple species (zoonotic diseases) pose significant risks to both wildlife and human health due to their unpredictable infectivity and lethality. Early detection and monitoring of potentially zoonotic pathogens in wildlife is essential in understanding their spread and determining the risk of cross-species transmission. This study investigates the serological immune responses in Baltic Sea grey seal pups (Halichoerus grypus grypus) to 27 viral antigens. These antigens include yeast expressed nucleocapsid (NP) and capsid (CP) recombinant proteins of various zoonotic (Prospect Hill virus (PHV), rat hepatitis E virus (RHEV), Puumala Vranica virus (PUUV), etc.), potentially zoonotic (Schmallenberg virus (SBV), tent-making bat hepatitis B virus (TBHBV), Thottapalayam thottimvirus (TPMV)) and species-specific (Merkel cell polyomavirus (MCPyV), norovirus (NoroV), porcine parvovirus (PPV), hamster polyomavirus (HaPyV), etc.) viruses. Enzyme-linked immunosorbent assay and Western blot were used for blood plasma and precipitated antibody analysis, respectively. A majority (> 60 %) of seal pup blood plasma showed significant antibody concentrations against polyoma (MCPyV, NJPyV, WUPyV, APyV, HaPyV, MarPyV2), measles, mumps, influenza viruses, while some (25-60 %) seal pup plasma showed antibody presence against orthohantaviruses (PUUV, PHV, SEOV, HTNV, TPMV, SNV). Our findings suggest that seals are exposed to orthohantaviruses infected rodents throughout their lifespan, as rodents serve as the primary reservoir of these viruses. This highlights the need for further research to understand potential water-borne transmission routes of orthohantaviruses via seals across the Baltic Sea.

1.15. MACROPHAGE INFLAMMATORY RESPONSE TO PHAGOCYTOSED VIRAL ANTIGENS

Asta Lučiūnaitė[1,2], Kristina Mašalaitė[1], Vincentas Maciulis[2], Mantvydas Usvaltas[2], Milda Norkienė[1], Ieva Plikusiene[2,3], Aurelija Žvirblienė[1]

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Macrophages play an important role in protecting our body from various pathogens. By recognizing these antigens, macrophages can initiate inflammatory reactions. One of the mechanisms is inflammasome

activation. Numerous factors, such as pathogens or endogenous molecular patterns, can activate NLRP3 inflammasome. However, there is a limited understanding of inflammasome activation mechanisms related to phagocytosis. Our study aims to investigate how phagocytosed structurally different antigens mediate inflammasome activation in macrophages. Our previous research showed that polyomavirus-derived virus-like particles (VLPs) mimicking native virus capsids activate NLRP3 inflammasome contrary to structurally different paramyxovirus-derived nucleocapsid-like structures. We also showed that the mechanism is linked to the phagocytosis process. Furthermore, we demonstrated that structurally different immune complexes (ICs) formed by specific IgG antibodies and VLPs induce distinct patterns of inflammatory response, including inflammasome activation and secretion of chemokines. We characterized the ICs using advanced biosensing surface-sensitive techniques and confirmed the differences between ICs regarding their relative size, viscoelasticity, and antibody affinity. To extend our research, we investigate macrophage phagocytosis and activation by either polyomavirus VLPs, SARS-CoV-2 S protein or VLPs composed of structural viral proteins and their ICs formed by different human IgG antibodies. As an in vitro cell culture model, we use human THP-1 macrophage-like cells. We evaluated the pattern of inflammatory response to phagocytosed antigens, focusing on inflammasome activation, chemokine secretion, and phagocytosis profile. Our study demonstrates that the structural properties of phagocytosed multimeric antigens and their ICs can define the extent and profile of the inflammatory response.

1.16. THE IMPACT OF ASC SPECKS ON MACROPHAGE ACTIVATION BY VIRAL ANTIGENS

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Inflammatory signals and cell death characterise the activation of innate immune component inflammasome. Inflammasome is a multiprotein complex made of receptor and adaptor (ASC) proteins. ASC proteins form micrometer-sized structures called ASC specks. The inflammasome or ASC specks enter extracellular space and exacerbate inflammation. This research investigates how ASC specks affect macrophage activation by viral antigens.

We treated THP-1 macrophage-like cells with human KI polyomavirus virus-like particles (KIPyV VLPs) and ASC specks gathered from THP1-ASC-GFP cells. Cell viability was assessed using a lactate dehydrogenase (LDH) assay. Inflammatory response was evaluated via cytokine (IL-8, IL-1β, and CCL2) secretion using ELISA.

Our findings showed that ASC specks, alone or combined with KIPyV VLPs, significantly increased LDH release, indicating pyroptosis. KIPyV VLPs alone caused an insignificant LDH increase. We also found that KIPyV VLPS induced a higher IL-8 and CCL2 secretion in macrophages than ASC specks. However, macrophages treated with ASC specks released more IL-1β than viral antigen-activated cells. Interestingly, ASC specks significantly reduced the KIPyV VLPs-induced effect on macrophages regarding CCL2 secretion. A mixture of ASC specks and KIPyV VLPs induced different secretion of CCL2 and IL-1β than KIPyV VLPs alone. However, a mixture of ASC specks and viral antigens had a bigger impact on IL-8 secretion than ASC specks alone.

Our results suggest that ASC specks promote inflammasome assembly and pyroptosis in macrophages. KIPyV VLPs stimulate the release of pro-inflammatory cytokines, and ASC specks modulate their secretion. In conclusion, ASC specks affect the KIPyV VLP-induced inflammatory response in macrophages.

1.17. CONSTRUCTION AND EVALUATION OF EXPRESSION VECTORS FOR THE HETEROLOGOUS BIOSYNTHESIS OF A PUTATIVE CAPSID PROTEIN OF TORQUE-TENO VIRUS

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The Torque-Teno virus (TTV) is a small, non-enveloped, circular single-stranded DNA virus encoding four open reading frames (ORFs) (Rezahosseini et al., 2019). The prevalence of the virus is almost 95 % depending on the population and there is enough evidence to consider TTV as a part of human virome (Focosi et al., 2016). Therefore, recent body of research is focusing on TTV as a robust marker in diagnostics to evaluate the immunity status of a patient after organ transplantation. The study performed by Kakkola and colleagues (2008) presented some evidence that ORF1 C terminal protein and ORF1 protein with reduced arganine – rich N terminus (ORF1dArg) are the most immunologically reactive viral antigen constructs. This research focuses on the expression and synthesis of the TTV-ORF1dArg antigen construct while using various expression vectors. Six vectors were employed in this study: pET28a+, pET44a+, pET43.1a+, pGEX-2TK, pRSFDuet-1_VNp-LZ-mNeongreen, and pET-MBP-TEV. Each vector was transformed into several E. coli strains and protein expression was induced under varying conditions. The expression of the TTV ORF1dArg protein was analysed in SDS-PAGE and Western blot. It is relatively difficult to obtain the intact form of TTV ORF1dArg protein and broader optimisation protocol is needed for its synthesis.

1.18. PUTATIVE TORQUE TENO VIRUS CAPSID PROTEIN PRODUCTION IN *E.coli*

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Torque teno virus (TTV) is a part of the human virome and is under active investigation as a marker of immunocompetence. After a solid organ transplant (SOT), patients receive immunosuppressants to reduce the immune response. However, inadequate immunosuppression increases the risk of infection or organ rejection. Therefore, a reliable molecular marker of immunosuppression is needed. Previous studies in this area have reported that TTV levels are elevated in the blood of SOT patients, making it a potential marker for monitoring the level of immunosuppression.

TTV is a small, non-enveloped, single-stranded DNA virus of the *Anelloviridae* family. Its genome has at least six open reading frames (ORFs), with ORF1 encoding the putative TTV capsid protein. This protein exposes antigenic peptides in its C-terminus and is recognized by antibodies. The aim of this study is to achieve bacterial synthesis of recombinant putative TTV capsid protein. A gene sequence was selected and the gene encoding the required protein was acquired based on the epitopes identified in the literature. The gene construct was chosen for its ability to allow gene expression in bacteria, followed by potentially soluble protein synthesis. A gene expression vector was selected, and protein synthesis was confirmed by Western blot.

In conclusion, the synthesis of a putative TTV capsid protein has been achieved. Future plans include protein purification, mouse immunization, and the development of monoclonal antibodies specific to this protein as universal tools for detection of TTV in patient samples.

1.19. BROADLY REACTIVE MONOCLONAL ANTIBODIES FOR IMMUNODETECTION OF CLASS C BETA-LACTAMASES

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Bacterial resistance to antibiotics is a growing threat to human health globally, requiring rapid and accurate diagnostics for effective treatment of infectious diseases. This study aimed to develop monoclonal antibodies (MAbs) against bacterial class C β-lactamases (also termed as AmpCs), key enzymes in antibiotic resistance, for potential diagnostic use. For MAb generation, the bacteriophage vB_EcoS_NBD2 tail tube protein gp39-derived nanotubes were used as immunogenicity enhancing carriers displaying a highly conserved 17 amino acid-long peptide of AmpC β-lactamases. These nanotubes were produced in yeast and used as an immunogen for MAb generation by hybridoma technology. In total, thirteen hybridoma clones producing target peptide-specific MAbs were developed. Selected MAbs were comprehensively characterised using different immunoassays, total internal reflection ellipsometry and computational modelling. Epitope analysis revealed that the group of broadly reactive MAbs recognise a highly conserved 11 amino acid-long epitope of AmpC enzymes. Therefore, these novel MAbs represent a promising tool for improving diagnostics and monitoring of antibiotic resistance profiles.

1.20. CHARACTERISATION OF MONOCLONAL ANTIBODIES AGAINST STREPTOLYSIN O

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Streptolysin O (SLO) is a cholesterol-dependent cytolysin (CDC) produced by Streptococcus pyogenes, also known as group A Streptococcus (GAS). It is a main virulence factor of S. pyogenes that causes mild human infections such as pharyngitis, scarlet fever or severe infections such as necrotizing fasciitis and streptococcal toxic shock syndrome. In addition, recurrent GAS infections can lead to autoimmune diseases, including acute rheumatic fever and rheumatic heart disease. The pathogenic effect of SLO is based on its ability to form large pores approximately 30 nm in the cholesterol-containing cell membrane and induce host cell lysis. Therefore, the SLO-neutralising antibodies may provide valuable tools for reducing S. pyogenes pathogenic effects.

This study aimed to characterize a collection of monoclonal antibodies (MAbs) (39 clones) against SLO, evaluating their specificity, affinity, and potential applications in research. The MAbs were purified from a hybridoma growth medium using affinity chromatography. The affinity-purified MAbs were further characterized for specificity using enzyme-linked immunosorbent assay (ELISA), western blot (WB) and neutralizing activity determination methods. 18 of 39 MAbs were exclusively reactive with SLO, 20 MAbs were cross-reactive with perfringolysin O (PFO) and 22 MAbs were able to neutralize the cytolytic activity of SLO on a lung epithelial cell line. The results of competitive ELISA, WB and neutralizing activity revealed four groups of MAbs directed to different immunogenic regions of SLO. Further the selected MAbs from different groups will be used for SLO epitope determination. In conclusion, the characterized MAbs in this study may be useful for functional and structural studies of SLO and for the development of therapeutic or detection tools.

2. INBORN ERRORS OF IMMUNITY

2.1. COMMON VARIABLE IMMUNODEFICIENCY: CLINICAL MANIFESTATIONS AND PULMONARY COMPLICATIONS – A RETROSPECTIVE STUDY AT THE LITHUANIAN UNIVERSITY OF HEALTH SCIENCES KAUNO KLINIKOS

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Common variable immunodeficiency (CVID) is a rare disorder, affecting approximately 1 in 25,000 individuals, characterized by low IgG and IgA levels, often accompanied by decreased IgM. CVID can manifest as recurrent infections, autoimmune diseases, malignancies, lymphoproliferative disorders. Patients with CVID often have pulmonary complications. This study aims to analyze the clinical manifestations, and pulmonary complications, in CVID patients at the Hospital of the Lithuanian University of Health Sciences (LUHS) Kauno Klinikos.

Methods. A retrospective analysis of medical records from 2013 to 2025 was conducted using data from the Hospital of the LUHS Kauno Klinikos Primary Immunodeficiency Center. Data analysis was performed using Microsoft Excel

Results. The study included 33 CVID patients (10 women, 21 men, 2 children) with a mean age of 39.53 ± 16.4 years. Three patients (9.09%) had died, and one (3.03%) was lost to follow-up.

Recurrent infections were the most common phenotype of 26 patients (81.25%), followed by autoimmunity 15 patients (46,88%), including autoimmune thyroiditis (13 patients) and autoimmune thrombocytopenia (2 patients). Malignancies were identified in 3 patients (9.38%).

Pulmonary complications were frequent, with bronchiectasis in 6 patients, non-allergic asthma in 4, granulomatous-lymphocytic interstitial lung disease in 2, chronic obstructive pulmonary disease in 1, and respiratory failure in 2, chylothorax in 1 and hydrothorax in 1. One patient required lung transplantation due to progressive respiratory failure.

Conclusions. Our findings underscore the predominance of recurrent infections in CVID patients. Additionally, a significant proportion of patients experienced pulmonary complications, especially bronchiectasis. Our study reveals the importance of continuous patient follow-up, and further research.

2.2.THE DIAGNOSTIC DILEMMA IN DISTINGUISHING PRIMARY IMMUNODEFICIENCIES: A CASE REPORT

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Primary immunodeficiencies (PIDs) are a heterogeneous group of inborn errors of immunity, presenting with recurrent, severe, or unusual infections, as well as autoimmunity and malignancies. The diversity of PIDs, combined with overlapping symptoms with other conditions, makes the diagnostic process

particularly challenging. Despite their rarity, accurate diagnosis is essential, as appropriate treatment can significantly improve prognosis. However, many cases go undiagnosed or are misdiagnosed as PIDs, resulting in years of inappropriate treatment or failure to implement beneficial treatment.

Case report: A 10-year-old female was referred for immunological evaluation due to recurrent sinopulmonary and gastrointestinal infections, requiring monthly antibiotics. Medical history included premature birth, atypical phenotypic features and central cyanosis noted from birth. She was diagnosed with tetralogy of Fallot, which was surgically corrected at 4 months. Immunological workup revealed persistent lymphopenia, primarily affecting CD3+ T cells, with detectable TREC/KREC and adequate vaccine responses. Genetic testing identified a PIK3CD mutation, associated with APDS1.

Summary: This case report highlights the need for heightened clinical suspicion and a thorough diagnostic workup in patients presenting with recurrent severe infections and persistent lymphopenia.

Conclusions: Primary immunodeficiencies encompass a diverse spectrum of heterogeneous disorders that pose significant diagnostic challenges. A comprehensive evaluation of clinical manifestations and diagnostic investigations is essential. Genetic findings, especially variants of uncertain significance, must be interpreted within the broader clinical context. Supplementary functional studies and further clinical monitoring are often required to establish a definitive diagnosis and guide treatment decisions.

2.3. HYPOMORPHIC BTK VARIANTS IN AN ADULT: A RARE CASE OF LATE-DIAGNOSED X-LINKED AGAMMAGLOBULINEMIA

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X-linked agammaglobulinemia (XLA) is a rare primary immunodeficiency usually diagnosed in childhood due to BTK (Bruton's tyrosine kinase) gene mutations. Hypomorphic BTK variants can cause milder immunodeficiency, delaying diagnosis until adulthood. These cases pose diagnostic challenges due to variable presentations and limited awareness.

Case Presentation. A 1-year-old girl was evaluated for transient hypogammaglobulinemia and dilated cardiomyopathy. Genetic testing unexpectedly revealed a hypomorphic BTK variant in her asymptomatic 36-year-old father, prompting further immunological assessment.

Objective. To present a rare case of late-diagnosed XLA in an adult with a hypomorphic BTK mutation, analyze clinical implications, and review the literature.

Methods. The patient had recurrent respiratory infections (purulent otitis, two pneumonias) and bacterial meningitis at 15 years. Immunological testing showed normal IgG and IgA but critically low IgM (<0.2 g/L), absent isohemagglutinins (blood group A [II]), and equivocal varicella antibodies. Flow cytometry revealed 0.5% B-lymphocytes, low T-helper (400 cells/µL), and T-cytotoxic (280 cells/µL) counts. BTK sequencing identified a hypomorphic variant (c.1685G>C (p.Arg562Pro).

Discussion & Conclusion. Hypomorphic BTK mutations can cause milder immunodeficiency, delaying diagnosis. Similar cases in literature highlight the importance of family screening. Early recognition and immunoglobulin therapy improve outcomes. Increased physician awareness is essential.

2.4. HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF SEVERE COMBINED IMMUNODEFICIENCY(SCID): EXPERIENCE FROM LITHUANIA

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Severe Combined Immunodeficiency (SCID) is a group of rare, life-threatening genetic disorders with profound immune defects. Allogeneic hematopoietic stem cell transplantation (HSCT) is curative for most SCIDs. We aimed to analyze neutrophil engraftment, chimerism, immune reconstitution at 1 year, event-free and overall survival (OS) in SCID patients transplanted at Santaros Klinikos.

Patients and methods. This retrospective single-center study included 12 children (9 Lithuanian and 3 Latvian) who underwent HCST in 2010-2023. The data were evaluated on January 1st, 2025. Donors were HLA-identical siblings (n=2) and MUD (n=10).

Results: Twelve patients underwent 13 transplants due to non-engraftment in one recipient. Median age at HSCT was 8.5 months (3 months – 6.6 years). Ten out of twelve recipients survived over 1 year with a median follow–up of 6.2 years (12 months–10 years). The median time for neutrophil engraftment was 18 days (9-37 days). After one year, the median number of CD4 cells was 685 (121–3014 cells/μL). Full chimerism was observed in 4 patients, while mixed chimerism in 6 patients. The OS of the entire cohort was 83.3% at 1 and 5 years. A non-significant difference in OS was observed between HLA-identical siblings (100%, n=2) and MUDs (80%, n=10) (p=0.516). Event-free survival of this cohort was 75% due to two toxic deaths and one non-engraftment.

Conclusion. Santaros Klinikos serves as a referral center for pediatric stem cell transplantation in two Baltic countries, but the number of SCID-HSCTs remains small. Raising awareness of SCIDs, early diagnosis, timely referral to the HSCT program, and accumulating experience have contributed to excellent OS from MUD during the last five years.

2.5. INCREASED LEVEL OF TYPE II INTERFERON IN A PATIENT WITH RAS-ASSOCIATED AUTOIMMUNE LEUKOPROLIFERATIVE DISEASE: A CASE REPORT

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RAS-associated autoimmune leukoproliferative disease (RALD) is a rare inborn error of immunity caused by pathogenic variants in either the NRAS or KRAS gene in hematopoietic cells. It is characterized by lymphoproliferation, autoimmunity, granulocytosis, and monocytosis (Tangye et al., 2024). Activation of the type I interferon pathway has been observed in these patients (Volpi et al., 2021); however, no publications on type II interferons in RALD are available in the literature.

Objective: To report an increased level of type II interferon (IFN-γ) in a patient with RALD.

Results: We present the case of a 30-year-old patient with genetically confirmed RALD due to a pathogenic KRAS variant (c.64C>A, p.Gln22Lys). The patient exhibited hypogammaglobulinemia, multiple autoimmune diseases (type 1 diabetes, autoimmune thyroiditis, celiac disease), and recurrent infections. To assess the response of SARS-CoV-2 spike protein-reactive T cells, an IFN-γ release assay (QuantiFERON SARS-CoV-2, Qiagen, Germany) was performed. The test revealed a high baseline IFN-γ level of 3.04 SV/ml. While no defined normal reference range exists for the baseline IFN-γ level in this assay, the median IFN-γ level in a cohort of 15 healthy controls was 0.064SV/ml (IQR = 0.074), and in a cohort of 36 patients with predominantly antibody deficiencies, the median level was 0.060 SV/ml (IQR = 0.088).

Conclusion: This report highlights the potential role of a broader spectrum of interferons in the inflammatory manifestations of RALD.

2.6. RECURRENT INFECTIONS IN CHILDREN WITH ASTHMA AND ALLERGIC RHINITIS: A DIAGNOSTIC CHALLENGE

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Background. Recurrent respiratory infections in children with asthma and allergic rhinitis (AR) often raise concerns about underlying immunodeficiency. However, it is essential to differentiate between allergic inflammation and true immune dysfunction to avoid unnecessary investigations and ensure appropriate management. Primary immunodeficiencies are extremely rare, and in children with an atopic history, attention should always be given to allergic rhinitis and asthma. This highlights the need to reconsider the possibility of overdiagnosis of pneumonia in the country

Methods. Sixty-four children with recurrent respiratory infections and suspected asthma or AR were evaluated in Ternopil, Ukraine, over a period of 6 months. The children were divided into two groups: those under 5 years (n=26) and those aged 6–12 years (n=38). The diagnostic approach included spirometry for children aged 6 years and older (except for one child who could not perform the maneuver), therapeutic trials for younger children, skin prick testing (SPT), specific IgE testing, and an immunological assessment of both humoral and cellular immunity. Results In terms of clinical history, 84,4% (n=54) of the children had a history of at least two pneumonia episodes, 75% (n=48) had recurrent secretory otitis media, and 56,3% (n=36) had recurrent purulent otitis media. Sensitization to aeroallergens was observed in 84,4% (n=54) of the children, with the most common allergens being house dust mites (90,7%, n=49), Alternaria (14,8%, n=8), and grass and birch pollen (20,4%, n=11). The immunological assessment revealed normal findings in all children, excluding primary immunodeficiency. Regarding the diagnosis of asthma, spirometry confirmed asthma in 86,5% (n=32) of children aged 6 years and older, while a positive therapeutic trial confirmed asthma in 33,3% (n=9) of children under 5 years.

Conclusion. Recurrent infections, such as wheezing and otitis, may be manifestations of bronchial asthma and allergic rhinitis. It is essential to conduct a thorough differential diagnosis, as these symptoms can also be associated with immunodeficiencies and other conditions, although these are extremely rare. Furthermore, it is important to carry out additional studies to assess the extent of pneumonia overdiagnosis in Ukraine, to ensure accurate diagnosis and appropriate management.

2.7. RAS-ASSOCIATED AUTOIMMUNE LEUKOPROLIFERATIVE DISEASE IN LATVIA – A REPORT OF TWO CLINICALLY DISTINCT CASES

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RAS-associated autoimmune leukoproliferative disease (RALD) is a rare immune dysregulation syndrome caused by somatic gain-of-function mutations in NRAS or KRAS genes.

Objective. To report our experience in diagnostics, clinical manifestations, management of RALD patients.

Methods. Patient 1 is 6-year-old female with initial complaints of generalized lymphadenopathy, hepatosplenomegaly that developed 2 years after? suspect EBV infection, multiple thyroid nodules (TIRAD 4b-c) were also found in further examinations. Biopsies of the cervical lymph nodes and one thyroid nodule revealed no evidence of malignancy. Diagnostic exome sequencing of the lymph node biopsy material and blood was performed and a gain of function variant in the KRAS gene (c.64C>A,p.Gln22Lys) in 33% of the reads was found. The variant is known as oncogenic in tumours, considering that malignancy was excluded, the diagnosis of RALD was made.

Patient 2 is a 27-year-old female with type 1 diabetes, celiac disease, chronic autoimmune thyroiditis and recurrent infections. Due to Decreased IgG and IgA levels, she was diagnosed with common variable immunodeficiency (CVID) at the age of 12 years and received immunoglobulin replacement therapy, she continued to experience recurrent upper airway infections, which were primarily managed in outpatient settings. At the age of 27, next-generation sequencing (NGS) and genetic testing identified a pathogenic somatic NRAS variant (NM_002524.5:c.35G>T, p.(Gly12Val)) with an allele frequency of 30%. This led to a revised diagnosis of RALD.

Conclusions. These 2 cases demonstrate different phenotypes of and highlights the role of genetic testing in the diagnosis of PID.

2.8. FAMILY CASE OF HEREDITARY ANGIOEDEMA

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Hereditary angioedema (HAE) is a rare genetic disorder that causes recurrent episodes of severe swelling in the skin, gastrointestinal tract, and airways. It is usually caused by a deficiency or dysfunction of the C1 inhibitor protein, leading to excessive bradykinin production. Swelling attacks can be painful and potentially life-threatening if they affect the airways.

Case Presentation: A 65-year-old woman consulted an immunologist-allergist with complaints of recurrent swelling all over her body without a clear trigger. Antihistamines and systemic steroids were ineffective in treating these episodes, which typically resolved within 2-3 days after onset. She had experienced these symptoms since childhood, but they had become more pronounced with age. Comprehensive allergy testing did not identify any causative factors. Additionally, the patient reported episodes of acute abdominal pain occurring spontaneously. The pain syndrome did not respond to nonsteroidal anti-inflammatory drugs

(NSAIDs) or antispasmodics. She had been hospitalized in a surgical ward on multiple occasions; however, no acute surgical pathology was found, and surgical intervention was deemed unnecessary.

The patient's daughter, who is 46 years old, was present during the consultation. During the medical history interview, it was found that she also experienced periodic swelling throughout her body, including on her face. There was one episode of acute abdominal pain for which no cause could be identified. Standard treatment for the described symptoms had also proven ineffective. Both mother and daughter were referred for laboratory testing of hereditary angioedema. Based on the results showing low levels and activity of the C1 inhibitor protein, clinical information, and family history, they were diagnosed with hereditary angioedema type 1.

They are currently receiving targeted treatment for their symptoms with the C1 inhibitor protein, administered intravenously twice a week. Their quality of life has significantly improved, with complaints occurring on rare occasions.

Conclusion: This clinical case underscores the importance of physicians maintaining a high index of suspicion for hereditary angioedema (HAE) in patients with recurrent angioedema, mainly when standard therapy is ineffective. A history of acute abdominal pain and similar symptoms in close relatives should further raise suspicion. Early diagnosis of HAE significantly improves patients' quality of life. It reduces the risk of fatal airway edema, as effective targeted therapies are available, enabling patients to achieve near-complete symptom control.

2.9. SEVERE CONSEQUENCES OF CONCOMITANT B. PERTUSSIS AND M. PNEUMONIAE INFECTIONS IN A TWO-MONTH-OLD PREMATURE INFANT: THYMIC INVOLUTION OR CLINICAL MANEFASTATION OF INNATE ERRORS OF IMMUNITY?

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This report discusses a lethal case of a two-month-old premature infant battling dangerous simultaneous infections caused by B. pertussis and M. pneumoniae. This situation raises critical questions about the link between acute thymic involution and the potential for underlying innate errors of immunity (IEI) in premature infants.

Background: in critically ill premature infants, it is crucial to differentiate between thymic involution resulting from infections and the possibility of IEI. Understanding these distinctions is key to providing accurate diagnoses and effective management.

Methods: the evaluation of the infant involved comprehensive clinical assessments, laboratory testing, imaging studies, and advanced diagnostic techniques, including ELISA and PCR for B. pertussis and M. pneumoniae. Morphological analyses were conducted to identify any structural abnormalities in the thymus and spleen.

Results: At the time of admission, the infant exhibited severe symptoms, including intense coughing and significant breathing difficulties. Pertussis was diagnosed with complicated course by pneumonia. Laboratory tests confirmed B. pertussis with an IgM level of 1.35 IU/l and M. pneumoniae at 2.3 x 10^3 copies/l. Despite following clinical protocols, the infant's condition deteriorated after 19 days, leading to

respiratory failure and other severe complications. Resuscitation efforts were unsuccessful, and the post-mortem examination revealed significant structural abnormalities in both the thymus and spleen.

Conclusions: This case underscores the serious effects of simultaneous bacterial infections in a premature infant, potentially worsened by underlying T-cell insufficiency. It emphasizes the need for thorough screening for IEI in similar cases to improve patient management and outcomes.

2.10. NEWBORN SCREENING FOR SEVERE T- AND B-CELL INBORN ERRORS OF IMMUNITY IN UKRAINE: 2-YEAR EXPERIENCE

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The expanded neonatal screening (NS) for 21 diseases including SCID and severe B-cell lymphopenias, started in Ukraine in October 2022 in two centers (Lviv, Kyiv), and since April 2023 after other two centers (Kharkiv, Kryvyi Rih) joined, the entire territory of Ukraine has been covered, with the exception of the territories occupied by Russia. The study aimed to present the results of implementing NS for severe T- and B-lymphocyte deficiencies in Ukraine over the first 2 years.

Methods. Data on screening results were collected from four centers of NS in Ukraine. Each of the centers is responsible for conducting screening from several regions of Ukraine, thus, the distribution is carried out in accordance with the geographical location, logistical capabilities and corresponding capacities of the screening centers. In 2 years, 350 thousand babies were born in Ukraine. TREC/KREC/SMN1 marker was used to determine T-cell and B-cell deficiency. New sample tests were needed for 0.13% of neonates. In patients with positive screening after two tests the immunological investigation and the whole genome sequencing are performed.

Results. In total, 31 newborns had positive results and needed referral to confirm the diagnosis: 7 neonates with low TRECs, 17 – KRECs, and 8 – TRECs/KRECs. SCID was confirmed in two neonates: IL2RG deficiency (HSCT at 2.5 months, uneventful) and RAG1 (HSCT at 3.5 mon, uneventful). In one child infantile T-cell lymphopenia (FOXN1) was detected and one patient with severe T-lymphocytopenia and neutropenia suspected for reticular dysgenesis is in process. In patients with low KRECs, 2 cases of XLA were diagnosed, 2 – ARA, and 1 patient is born from the mother with B-cell lymphoma who received rituximab during pregnancy with negative genetic result. In addition, three cases of Nijmegen breakage syndrome were confirmed. Eighteen infants are being followed, mostly with low KRECs.

Conclusions. The implementation of NS in Ukraine using TREC and KREC assay made it possible an early diagnosis of IEI in 10 children, not only SCID but also other severe immunodeficiencies with T- and/or B-lymphopenia allowing early observation and treatment.

2.11. BRIDGE OR ABYSS? CHALLENGES IN THE TRANSITION OF PATIENTS WITH INBORN ERRORS OF IMMUNITY TO ADULT CARE

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INTRODUCTION. Inborn errors of immunity are a heterogeneous group of disorders with various complications, comorbidities, and a need for long-term therapy, requiring special attention during care transfer. The lack of a coordinated transition care model increases the risk of treatment discontinuation, complications, and higher hospitalization rates.
The aim of this study was to identify the main challenges encountered by patients and their families in Poland during the transition from pediatric to adult immunology outpatient clinics and to develop recommendations for optimizing this process.
MATERIAL AND METHODS. The analysis included data collected on the basis of questionnaires completed by adult patients after transitioning to adult care (n=23, aged 20 to 40 years)) and their parents (n=11).
RESULTS. While 60% of patients and 75% of parents confirmed that they had been prepared for the transition in their pediatric center, none of the respondents participated in a structured transition program. The most frequently reported challenges included: lack of coordinated medical documentation transfer (39% of all answers complained about receiving only fragmentary medical records); insufficient collaboration between healthcare centers (30% of all answers); long waiting times for the first appointment (18% of patients, 27% of parents).
CONCLUSION. Based on the study findings, optimizing the transition care process in Poland requires systemic changes, including: standardization of patient transition, improve documentation transfer and development of individualized transition plans, ensuring direct consultation between pediatric and adult physicians during the critical transition period, reducing waiting times for the first appointment in adult clinics.

3. STERILE INFLAMMATION AND AUTOIMMUNITY

3.1. CHARACTERISTICS OF KAWASAKI DISEASE IN UKRAINE: A SINGLE-CENTER EXPERIENCE IN 2024

Yuriy Stepanovskyy[1], Yuliia Klymyshyn[2], Lina Kostenko[3]

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Kawasaki Disease (KD) is acute pediatric vasculitis and a leading cause of acquired heart disease in children. It commonly presents with fever of unknown origin, mucocutaneous inflammation, and a risk of coronary artery complications if untreated. KD remains underdiagnosed in Ukraine, with an estimated incidence of 5–10 cases per 100,000 children under five years old. Kyiv Children's Hospital #1 (KCH1) plays a key role in KD awareness, timely diagnosis, and treatment, making its data valuable for national extrapolation.

Methods: A retrospective analysis of 14 patients diagnosed with KD and admitted to KCH1 in 2024 evaluated demographics, clinical presentation, treatment, and outcomes.

Results: The median age at diagnosis was 39 months (range: 1–93), with 7 boys and 7 girls. All received intravenous immunoglobulin (IVIG) 2 g/kg, with one requiring a second dose and one experiencing self-limited KD. The average hospital stay was 7 days. Severe cases were more frequent in infants. Atypical presentations mimicked urinary tract infection, acute abdomen, retropharyngeal phlegmon, and infective endocarditis. Coronary artery lesions (CAL) were observed in 50% (7/14), including dilation and small-to-medium aneurysms, but no giant aneurysms. After six months, CAL resolved in 43% (3/7) of these patients.

Conclusion: The estimated KD incidence in Kyiv in 2024 was 10–15 per 100,000 children under five, aligning with European data. Larger cohort studies and long-term follow-up are needed to understand KD in Ukraine further. Increased awareness and early diagnosis remain crucial for improving outcomes.

3.2. GRAVES' DISEASE IN A 62-YEARS-OLD MAN WITH MILD OPHTHALMOPATHY: IS IT POSSIBLE TO GET DISEASE COMPENSATION?

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Introduction: This case report describes a 63-year-old man diagnosed with Graves' disease and mild endocrine ophthalmopathy, which is more common in women. The patient first visited an ophthalmologist in November 2023 after his family noticed that his left eye was bulging more than the right. The ophthalmologist diagnosed unilateral exophthalmos of unknown cause and recommended further tests. The patient was referred to an endocrinologist for evaluation.

Background: Graves' disease is an autoimmune disorder that affects the thyroid and can cause eye problems due to immune-related inflammation. Thyroid diseases are more frequent in women, but when they occur in men, they can be more severe. Understanding the role of thyroid-stimulating antibodies (TSAb) and inflammation in Graves' disease is important for proper treatment, especially when eye involvement is present.

Case Presentation: During the visit to the endocrinologist, the patient reported a weight loss of 18 kg over six months, which he linked to dietary changes. He also mentioned occasional dizziness when changing position.
Physical examination showed no major abnormalities except for protrusion of the left eye. Further laboratory and imaging tests were done, including: Thyroid-stimulating hormone (TSH), Free thyroxine (T4 free), TSH receptor-stimulating antibodies (TRAb), Thyroid ultrasound.

Results and Diagnosis: The results confirmed Graves' disease and mild endocrine ophthalmopathy.

Treatment and Follow-up: The patient was treated with thiamazole, with a gradual dose reduction under T4 free monitoring, along with sodium selenite for six months.
At the December 2024 follow-up, his condition was much improved: No signs of exophthalmos in the left eye, Normal thyroid function (TSH, T4 free, and TRAb within normal limits), Weight increased by 10 kg compared to the initial visit.
Thiamazole treatment was stopped, and after two months, follow-up tests showed stable thyroid function.

Conclusion: This case highlights the importance of early diagnosis and proper evaluation in patients with unexplained eye protrusion. Graves' disease is an autoimmune condition where antibodies overstimulate the thyroid, leading to thyroid overactivity and eye inflammation. While thyroid diseases are more common in women, this case shows that men can also experience serious complications. Correct treatment led to full recovery of eye symptoms and stable thyroid function. Long-term follow-up is important to prevent relapse.

3.3. SLPI PROTEIN AS A POTENTIAL REGULATOR OF NEUTROPHIL MATURATION AND MIGRATION

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Neutrophils are key effectors of the innate immune system, and their development and function are tightly regulated by various factors, including Secretory Leukocyte Protease Inhibitor (SLPI). This multifunctional protein is primarily recognized for its anti-inflammatory properties. However, our research demonstrates that SLPI also plays a significant role in regulating neutrophil maturation and migration.

Methods: SLPI knockout (KO) mice from two age groups (8–10 weeks and 14–16 weeks) were used in this study. Neutrophil maturation was analyzed using flow cytometry with surface markers c-kit and Ly6G to distinguish five developmental stages: myeloblast (MB), promyelocyte (PM), myelocyte (MC), metamyelocyte (MM), and mature neutrophil (PMN). Additionally, CD16/32, CD34, and CD48 markers were used to examine the Granulocyte-Macrophage Progenitor (GMP) population. Neutrophil migration was first assessed in fresh-frozen mouse skin tissue via fluorescence microscopy, utilizing specific markers: Ly6G (neutrophil marker), CD45 (leukocyte marker), and Hoechst (nuclear dye). These data were complemented by intravital microscopy-based skin imaging in early stages of psoriasis development.

Results and Conclusions: SLPIKO mice aged 14–16 weeks exhibited an increased number of mature neutrophil forms (PMN and MC) compared to wild-type (WT) mice, whereas no differences were observed in the 8–10-week-old group. Although both age groups of SLPIKO mice showed a trend toward an

increased number of GMP cells, this difference did not reach statistical significance. The enhanced granulopoiesis observed in SLPIKO mice may be attributed to various factors, including altered bone marrow egress efficiency and compensatory mechanisms. Furthermore, under chronic inflammatory conditions in early stages of psoriasis, SLPI KO mice exhibited a delayed influx of neutrophils into the skin compared to WT mice. This aberrant neutrophil migratory behavior in SLPIKO mice may be associated with the role of SLPI in facilitating neutrophil migration across the vascular barrier.

3.4. RECOMBINANT FIBRINOGEN BN-DOMAIN: IMMUNOMODULATORY PROPERTIES AND INFLUENCE ON LEUKOCYTE TRANSENDOTHELIAL MIGRATION

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The βN-domain of fibrinogen has been linked to immune regulation, particularly its potential role in leukocyte transendothelial migration. Its inhibitory properties suggest involvement in modulating inflammatory responses, though the underlying molecular mechanisms remain unclear. Current hypotheses propose that βN-domain peptides may interact with endothelial receptors, such as VE-cadherin, or influence VLDLR-dependent signaling, potentially affecting immune cell trafficking. Understanding these interactions is essential for advancing immunomodulatory therapeutic strategies targeting chronic inflammatory conditions.

This study aimed to obtain and characterize a recombinant βN-domain fragment to facilitate further research into its immunological properties. Competent BL21 Rosetta cells were transformed with pCAL-n_β1-66 plasmid DNA via electroporation, followed by selection and expression under optimized conditions. After ultrasonication, the lysate underwent SDS-PAGE and Western blot to confirm molecular weight and specificity. A turbidimetric assay at 350 nm assessed the effect of β1-66 on fibrin clot formation.

Analysis confirmed successful expression of the β1-66 fragment, with electrophoresis revealing a band of the expected molecular weight and Western blot verifying specificity. Turbidimetric analysis indicated a negative impact on fibrin clot formation.

The production and initial characterization of β1-66 provide a foundation for further research into its immunological functions. Its interactions with endothelial receptors require deeper investigation to clarify its regulatory effects. Future studies will assess its potential anti-inflammatory properties and therapeutic relevance in experimental models.

3.5. SYK SIGNALING IN ANTIGEN PRESENTATION AND NLRP3 INFLAMMASOME ACTIVATION IN MACROPHAGES STIMULATED BY VIRUS-LIKE PARTICLES AND THEIR IMMUNE COMPLEXES

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The inflammasome is a key component of innate immunity, with NLRP3 being the most well-characterized. NLRP3 inflammasome activation triggers the release of proinflammatory cytokines, such as IL-1β, and induces pyroptosis. Spleen tyrosine kinase (SYK), a non-receptor kinase involved in immunoreceptor signaling, has been implicated in regulating NLRP3 inflammasome activation. Our previous research demonstrated that viral antigens and immune complexes (ICs) composed of these antigens and specific antibodies activate the NLRP3 inflammasome in macrophages. This study aimed to determine the role of SYK in IC-induced NLRP3 inflammasome activation in microglia.

Primary murine microglia were treated with spherical virus-like particles (VLPs) of human WU polyomavirus and their ICs formed with murine IgG of different subtypes. NLRP3 inflammasome activation was assessed by measuring cell viability, IL-1β and TNF-α secretion, and ASC speck formation. SYK activity was inhibited using R406 to evaluate its role. Protein expression and activation were analysed via Western blot and flow cytometry. IC phagocytosis was confirmed with fluorescent microscopy.

Our results show that VLPs and their ICs activate SYK, while R406 effectively blocks SYK activation, NLRP3 expression, ASC speck formation and cytokine secretion, indicating inhibition of NLRP3 inflammasome activation. ICs induced a stronger inflammatory response than VLPs alone. In conclusion, our findings suggest that ICs amplify the inflammatory response in microglia via a SYK-dependent pathway.

3.6. SYK-DEPENDENT ACTIVATION OF MICROGLIAL CELLS WITH VIRAL ANTIGENS AND THEIR IMMUNE COMPLEXES

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Viral antigens and immune complexes (IC) of viral antigens and their specific antibodies can activate innate immune cells and induce inflammation, but the mechanisms are poorly understood. Splenic tyrosine kinase (SYK) is a non–receptor tyrosine kinase that plays an important role in immune receptor signaling in immune and inflammatory responses. SYK has been linked to the formation and activation of the NLRP3 inflammasome. SYK causes ASC phosphorylation and oligomerization, which drives the assembly of the NLRP3 inflammasome. In this research, we investigated macrophage activation by IC and its association with SYK. We used primary murine microglia as a cell model, virus-like particles (VLPs) of polyomavirus (PyV) capsid protein as antigens, and a collection of murine monoclonal antibodies against VLPs. The inflammatory response was investigated by analyzing viability, inflammatory cytokine secretion, synthesis, and activation of the main test proteins (SYK and NLRP3). To examine the influence of SYK on the cellular response of primary microglia we used specific SYK inhibitor R406. After exposure of cells to viral antigens or IC their viability did not change, but the secretion of inflammatory cytokines (i.e. TNF-α and IL-1β) was stimulated. Moreover, NLRP3 protein was expressed and ASC particles were formed, which confirmed that microglial cells were activated, an inflammatory response – NLRP3 inflammasome activation was stimulated. While using R406, the opposite effects of microglia activation were observed. This proved that VLPs and IC induced SYK-dependent inflammatory response signaling pathways, secretion of inflammatory cytokines, and activation of NLPR3 inflammasome in murine microglia.

3.7. THE PATHOPHYSIOLOGICAL LINK BETWEEN THYROID AUTOIMMUNITY AND FEMALE INFERTILITY: A LITERATURE REVIEW

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Thyroid autoimmunity (TAI), characterized by the presence of thyroperoxidase (TPO) and/or thyroglobulin (Tg) antibodies, affects nearly 10% of reproductive-aged women and is linked to infertility — the inability to conceive after 12 months of regular unprotected sexual intercourse.

The objective was to summarize literature from the past five years on the association between autoimmune thyroid diseases and female infertility, examining potential pathophysiological mechanisms. A keyword search for relevant literature was performed in PubMed database and Google Scholar.

According to recent literature, TAI-induced infertility is explained by two pathophysiological immune dysfunctions: humoral and cellular. In humoral pathway, anti-thyroglobulin (TgAb) and anti-thyroperoxidase (TPOAb) cross the blood-follicle barrier during ovarian maturation, damaging follicles via antibody-mediated cytotoxicity. Additionally, structural similarities between zona pellucida and thyroid tissue might provoke cross-reactivity of TPOAbs with human chorionic gonadotropin receptors, affecting oocyte fertilization and embryo implantation. Moreover, according to Zhu et al., infertile women with TAI generally have elevated non-organ-specific antibodies, such as antiphospholipid antibodies, which reduce trophoblast viability and syncytialization. Alternatively, cellular mechanism of TAI disrupts T cells, where due to exacerbated Th1/Th2/Th17 inflammatory responses excessive cytokines are produced, leading to implantation failures, overreactive natural killer (NK) cells' further activation and endometrial inflammation caused by enhanced cytolytic activity.

Latest medical literature suggests two main pathophysiological pathways for TAI-induced infertility, both contributing to a hostile environment for conception, making TAI a significant factor for female infertility.

3.8. ANTI-OVARIAN ANTIBODIES: IMPACT ON REPRODUCTIVE HEALTH AND FERTILITY. A SYSTEMATIC REVIEW

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Anti-ovarian antibodies (AOA) are a diverse class of antibodies against the granulosa membrane, theca interna, ooplasm, zona pellucida, and lutein cells of the ovary. AOA play an important role in reproductive disorders, such as premature ovarian failure (POF) and infertility.

This systematic review examines the association between anti-ovarian antibodies and female reproductive disorders. The literature search was performed using the PubMed database.

Some studies suggest that AOA play a role in autoimmune-mediated POF. A prospective controlled trial (2013) detected AOA in 20% of POF patients but none in controls, while another study (2005) identified AOA targeting the zona pellucida in 66.6% of idiopathic POF cases. However, other findings suggest that AOA may not be a definitive marker of autoimmune POF but rather a consequence of the disease.

Moreover, evidence suggests that AOA is associated with infertility. Higher AOA levels have been observed in in-vitro fertilization patients compared to fertile women. Evidence on the association between AOA and poor ovarian response in assisted reproductive technology cycles remains inconsistent. While some studies report a higher prevalence of AOA in poor ovarian response patients and suggest immune-inflammatory changes induced by AOA, others find no significant difference in AOA levels between poor ovarian response and control groups.

Evidence suggests that AOA may significantly contribute to POF and infertility. Further studies are needed to clarify the role of AOA in female reproductive disorders, which could improve diagnostic and therapeutic approaches in reproductive medicine.

3.9. COMPARATIVE IMMUNE PROFILING OF BLOOD AND UTERINE SAMPLES IN FEMALE UNEXPLAINED INFERTILITY

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Unexplained infertility remains a major challenge in reproductive medicine, with emerging evidence suggesting an immunological component, which may interfere with implantation and early pregnancy. This study aimed to evaluate immune cell profiles in blood and endometrial samples to determine their diagnostic potential in unexplained female infertility.

Methods: Samples were obtained from three groups: women with unexplained infertility (GR1M), women with defined non-immunological infertility (GR2M), and healthy controls (GR3M). Multi-parameter flow cytometry was utilized to characterize immune cell phenotypes of blood and endometrial tissue, focusing on helper and cytotoxic T-lymphocytes, NK and NKT cells, monocytes, B-lymphocytes and granulocytes (neutrophils and eosinophils).

Results: A total of 43 women were included in the study: GR1M (N=20), GR2M (N=6), GR3M (N=17). An AI-driven classification model incorporating up-sampled data managed to achieve 95% accuracy, identifying CD8+ T cells and NKT cells as key predictors of infertility based on blood cytometry. However, endometrial samples exhibited suboptimal quality and inconsistent cytometry results that neither correlated with blood cytometry findings, nor contributed to a reliable diagnostic model.

Conclusion: While blood-based immune profiling integrated with AI modeling offers diagnostic potential for identifying immunological signatures in unexplained infertility, endometrial cytometry currently lacks reliability. Further research should focus on optimizing uterine sampling techniques, refining storage conditions, and advancing expertise in endometrial cytology to enhance its diagnostic utility.

3.10. OPTMIZING A T-CELL PANEL FOR A SPECTRAL FLOW CYTOMETER

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T-cells play a critical role in maintaining immune system function, protecting the body from infections and diseases. Broadly, T-cells are divided into two categories: cytotoxic T-cells, which can induce apoptosis in infected cells, and helper T-cells, which activate other immune cells to coordinate the immune response. These categories can be further subdivided into smaller subpopulations, each with specialized functions. These subpopulations can be distinguished from one another by detecting the receptors and proteins on their surface and within, through immunophenotyping.

For years, conventional flow cytometry has been the standard for immunophenotyping, but its limited in the number of fluorochromes it can simultaneously detect. Spectral flow cytometry overcomes this by using an advanced optical system that captures the full emission spectrum of each fluorochrome, enabling better differentiation. This allows the construction of larger, more informative panels. The data gained from a single sample using spectral flow cytometry could significantly improve the diagnosis and monitoring of various diseases.

This project aimed to develop a comprehensive T-cell panel for spectral flow cytometer. Peripheral blood mononuclear cells were used to create and optimize two panels—one for surface markers and another for intracellular markers. The optimization process involved determining the binding preferences of 68 antibodies and identifying the optimal titers for 50 of them. In total, the panels enable the identification of 35 distinct T-cell populations.

These comprehensive T-cell panels have great potential in both clinical and research settings, enhancing understanding of immune responses in different conditions and contributing to advancements in immunological diagnostics.

3.11. PRECLINICAL EVALUATION OF RECOMBINANT HB-EGF GEL: SAFETY AND BIOCOMPATIBILITY

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Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a multifunctional protein with potent anti-inflammatory and regenerative properties. As a key modulator of the resolution process of sterile as well as non-sterile inflammation, HB-EGF influences immune cell activation, mitigates excessive inflammatory responses, and facilitates tissue repair. Its therapeutic potential is particularly relevant in conditions driven by non-infectious inflammation, such as chronic ulcers and autoimmune dermatological disorders.

This study assessed the regenerative efficacy of recombinant HB-EGF in an in vitro fibroblast model (NIH-3T3, L929) and evaluated the safety and biocompatibility of an HB-EGF-containing gel in preclinical models.

Recombinant HB-EGF was expressed in E. coli, purified by affinity chromatography, and incorporated into a hyaluronic acid-based gel. Biological activity was assessed through fibroblast proliferation assays. The gel's safety was tested in rabbits, mice, and guinea pigs, monitoring for irritation, redness, and swelling. All animal experiments followed institutional, national, and EU guidelines.

The HB-EGF-containing gel demonstrated excellent biocompatibility. The primary irritation index was negligible (0.333 in rabbits, 0.121 in mice). Sensitization was evaluated using the Buehler test and scored by the Magnusson and Kligman scale (ISO 10993-10), with all guinea pigs receiving a 0 – no visible signs.

These findings confirm the safety and compatibility of recombinant HB-EGF for topical use, supporting its non-toxic nature in preclinical settings. This study lays the groundwork for further research into HB-EGF's clinical applications in regenerative medicine and inflammatory skin treatments. Further research is warranted to explore its potential in autoimmune and inflammatory skin disorders.

3.12. DRUG ALLERGY TO ANTIBIOTICS

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Drug allergy is part of adverse drug reactions and belongs to those pathological reactions that are unpredictable, do not depend on the dose of the drug (type B adverse reactions) and are based on immunological mechanisms. It is an extremely heterogeneous phenomenon characterized by etiological, pathogenetic and clinical polymorphism. Among the population, drug allergy is observed in 2% of cases, among categories that are frequently and long-term treated - in 5-15%, and among medical workers - up to 30%. More often, drug allergy is caused by antibiotics, among which the undisputed leaders are β-lactams. Other antibiotics (macrolides, fluoroquinolones, tetracyclines, etc.) cause drug allergy much less often. The problem of cross-reactivity between antibiotics with similar antigenic determinants is especially relevant for penicillins and cephalosporins of the first generation, as well as the aminoglycoside group. This problem is not relevant for penicillins, monobactams and carbapenems and is not well studied for macrolides and fluoroquinolones. Clinical manifestations of drug allergy depend on the mechanisms of development of hypersensitivity reactions to antibiotics. It is necessary to collect a pharmacological history from patients before prescribing any drugs, especially antibiotics. The doctor should carefully maintain medical documentation at all stages of the treatment of a patient with adverse drug reactions, and the optimal solution for the doctor in the presence of an allergic reaction in the anamnesis should be the appointment of an alternative drug with similar pharmacological properties, but with a different chemical structure. It is not necessary to establish a diagnosis of drug allergy solely on the basis of laboratory test data, a significant part of which is not validated in Ukraine, condemn patients to a lifelong "label" of such a diagnosis and deprive them of the opportunity to be effectively treated with drugs that are not causally significant.

3.13. UNCOVERING ALLERGY TRENDS: SENSITIZATION TO NUTS AND LEGUMES IN THE LITHUANIAN POPULATION

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The molecular sensitization patterns to nuts and legumes in Lithuania have not been extensively studied.

Method: A retrospective analysis of specific IgE results was conducted on 1715 patients using the ALEX2 macroarray, assessing sensitization to tree nuts and legumes (soy and peanuts).

Results: Sensitization to at least one allergen was identified in 1190 (69.39%) patients. Bet v 1 sensitization was observed in 527 (44.29%). Stable molecular allergens (MA's) of tree nuts and/or legumes were detected in 252 (21.18%) patients, with 218 (18.32%) sensitized to stable tree nut MA's and 153 (12.77%) to stable legume MA's. Co-sensitization to both tree nut and legume MA's was present in 118 patients, while 34 were sensitized only to legume MA's and 100 only to tree nut MA's.

Among the 252 sensitized patients, the most common sensitizations were walnut (66.27%), hazelnut (57.54%), peanut (51.59%), cashew (34.92%), pistachio (34.13%), soy (29.37%), macadamia (13.10%), and Brazil nut (7.14%). A significant difference in the frequency of sensitization to stable MA's was found between adults and children ($p<0.001$), with children more often affected. Pearson's correlation indicated a significant negative association between age and sensitization to stable MA's of peanut, soy, cashew, Brazil nut, hazelnut, walnut, macadamia, and pistachio ($p<0.001–0.002$), suggesting younger individuals are more likely to be sensitized.

Conclusion: While birch remains the primary sensitizer in Lithuania, stable tree nut and legume MA's play a crucial role, particularly in children, highlighting the need for further investigation.

3.14. UKRAINIAN MOTHERS' AWARENESS OF ALLERGENIC FOOD INTRODUCTION

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Food allergies affect 4–6% of children, with many reacting to multiple allergens. This study examines Ukrainian mothers' awareness of allergenic food introduction and its long-term effects.

Materials and Methods: A survey of 165 mothers assessed knowledge on allergenic food introduction, first aid, information sources, and personal experiences.

Results: Most first-group mothers (77.1%) were under 35, while 60.7% in the second group were over 36. Allergies were more common in the second group (32.1% vs. 3.2%, $p<0.01$). Social media influenced 69.7% of first-group mothers, with 23.9% adjusting diets, while 58.9% of second-group mothers used books and 73.2% made independent decisions. 82.6% of first-group mothers introduced allergens between 6–12 months (vs. 58.9% in the second group, $p<0.05$); 8.9% of second-group mothers avoided allergens. First-group mothers introduced cow's milk (83.5%), seafood (69.7%), and nuts (68.8%) more often than the second group ($p<0.05$). Cow's milk allergy was higher in the second group (19.6% vs. 4.6%, $p<0.05$). 83.5% of first-group mothers introduced peanuts before one year, compared to 58.9% in the second group ($p<0.05$). In emergencies, 23.2% of second-group mothers preferred dexamethasone, while 10.1% of the first group did. For severe reactions, 67% of first-group mothers and 50% of the second sought medical help.

Conclusions: Maternal age and family structure affect allergenic food introduction. Younger mothers are more influenced by social media, while older mothers tend to be more cautious. Education strategies tailored to maternal experience are needed to improve allergenic food introduction practices.

3.15. GENERATION OF RECOMBINANT ANTIBODY TARGETING THE HONEYBEE VENOM ALLERGEN Api M 3 USING THE GENETIC MATERIAL DERIVED FROM A SINGLE B CELL

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In clinical allergy diagnosis, serological tests measuring allergen-specific IgE levels play a crucial role. However, quantitative serological diagnostic tests have drawbacks in reproducibility. Better standardised and cheaper calibrators are required. Recombinant chimeric antibodies are homogenous in specificity and affinity and can be reproducibly generated in unlimited quantities. Thus, they would make for great calibrators of such systems. Using genetic information from a single B cell would greatly increase their production efficiency, owing to the omission of the time-consuming hybridoma generation part used traditionally.

Considering the importance of novel calibrators, this study aimed to produce chimeric antibodies substituting natural human antibodies against bee venom allergen Api m 3 utilising genes encoding immunoglobulins from a single mouse B lymphocyte.

To achieve this, antibody variable sequences from Api m 3 specific mouse single B cells were determined by PCR and sequencing. Their sequences were cloned into expression vectors coding genes for the domains of human constant heavy IgE and light kappa chains and transfected into HEK293 cells. Expression of recombinant chimeric antibodies to Api m 3 was successfully confirmed by ELISA using recombinant allergen and secondary antibodies against the constant domain of human IgE.

This study successfully determined Api m 3 specific antibody genes from a single B cell (3C6B7). The mouse-human chimeric antibodies of IgE class against Api m 3 allergen were developed in this study from single B cells genetic material. This type of antibody is a potential candidate for further research in developing novel calibration systems for serological allergy assays.

3.16. DEVELOPMENT OF CHIMERIC ANTIBODIES MIMICKING HUMAN IgE AGAINST HOUSE DUST MITE MAJOR ALLERGENS Der P 2 AND Der P 23

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House dust mite (HDM) allergy affecting approximately 65-130 million people worldwide ranks HDM as one of the leading indoor allergens. *Dermatophagoides pteronyssinus* is a prominent HDM species found in Europe. Proteins Der p 2 and Der p 23 are its major allergens. Quantitative serological assays measuring allergen-specific IgE levels are important tools for allergy diagnosis. However, quantifying allergen-specific IgE using human IgE antibodies derived from serum or plasma as calibrators presents several challenges. These include low quantities of source material, specificity variability, characterization constraints, etc. Thus, reliable calibrators for these diagnostic systems are required. Considering the importance, this study aimed to produce chimeric antibodies (CAbs) resembling natural human antibodies against Der p 2 and Der p 23.

In this study, previously generated murine hybridomas producing monoclonal antibodies (MAbs) against Der p 2 and Der p 23 were used. Four MAbs 10C12, 4G7, 5E12 and 2B4 against Der p 2 were characterized. All of which recognized both natural and recombinant Der p 2. Additionaly, MAbs 10C12, 4G7 and 5E12 were cross-reactive with Der f 2. MAb 4G7 against Der p 2 and MAb 18E4 against Der p 23 were chosen for CAb development. The variable regions of the MAb heavy and light chains were amplified, and sequenced. Vectors containing these sequences fused with human IgE or κ chains constant regions were constructed and transfected into mammalian cells for CAb expression and validation.

CAbs generated in this study can be further investigated for their potential application as calibrators for allergen-specific human IgE in allergy diagnostic systems.

3.17. TWO NOVEL, RAPID, AND SIMPLE ENZYME-LINKED IMMUNOASSAYS FOR C-REACTIVE PROTEIN, A CARDIOVASCULAR MARKER

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Blood circulating C-reactive protein (CRP) levels are prominently associated with inflammation. CRP is considered a pathogen recognition receptor that activates the complement immune pathway by triggering the C1q molecule. IL-6 regulates CRP expression, and it is involved in phagocytosis. More recent research suggests that CRP level plays a role in cardiovascular disease risk management, and early detection helps lower the cardiac failure event risk. Here, we present two simple and rapid ELISA methods developed for CRP quantification with high sensitivity. Because the methods presently used in clinical labs are not sensitive enough. Hence, direct and competitive ELISA has been developed to provide a broad and sensitive standard range of CRP.

The standard range for developed direct ELISA is 3.12-100 ng/mL with a sensitivity of 0.31 ng/mL. Using the sodium periodate oxidation method, the anti-CRP antibody was conjugated with horseradish peroxidase (HRP). The analytical validation included linearity, limit of detection, precision, repeatability, and accuracy were investigated and found within permissible limits. Competitive ELISA was developed using a magnetizable cellulose particle separation system. Forming a stable complex between cellulose and antibody. This makes separation easy and reduces the assay incubation period. The assay standard ranged from 3.12-100 ng/mL with a detection sensitivity of less than 2 ng/mL; standards were calibrated with WHO international standards. Assay parameters like recovery, cross-reactivity, parallelism, precision, inter-assay, and intra-assay variation were checked for analytical validation. The analyzed samples were compared with a commercial CRP kit, which showed similar results.

3.18. THE ASSOCIATION OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES WITH INFLAMMATORY PROTEINS IN RHEUMATOID ARTHRITIS PATIENTS

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Aim of the study – to identify the possible association between rheumatoid arthritis markers – autoantibodies to cyclic citrullinated peptide (anti-CCP) and inflammatory serum proteins.

Material and methods. Serum samples of 30 patients (57.5±14.9 years) with rheumatoid arthritis was studied and analyzed. Determination of the concentration of autoantibodies to cyclic citrullinated peptide (anti-CCP) was performed by ELISA. Serum samples with positive anti-CCP test result were analyzed for determination of protein fractions: agarose gel electrophoresis and NDS-PAGE gel electrophoresis were performed in order to assess anti-CCP interactions with inflammatory proteins. 10 subjects' blood serum samples with a negative anti-CCP test result were selected as the control group.

Results. Agarose gel electrophoresis fractionation of serum proteins showed a lower expression of the 60 kDa protein in anti-CCP-positive samples compared with anti-CCP-negative controls. Single proteins of ~90 kDa, 110–118 kDa and above 200 kDa were detected in several fractions. NDS-PAGE also detected a lower expression of the 60 kDa protein in anti-CCP-positive samples. Higher protein variation below 20 kDa was detected. NDS-PAGE data show that proteins with a molecular mass of 15 kDa (it is known as serum amyloid A molecular mass) appeared in anti-CCP positive samples.

Conclusions. Agarose gel electrophoresis showed no anti-CCP link to inflammatory proteins; NDS-PAGE gel electrophoresis showed a possible anti-CCP association with the acute-phase inflammatory protein – serum amyloid A.

3.19. THE SEROPREVALENCE AND ASSOCIATION OF ANTI-DFS70 ANTIBODIES WITH ANTINUCLEAR ANTIBODIES

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Objective and aim. Antinuclear antibodies (ANA) are used in diagnostics of systemic autoimmune rheumatic diseases (SARD). Evaluated by indirect immunofluorescence (IIF) dense fine speckled (DFS) fluorescence pattern is associated with antibodies to DFS70 (70kDa protein). A distinctive clinical association of anti-DFS70 antibodies is still unclear. The aim of this study was to compare prevalence of anti-DFS70 antibodies between groups of patients with SARD and with non-autoimmune inflammatory conditions also to evaluate anti-DFS70 antibodies in association with other ANA.

Methods. 235 patients (41,7 ± 23,5 years old) were included in the study; two groups were distinguished based on the established diagnosis: patients with SARD (n = 117) and group of patients with non-autoimmune inflammatory conditions (n = 118). The blood serum of the patients was investigated for ANA by IIF and line immunoassay (LIA) (Euroimmun AG, Lubek, Germany).

Results. The prevalence of anti-DFS70 antibodies in patients with SARD and with inflammatory conditions was 24.8% and 18.6 %, respectively. There was no statistically significant difference in the prevalence of anti-DFS70 antibodies between compared groups. Concomitant ANA were found in 62.1 % of anti-DFS70 positive SARD patients group. Compared anti-DFS70 negative and anti-DFS70 positive patients with SARD, only anti-Jo-1 (Histidyl-tRNA synthetase) antibodies were significantly more prevalent in anti-DFS70 positive patients group (p = 0,013).

Conclusion. There was no statistically significant difference in the seroprevalence of anti-DFS70 antibodies between groups of patients with systemic autoimmune rheumatic diseases and with other inflammatory conditions. Statistically significant association was found between anti-DFS70 and anti-Jo-1 antibodies in patients with systemic autoimmune rheumatic diseases.

4. TUMOR IMMUNOLOGY

4.1. TRANSCRIPTOMIC PROFILING OF THE IMMUNE TUMOR MICROENVIRONMENT FOR ADVANCED CHARACTERIZATION OF DESERT, EXCLUDED, AND INFLAMED MELANOMA TUMORS

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The spatial distribution of tumor-infiltrating lymphocytes (TILs) defines several histologically and clinically distinct immune subtypes—desert (no TILs), excluded (TILs in stroma), and inflamed (TILs in tumor parenchyma). While our previous research has established a framework for such immune subtyping in melanoma, further validation in real-world datasets is essential. In this study, we aimed to provide a comprehensive description of the immune tumor microenvironment and transcriptome profiles in a previously subtyped melanoma cohort. We performed BRB-seq on tumor samples from 48 real-world melanoma patients. Using CIBERSORTx deconvolution algorithm, we analyzed the transcriptomic data to quantify the proportions of different infiltrating immune cell subpopulations. These immune signatures were then compared with previously defined immune subtypes (desert, excluded, inflamed) and correlated with clinical parameters and outcomes of the patients. The immune cell deconvolution algorithm identified distinct immune cell populations within the tumor microenvironment of each subtype. Differential gene expression analysis revealed unique transcriptomic signatures with specific pathways enriched in desert, excluded, and inflamed tumors. These results support and refine the classification of melanoma into distinct immune subtypes and highlight the importance of immune cell composition in predicting clinical outcomes. The findings support using immune subtypes as biomarkers to improve patient stratification in clinical practice.

4.2. NOVEL ISOLATION AND ACTIVATION PLATFORM WITH ACTIVE-RELEASE TECHNOLOGY FOR SCALABLE CELL THERAPY MANUFACTURING

Laura Kaščicaitė-Radzivilova, H. Meås, M. T. Gabriel, S. Kjær, K. Bernstrøm, N. Guadagno, R. Hartberg, D. T. Gjølberg, I. N. Moen, J. M. Rasmussen, N. N. Moharrami, S. Metavne, N. R. Nilssen, I. C. Schrøder, A. Javmen, L. Zaliauskienė, J. Kern, L. Kapus, H. Adams, P. Hermans, E. Klijs, L. Sierkstra, E. Kang, H. C. Vebø, T. H. Hereng, and H. Almåsbak.

Thermo Fisher Scientific

Gibco™ CTS™ Dynabeads™ CD3/CD28 are regarded as the gold standard in clinical CAR-T cell manufacturing for simultaneous isolation, activation, and expansion of T cells. They enable research, development and manufacturing of commercial CAR-T cell drugs, including the first FDA approved CAR-T immunotherapy Kymriah™.

Dynabeads CD3/CD28 selectively target and bind cells co-expressing CD3 and CD28 in heterogenous cell populations. In addition to the antibody-coated Dynabeads providing the activation and co-stimulatory signals required for T cell activation and expansion, their paramagnetic property allows the Dynabead-bound cells to be captured on a magnet while unwanted cells are removed. The isolated Dynabead-bound

cells have then traditionally been placed in culture for a minimum of 5 days to allow for the passive dissociation of the cells from the Dynabeads before their subsequent magnetic removal. Removing the Dynabeads at earlier time points is possible but can result in reduced cell yields as the Dynabeads are not fully dissociated from the cells. This presents a challenge for manufacturing processes that require downstream applications to be carried out shortly after isolation. Building upon our well established Dynabeads and CaptureSelect™ offerings, our new CTS Detachable Dynabeads CD3/CD28 can similarly provide simultaneous isolation and activation of T cells while also allowing full flexibility over the timing of bead removal.

The new Detachable Dynabeads technology platform employs an innovative and highly effective active release mechanism. The Detachable Dynabeads are coated with streptavidin and conjugated with the variable domain of camelid heavy-chain only antibodies (VHH ligands) that target and efficiently bind to specific surface markers. The active release mechanism is subsequently based on the VHH ligands being conjugated to a low-affinity biotin derivative, which allows the beads to be actively detached from target cells at any desired timepoint through competition with a biotin buffer.

Detachable Dynabeads CD3/CD28 are optimized for use with Gibco™ CTS™ DynaCellect™ Magnetic Separation System, thus allowing for full automation and scalability of the protocol in process development and clinical manufacturing.

4.3. EXERCISE-INDUCED MODULATION OF TUMOR BIOLOGY ENHANCES CHEMOTHERAPY OUTCOMES IN BREAST CANCER

Diana Skorinkina[1], Kristaps Eglītis[2], Pawel Zayakin[1], Agata Mlynska[3], Beatriz Martin-Gracia[4,5], Eva Bassols-Citores[4,5], Lilite Sadovska[1], Agnese Brokāne[1], Fabian Rise[4,5], Krizia Sagini[4,5], Silvana Romero[4,5], Konstantinos Gkelis[6], Marija Boguševiča[1], Signe Folkmane[1], Mārtiņš Čampa[7], Rūdolfs Cešeiko[7], Aija Kļaviņa[7], Inta Liepniece-Karele[8], Jānis Eglītis[2], Karina Silina[6], Alicia Llorente[4,5,9], Aija Linē[1,10]

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A growing number of studies show that exercise is safe for cancer patients, improving physical and cognitive functions, quality of life, and disease outcomes. However, the molecular mechanisms remain poorly understood. We investigated the effects of high-intensity interval training (HIIT) in breast cancer (BC) patients undergoing neoadjuvant chemotherapy (NAC).

The HIIT group had a significantly higher response rate than both prospective (80% vs. 47%, p = 0.036) and retrospective controls (80% vs. 50%, p = 0.047). Proteomics identified 2 plasma proteins induced by exercise at diagnosis and 20 after completing HIIT/NAC, five of which had not been previously linked to exercise. RNA sequencing revealed exercise-induced tumor gene expression changes favoring less aggressive phenotypes, improved outcomes, and a reprogrammed wound-healing process. Stromal cells, including fibroblasts and endothelial cells, played key roles in sensing and responding to exercise. CIBERSORTx analysis showed HIIT tumors were infiltrated with activated NK cells, unlike the resting

NK cells in controls. Additionally, decorin protein levels in plasma and tumor tissue were upregulated in HIIT patients, correlating with better NAC response and prognosis.

Our study provides novel insights into the molecular mechanisms behind exercise's protective effects on BC progression. These findings support incorporating exercise into BC treatment and highlight the need for clinical trials on HIIT's impact on immunotherapy efficacy.

4.4. THE LINK BETWEEN HYPOXIA AND PARPS IN COLORECTAL CANCER CELLS AFTER EXPOSURE TO IONIZING RADIATION

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Colorectal cancer is the second leading cause of cancer-related deaths worldwide. Radiotherapy, often combined with chemotherapy, is a common treatment strategy for this disease. However, its effectiveness is limited by therapy resistance and damage to adjacent normal tissues. Targeting specific molecular pathways could enhance treatment efficacy.

Poly (ADP-ribose) polymerase (PARP) proteins are of particular interest in cancer therapy. The human PARP family consists of 17 proteins that regulate various cellular processes, such as DNA repair and immune response. While several PARP inhibitors are already used in clinical settings, they have not yet been approved for the treatment of colorectal cancer.

Our previous studies have shown that the expression of several PARP genes increases in 3D colorectal cancer cell models after exposure to fractionated-dose ionizing radiation. A similar effect was observed in rectal cancer tumor samples after patients underwent chemoradiotherapy. Some of these PARPs are known immune response regulators. Notably, the knockout of a specific PARP gene in colorectal cancer HT29 cell line activated the innate immune response and resulted in increased sensitivity to ionizing radiation.

Since both 3D cell models and the tumor microenvironment exhibit hypoxia, this study aimed to determine a possible association between hypoxia and PARP gene expression after irradiation. We assessed hypoxia levels in clinical samples based on the expression of hypoxia signature genes. For cell models, colorectal cancer cell lines HT29 and DLD1 were cultured under normoxic or hypoxic conditions and exposed to ionizing radiation. Then, the link between hypoxia and PARP gene expression was determined.

4.5. THERAPEUTIC PROGRAMMING OF TUMOUR-ASSOCIATED MACROPHAGES BY ALPHAVIRAL VECTORS COMBINED WITH PHOTODYNAMIC TREATMENT IN MOUSE BREAST CANCER MODEL

Ksenija Korotkaja, Zhanna Rudevica, Dace Skrastiņa, Juris Jansons, Karina Spunde, Anna Zajakina

Latvian Biomedical Research and Study Centre

Reprogramming macrophages within the tumour microenvironment (TME) is a promising strategy for improving cancer treatment outcomes. Tumour-associated macrophages (TAMs) often support tumour progression, but their functional plasticity presents an opportunity for therapeutic reprogramming. Alphaviruses, such as Semliki Forest virus (SFV), are efficient vectors for gene delivery due to their broad tissue tropism, transient but strong transgene expression, and immunostimulatory properties. However, optimising their therapeutic efficacy remains a challenge. In this study, we investigated the combination of SFV-mediated IFNγ gene delivery with photodynamic therapy (PDT), a treatment modality that generates reactive oxygen species, induces immunogenic cell death, and enhances antitumor immune responses. We hypothesised that PDT could create a favourable immune environment, improving the efficacy of IFNγ gene delivery and TAM programming to M1 phenotype. Our results demonstrate that combining alphavirus therapy with PDT leads to a synergistic effect, significantly enhancing tumour growth inhibition in a murine model. Mice receiving the combined treatment exhibited markedly smaller tumours compared to those treated with either therapy alone. The combination therapy also led to increased immune activation, suggesting enhanced antitumor immunity. These findings highlight the potential of integrating alphavirus-based gene delivery with PDT to improve cancer immunotherapy. Further investigations will help refine this combinatorial approach and explore its translational potential in clinical settings.

4.6. DEVELOPMENT OF ALPHAVIRAL VECTORS EXPRESSING HTLV-1 ENV AND THEIR SYNCYTIA-INDUCING EFFECTS IN TUMOUR CELLS

Darija Lapina, Zhanna Rudevica, Anna Zajakina, Karina Spunde

Latvian Biomedical Research and Study Centre

The vector-based expression of immunomodulatory genes within the tumour is a promising therapeutic strategy. Semliki Forest Virus (SFV) recombinant vectors infect tumour cells, induce apoptosis, and promote their lysis, releasing antigens that enhance immune recognition. However, the SFV efficacy is limited by challenges related to targeted delivery, distribution, and expression within tumour tissues. Human T-lymphotropic virus type I (HTLV-1) elaborates the glucose transporter GLUT1 for cell adsorption and fusion, leading to HTLV-1 distribution through syncytium formation. The overexpression of GLUT1 is discovered in most cancers, therefore can be utilised for cancer cell fusion . In this study, we propose a novel approach using SFV-mediated gene delivery and distribution by cell fusion mechanism (syncytia) induced by the HTLV-1 hyperfusogenic envelope (env) gene variant. This strategy aims to enhance SFV replicon spread and immunogenic cell death in tumour. The SFV-1 vector was utilized for the insertion and expression of both the wild-type (WT) HTLV-1 env gene and its hyperfusogenic truncated mutant (D8). The specific fusogenic effect induced by the HTLV-1 env glycoprotein was assessed by infecting HeLa and 4T1 cell lines with recombinant viruses. Crystal violet staining was used to assess syncytia formation, revealing that SFV/HTLV-env D8 variant exhibited greater cell fusion ability than the WT. The study demonstrates that alphavirus-mediated delivery of hyperfusogenic env gene induces syncytia formation in cancer cells and potentiates cytotoxic effect of SFV infection.

4.7. THE ROLE OF AUTOPHAGY IN REGULATING THE SURFACE EXPOSURE OF B7 FAMILY PROTEINS IN COLORECTAL CANCER CELLS

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Colorectal cancer (CRC) is the third most common malignancy worldwide and is the second leading cause of cancer-related deaths. Regardless of enhanced screening and therapy, CRC remains a clinical challenge due to chemoresistance and immune evasion driven by tumor adaptation mechanisms.

Macroautophagy (hereafter autophagy) is a cellular degradation and recycling process that maintains cellular homeostasis by eliminating misfolded proteins and damaged organelles. However, in cancer, specifically in the context of anticancer treatment, autophagy functions as a survival mechanism. Our research on chemoresistant CRC cells has shown an upregulation of key autophagy-related proteins, ATG7, ATG12, p62. Besides promoting cell survival, autophagy impacts immune evasion by regulating the surface exposure of immunomodulatory proteins. The B7 family proteins, such as PD-L1, CD80, ICOSLG, are essential signals for T cell activation and immune response modulation. Our preliminary results show that chemoresistant CRC cells exhibit increased expression of these proteins, suggesting a link between autophagy and immune escape.

In this study, we aim to determine the impact of autophagy inhibition on the exposure of PD-L1, CD80 and ICOSLG proteins on the surface of chemoresistant sublines of CRC cell line HCT116. Our preliminary findings suggest that the silencing of ATG7, ATG12, ATG14 or ATG16L expression is the most efficient in reducing autophagy. Therefore, we have derived chemoresistant sublines of HCT116 cells with reduced expression of these core autophagy genes by implementing lentiviral transduction to deliver specific shRNAs. The results will uncover the molecular details underlying autophagy's impact on the cell surface exposure of B7 family proteins.

4.8. DEVELOPMENT OF A 3D SPHEROID MODEL SYSTEM FOR IMMUNE RESPONSE STUDIES IN GLIOBLASTOMA

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Glioblastoma is classified as a WHO grade IV malignant glioma. This highly aggressive tumor is linked to a poor prognosis, with a median survival of around 15 months after diagnosis. Researchers are actively exploring potential biomarkers for diagnosis and prognosis to improve patient outcomes. The combination of 3D cell culture models and gene expression analysis allows for a deeper understanding of complex cellular regulatory networks, some of which may be key contributors to tumor progression and disease prognosis.

This study aimed to develop a 3D spheroid cell model system for immune response studies in glioblastoma cells. To achieve this, spheroids were cultivated from two glioblastoma cell lines: primary and secondary-U87 and A172. The expression of immune response genes was analysed on days 2 and 6 using RT-qPCR. The obtained data were analysed using GraphPad v.9.

The results indicate significant immune response gene expression differences between the U87 and A172 glioblastoma cell lines. Notably, IL1β expression was found to be upregulated in U87 cells, whereas it was

downregulated in A172 cells, showing different signalling pathways. Additionally, CCL20 expression on day 6 showed a statistically significant difference between U87 and A172 cells.

It hypothesizes that the U87 cell line is a suitable model for studying immune responses in glioblastoma, as it exhibits active cytokine expression. In contrast, A172 cells do not respond to inflammatory stimuli in the same manner and follow distinct signalling pathways. In conclusion, the U87 3D spheroid model demonstrates significant potential for immune response studies in glioblastoma research.

4.9. sPD-L1 AND sPD-1 INTERACTION WITH ANDROGEN RECEPTOR: IMMUNE REGULATION AND ITS ROLE IN PROSTATE CANCER DIAGNOSIS

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Membrane PD-L1 interacts with androgen receptor (AR) signaling, potentially influencing immune evasion mechanisms in prostate cancer (PCa). This study evaluates the diagnostic performance of urinary AR mRNA and its enhancement when combined with plasma sPD-L1 and sPD-1 for clinically significant PCa (csPCa) classification.

Methods: A cohort of 68 PCa patients was analyzed, with sPD-L1 and sPD-1 levels quantified by ELISA. Urinary mRNA transcripts of AR, PSMA, and PCA3 were measured using RT-qPCR. ROC curve analysis was performed to assess the area under the curve (AUC) for various biomarker combinations across ISUP grade and pathological stage risk groups.

Results: In ISUP-classified groups, AR mRNA alone demonstrated an AUC of 0.65, whereas in combination with sPD-L1 and sPD-1 together, increased the AUC to 0.97, achieving 100% sensitivity and 95% specificity. In the pathological stage groups, the combination improved the AUC from 0.65 to 0.81, outperforming PCA3 combination (AUC 0.78). Notably, sPD-1, likely for the first time, was shown to enhance the diagnostic performance of other parameters, as its combination with AR mRNA demonstrated significant improvement, while it did not enhance the performance of PCA3 or PSMA. No correlation was observed between sPD-L1/sPD-1 and AR mRNA, nor between soluble immune checkpoints and PSMA, PCA3.

Conclusion: The combination of urinary AR mRNA with plasma markers sPD-L1 and sPD-1 significantly enhances the detection of clinically significant PCa, offering high sensitivity and specificity. These findings highlight the crucial role of AR signaling and immune checkpoint molecules in PCa progression, and emphasize the potential of immune-related biomarkers to improve diagnostic strategies.

4.10. P62 PROMOTES COLORECTAL CANCER CELL CHEMORESISTANCE BY MODULATING PRO-SURVIVAL CYTOKINES

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Colorectal cancer (CRC) ranks third for incidence and second for mortality rate worldwide. Advanced CRC is treated with a combination of cytotoxic chemotherapeutic agents 5-fluorouracil (5-FU) and oxaliplatin (OxaPt). However, cancer cells can acquire resistance to chemotherapy, reducing treatment efficacy. The general mechanisms underlying chemoresistance are currently known, but the role of specific factors and proteins remains poorly characterized.

p62 is a selective autophagy receptor, but due to its multidomain structure, it also has role in tumorigenesis by regulating NF-κB signalling, inflammation, cell proliferation, survival, angiogenesis, and cytokine expression in the tumour environment. Elevated p62 levels have been detected in CRC patients. As p62 regulates both cell survival and death, it is a promising target of chemoresistance. The impact of p62 on the molecular causes of chemoresistance in CRC cells is insufficiently analysed.

Therefore, we aimed to investigate the role of p62 in resistance to 5-FU and OxaPt in human CRC cell lines. We used colorectal carcinoma cell line HCT116 and its chemoresistant sublines HCT116/FU and HCT116/OXA, which had acquired resistance to 5-FU and OxaPt. Our data revealed that p62 silencing decreased amount of HCT116, HCT116/FU and HCT116/OXA cells, reduced caspase-3 activation, changed amount of cytokines IL1A, IL6, CXCL8 and receptor CXCR2 transcripts, also reduced IL-8 and CXCR2 proteins expression. Our results show that p62 promotes chemoresistance in CRC cells by stimulating pro-survival cytokine signalling, suggesting p62 relevance as a therapeutic target.

4.11. INFLUENCE OF STEMNESS INHIBITORS ON THE IMMUNOMODULATORY PROPERTIES OF COLORECTAL CANCER

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Colorectal cancer (CRC) is a heterogeneous disease characterized by a diverse tumor microenvironment comprising immune and non-immune components. Acquired mutations and phenotypic plasticity in the tumor microenvironment create a diversity of cells whose interactions contribute to tumor progression and create resistance to anticancer therapy through various activated mechanisms. At the molecular level, four subtypes of colorectal cancer (CMS1-4) have been validated, differing in their aggressive characteristics and immune infiltration. With the rapid development of oncology science and the development of new cancer treatment strategies, more attention is given to the microenvironment surrounding cancer cells. Since the tumor is densely infiltrated by host immune cells, especially macrophages, it could be a promising target for developing new treatment strategies. This study aims to explore how inhibition of CRC cell stemness properties with specific inhibitors could modulate macrophage polarization, shifting the immune balance from protumoral M2 type to the antitumoral M1 type. We evaluated the effect of four stemness inhibitors - salinomycin, SB-431542, JIB-04, and napabucasin - on CMS4 CRC cell lines (HCT116 and SW620) and human peripheral blood-derived macrophages in an indirect co-culture model. Our results demonstrate that CMS4 CRC cell lines induced distinct macrophage polarization patterns, with HCT116 promoting M2 macrophages and SW620 leaning toward M1 profile. The combination of stemness inhibitors reduced stemness markers (CD133, CD44) in CRC cells and shifted macrophage polarization toward an M1 phenotype, particularly in co-culture with HCT116. These findings suggest that stemness inhibitors not only target cancer cells but also modulate the immune microenvironment by reprogramming macrophages.

4.12. ROLE OF B7 FAMILY PROTEINS IN THE CHEMORESISTANCE OF COLORECTAL CANCER CELLS

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Colorectal cancer is one of the most common malignancies worldwide and the third leading cause of cancer related deaths. Chemoresistance remains an unresolved problem in the anti-cancer treatment. It can be caused by a variety of reasons: changes in drug transport and metabolism, modification of drug targets, activation of DNA repair, changes in cellular death or survival signaling.

Immune checkpoint inhibitor therapy is one of the potential approaches to address the problem of chemoresistance. This therapy acts by different mechanisms than chemotherapy – it restores the immune system's ability to recognize cancer cells by blocking the immune checkpoint proteins that are overexpressed on the surface of cancer cells and inhibit their recognition. Cancer cells that are resistant to the initiation of the cell death may activate molecular pathways that not only confer chemoresistance but may also modulate the expression of immune checkpoint proteins.

In this study we have evaluated the changes in the expression of eight B7 family immune checkpoint proteins in the chemoresistant colorectal cancer cells. We have determined that the levels of several B7 family protein transcripts are differentially expressed in HCT116 and DLD1 cells which have acquired chemoresistance to 5-fluorouracil. Treatment with 5-fluorouracil or oxaliplatin also modulates the levels of B7 protein-coding transcripts in HCT116 and DLD1 cells. The importance of autophagy for immune checkpoint proteins exposition on the cell surface was determined by deriving cell lines expressing transcript-specific shRNA against various ATG proteins.

4.13. SIGNIFICANCE OF B7 FAMILY PROTEINS FOR COLORECTAL CANCER CELL SENSITIVITY TO CHEMOTHERAPY DRUGS

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Colorectal cancer is one of the leading types of cancer in terms of both the number of incidence and mortality worldwide. Despite the widespread practice of chemotherapy, the unresolved problem of chemoresistance encourages to look for alternative ways of colorectal cancer treatment. Immunotherapy has recently become one of the promising alternatives. Despite numerous improvements in this field, more research is still required to fully address its shortcomings.

Immune checkpoint inhibitors are one of the types of immunotherapy. They block the immune checkpoint proteins thus preventing inhibitory signaling from the cancer to immune cells. This inhibition returns the ability for the immune system to recognize tumor cells. It is known that in many cases of cancer there is an increased expression of these proteins. Furthermore, immune checkpoint proteins, including the ones belonging to B7 family, may have additional functions for cancer cells that are not related to immune suppression.

The aim of our study is to examine how the silencing of different immune checkpoint proteins belonging to B7 protein family affects chemoresistance of colorectal cancer cells. Silencing was achieved by introducing shRNA coding sequences targeting specific B7 protein transcripts into the genome of colorectal cancer cells HCT116. The efficiency of this method was evaluated on both transcript and protein levels. We have found that the silencing of several B7 family proteins increases sensitivity of colorectal cancer cells to chemotherapy drugs, suggesting the role of these proteins in chemoresistance.

4.14. EXPLORING THE TUMOR MICROENVIRONMENT THROUGH 3D OVARIAN CANCER SPHEROIDS

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The adoption of 3D cell culture models has significantly enhanced our understanding of tumour and its intricate microenvironment dynamics. While 2D models do not reflect tumour complexity, 3D models, such as spheroids, offer improved physiological relevance. However, they usually lack crucial tumour components like macrophages and fibroblasts. Innovative strategies, including co-culture systems and organoids, address this limitation by integrating diverse cell types, thereby offering a better representations. In our study, we optimized 3D culture conditions for four epithelial ovarian cancer (EOC) cell lines and their co-cultures with fibroblasts or THP1 monocytes. We noticed that the A2780 cell line did not form spheroids unless the fibroblasts were also present in the co-culture. Using qPCR, we compared gene expression profiles between 3D EOC cell models, co-cultures with fibroblasts, and conventional 2D cell culture models, focusing on stemness, epithelial-mesenchymal transition, and immune interaction genes. VEGF expression increased in SKOV3 and COV362 3D models, and POU5FA and SOX2 expression increased in SKOV3, COV362, and OV7 3D models. Additionally, flow cytometry analysis revealed variations in monocyte infiltration depending on the 3D culture model used, highlighting the importance of optimizing culture conditions for studying EOC and its microenvironment interactions.

4.15. MODELING THE IMMUNE TUMOR MICROENVIRONMENT IN SYNGENEIC 4T1 TRIPLE-NEGATIVE BREAST CANCER MOUSE MODEL

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Triple-negative breast cancer (TNBC) is the most aggressive subtype responsible for highest breast cancer related mortality. Lacking hormone receptors it consequently lacks a standardized treatment regimen. As a result, there is an urgent need for further research to improve therapeutic strategies.

Mouse models are valuable tools for studying cancer, however, their accuracy can be limited since the immune system plays a crucial role in tumor development. To address this, we hypothesized that injecting immunocompetent mice with syngeneic cancer cell spheroids could lead to formation of tumors with immune-enriched microenvironment in comparison to tumors generated using syngeneic cell suspension. Despite its potential, research on tumor modeling using spheroids remains limited, with most studies conducted in xenograft models.

In this study, we used the 4T1 TNBC cell line and syngeneic BALB/c mice to compare these injection methods. Spheroid formation was optimized, and preliminary in vivo data reveal differences in tumor growth patterns and immune cell infiltration, highlighting the potential for further investigation.

A more physiologically relevant tumour microenvironment could better reflect interactions between tumor cells and immune components, improving the predictive value of preclinical models. Our findings provide insights into how tumor injection methods influence the tumor microenvironment in an immunocompetent setting. By using a syngeneic model, this study could help improve the relevance of preclinical breast cancer research, particularly in understanding immune-tumor interactions.

4.16. IMMUNE CELL SUBPOPULATION CHANGES FOLLOWING ELECTROCHEMOTHERAPY: EFFECTS OF VARIOUS PROTOCOLS AND GOLD NANOPARTICLES

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Electrochemotherapy (ECT) is a pulsed power technique that facilitates the delivery of non-permeable drugs or other molecules into cells. This method involves the application of short, high-intensity electric pulses, which temporarily permeabilize the cell membrane (electroporation), increasing the cytotoxicity of drugs by 2-3 orders of magnitude. The primary focus was to characterize calcium and bleomycin ECT in vivo and determine the immune response elicited by these treatments. Additionally, the capabilities of gold nanoparticles to improve the treatment are investigated.

Balb/C mice with 4T1 tumors were used. Once the tumors reached the desired volume, calcium or bleomycin, with or without gold nanoparticles, was administered, followed by EP using various electric field protocols. At the end of the experiment, spleens and lymph nodes were collected for analysis using multi-color flow cytometry.

Our study demonstrated that both calcium and bleomycin ECT result in partial or complete response of 4T1 tumors. The induction of a systemic immune response after calcium ECT was indicated by an increased percentage of splenic central memory T cells, a decrease in CD4+ regulatory T cells in both the spleen and tumor-draining lymph nodes, and a reduction in myeloid-derived suppressor cells in the lymph nodes. In contrast, bleomycin ECT, with or without gold nanoparticles, resulted in an increased percentage of NK and NKT cells in the spleens and lymph nodes. ECT's immunomodulatory potential highlights its promise as a therapeutic strategy for metastatic disease and the development of durable anti-tumor treatments.

The research was supported by the Research Council of Lithuania (S-MIP-24-74).