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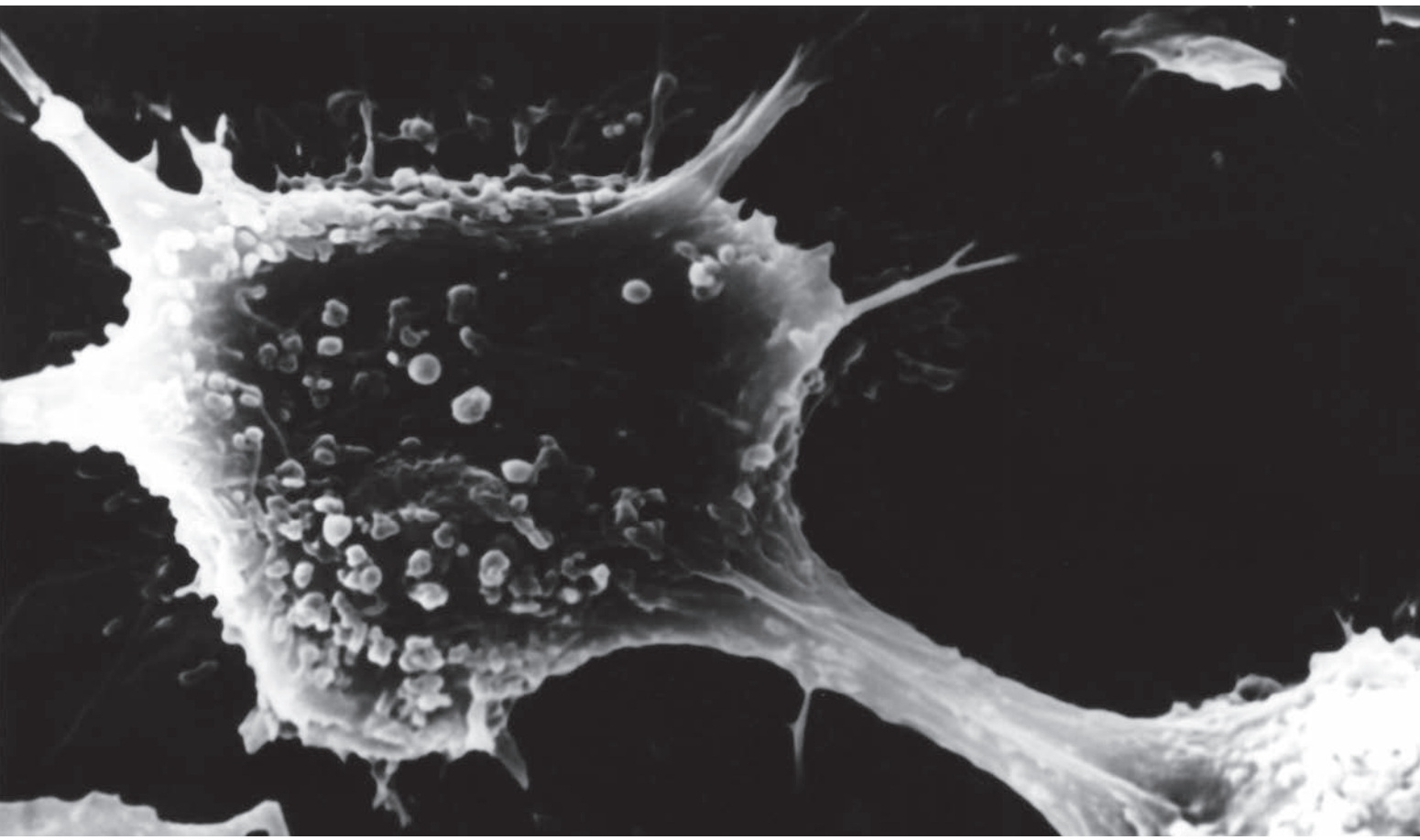

18th Congress of the Polish Society for Fundamental and Clinical Immunology
Białystok, 16-18 May, 2024

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18th Congress of the Polish Society for Fundamental and Clinical Immunology

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Dear Readers,

It is a great pleasure to present this special issue of the *Central European Journal of Immunology* (CEJI), dedicated to the 18th Congress of the Polish Society for Fundamental and Clinical Immunology, held in Białystok from May 16 to 18, 2024. This Congress marked another milestone in advancing immunological research, fostering collaboration among scientists, and translating discoveries into clinical practice.

The Congress contained a diverse array of topics, including, basic immunology, modern diagnostics, breakthroughs in biological treatment, innate and adaptive immunity, immuno-oncology, and the intricate interplay between the immune system and diseases of aging. These sessions highlighted cutting-edge research and encouraged lively discussions among leading experts, early-career researchers, and clinicians. Moreover, integrating advanced technologies, such as molecular cytogenetics, omics, spectral cytometry, and organoid models, emphasized the translational potential of innovation in immunology.

We are particularly proud to feature abstracts from oral and poster presentations, reflecting the vibrant and multidisciplinary nature of the Congress. These contributions exemplify the collaborative spirit of the Polish Society of Experimental and Clinical Immunology community and underscore the importance of knowledge exchange in tackling pressing immunological challenges.

A highlight of the event was the keynote lecture by Prof. Amiram Ariel, President of the Israeli Immunological Society, who presented groundbreaking insights into novel effectors for resolving inflammation and tissue fibrosis. Complementing the scientific program, the Congress also provided networking opportunities through social gatherings, fostering new collaborations and strengthening existing partnerships.

We extend our heartfelt thanks to the organizing committee, speakers, sponsors, and attendees who made this event a great success. We hope this special issue serves as a record of the Congress and a source of inspiration for future research endeavors.

On behalf of the scientific team, the organizing committee, and the editorial team, we invite you to delve into these abstracts and discover the innovative work that continues to shape the future of immunology.

Best regards,

Prof. Ewelina Grywalska, President of PTIDiK, Chair of the Scientific Committee
Prof. Maciej Kurpisz, Past President of PTIDiK, Chair of the Scientific Committee
Prof. Marcin Moniuszko, Chair of the Organizing Committee,
Rector of the Medical University of Białystok
Dr hab. Andrzej Eljaszewicz, Editor-in-Chief *Central European Journal of Immunology*

On behalf of the CEJI Editorial Team and the Polish Society of Experimental
and Clinical Immunology Congress Organizing Committee

Immunogenetics

Variations within immune checkpoint genes and soluble immune checkpoint isoforms as factors influencing bladder cancer risk and clinical outcome

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Introduction: Numerous data have shown associations between variants in genes encoding immune checkpoints (ICs) – crucial immune response modulators, with cancer risk, and worse clinicopathological features. Moreover, soluble forms of ICs have been documented to play an important role in cancer development and progression.

Aim: The aim of this study was to investigate the association between the presence of selected single nucleotide polymorphisms (SNPs) in genes coding for ICs with the risk of bladder cancer (BC) development and clinicopathological features, as well as to evaluate the levels of soluble forms of IC-related proteins in the serum samples of BC patients.

Methodology: Using TaqMan probes for 12 SNPs in 5 genes, including *TIM-3*, *LGALS9*, *BTLA*, *HVEM*, and *CD160*, we genotyped 314 BC patients and over 500 healthy controls (HC). Serum concentrations of sTIM-3, sGAL-9, sBTLA, sHVEM, and sLAG-3 were analyzed by the custom Human ProcartaPlex multiplex immunoassay in BC cases ($n = 47$) and HC ($n = 32$).

Results: Statistical analysis of genotype and allele distributions showed that specific *BTLA* SNPs may be associated with susceptibility to BC. In addition, selected SNPs in *BTLA*, *HVEM*, and *CD160* genes might increase BC risk in women, as well as correlate with worse clinicopathological features. Analysis of soluble ICs levels showed that levels of sTIM-3, sLGALS9, and sBTLA are significantly upregulated in BC cases compared to HC. Furthermore, sTIM-3 levels correlated with sLGALS9 and sBTLA serum levels in BC.

Conclusions: Our results indicate that SNPs within the IC genes may be risk and prognostic markers in BC. Moreover, we identified soluble IC-related proteins associated with the BC risk that could potentially be used as BC biomarkers in the future.

Basic immunology

Hemagglutination capacity of *Lagovirus europeaus* GI.1 – RHDV – strains Fr-2, ŻD, BLA, and selected indicators of natural immunity in experimentally infected rabbits

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Introduction: *Lagovirus europeaus* GI.1 – RHDV including its antigenic variant GI.1a – RHDVa, cause disease in rabbits worldwide, except in Antarctica, which has an acute course and almost 100% mortality and is similar to viral haemorrhagic fevers in humans. Among GI.1 – RHDV viruses, the majority are hemagglutination-positive (HA+), although there are also HA- and occasionally HA+/- strains, and which feature is considered a determining element on their pathogenicity. Within these viruses, immunotypes are also differentiated.

Aim: Effect of *Lagovirus europeaus* GI.1 – Fr-2, ŻD, BLA strains on the activity of selected indicators of natural immunity in experimentally infected rabbits.

Methodology: 40 rabbits (*Oryctolagus cuniculus*) were divided into 4 groups of 10 animals each and infected with each of 3 virus strains (Fr-2 – HA+, immunotype I; ŻD – HA+/-, immunotype III; BLA – HA-, immunotype II) prepared as lyophilisates that were dissolved in distilled water. The control group was 10 animals injected with distilled water. The 11 selected indicators of natural immunity associated with the peripheral blood PMN cell of rabbits were evaluated at: 0 h before infection and at 4, 8, 12, 24, 36, 48, 52, 56, 60 h after infection.

Results: Analyzing the changes within the studied indicators, 42 increases and 4 decreases were found with the Fr-2 strain, while 29 increases and 3 decreases were found with the BLA, and only 8 increases and 4 decreases were found with the ŻD.

Conclusions: The ability to hemagglutination (HA) of the 3 tested strains, confirms the influence of this feature on the indicators studied, as the Fr-2 (HA+) strain, caused more than 30% more changes compared to the BLA (HA-) and up to more than 70% compared to the ŻD (HA+/-). This would confirm the results of the Fr-2 (HA+) strain belonging to immunotype I of this virus.

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Viral infections and immunity

An inflammatory blast in the brain – SDAV, coronavirus story

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Introduction: Sialodacryoadenitis virus (SDAV) is a common etiological agent of infections in rats. As a synanthropic species, rats represent a potential source of pathogen transfer of viruses of the genus *Coronaviridae* causing potential zoonotic threat. The scant literature, consisting mainly of data from the last decades, has not sufficiently addressed the topic of SDAV infection, particularly CNS cell infection and the immune response to infection.

Aim: In the present study, we investigated the effect of Sialodacryoadenitis virus (SDAV) infection on mixed primary culture cells of microglia and astrocytes *in vitro*, emphasizing analysis of the expression levels of cytokines secreted in response to infection.

Methodology: The effect of SDAV infection on the production of reactive oxygen species, cytokines and chemokines in microglia and astrocyte culture cells were examined. The study was carried out using confocal microscopy and flow cytometry analyses, classic ELISA, and Luminex high-throughput method analysing 48 crucial cytokines and chemokines for neuroinflammation.

Results: We have noted that the level of pro-inflammatory cytokines – IL-2, IL-12, and IL-18 – remained relatively high after 4 days post-infection. Expression of anti-inflammatory cytokines, such as IL-4 and IL-10, was recorded only around day 4 after infection. Chemokine expression fluctuated throughout the infection period. The amount of secreted ROS was already high 2 hours after the virus entered the cell, 1000 times higher 24 hours after infection.

Conclusions: SDAV replication in mixed primary culture cells of microglia and astrocytes enhances the production of pro-inflammatory cytokines and inhibits the production of anti-inflammatory cytokines and leads to an increase in ROS expression.

This study was mainly financed by grant from the Ministry of Science and Higher Education of the Republic of Poland, decision no. 2021/41/N/NZ6/04383 and partially financed by grants decision no. 9506/E-385/R/2018 and 16/491568/SPUB/SP/2021.

Immunogenetics; Primary immunodeficiences; Immune disorders in pediatrics

Fifty shades of neutropenia: what we have learned about the molecular background of congenital neutropenia in 15 years of genetic studies

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Introduction: Congenital neutropenia (CN) is a group of rare primary immunodeficiences, characterized by impaired neutrophil maturation predisposing to life-threatening bacterial infections, myelodysplastic syndromes or acute myeloid leukemias. So far, nine types of severe congenital neutropenia (SCN) and numerous syndromes associated with chronic neutropenia have been identified, caused by defects in over 50 genes. Most often changes concern the ELANE gene, encoding neutrophil elastase.

Aim: The aim of the study was to explore the genetic background of CN in the polish pediatric patients and to demonstrate the impact of a nationwide information campaign on the efficiency of patient recruitment.

Methodology: The study included 122 patients with suspected congenital neutropenia recruited in 2008-2019 and 295 patients recruited in 2020-2023 as part of the project with a nationwide campaign. Molecular analyzes were performed using Sanger sequencing (87 patients) and targeted NGS (330 patients) in a panel of primary immunodeficiency-related genes.

Results: 142 patients (36%) received a molecular diagnosis, of whom 56 were diagnosed with SCN/CyN, 36 with other neutropenia-related syndromes and 50 patients were diagnosed with bone marrow failure or CGD,

ALPS, CVID and other inborn errors of immunity that may lead to autoimmune neutropenia. Anti-neutrophil antibodies were detected in 59 patients, but some of them also had a genetic defect. After opening the nationwide campaign, the number of recruited CN patients increased sevenfold.

Conclusions: The work summarizes 15 years of research on the causes of neutropenia and describes the largest group of patients with genetically confirmed neutropenia in Poland so far. The nationwide campaign opened the possibility of patient diagnostics and understanding the causes of neutropenia, which enabled appropriate treatment using GCSF or HSCT.

This study was supported by the Foundation for Polish Science (FNP) TEAM NET Programme, POIR.04.04.00-00-1603/18. Project title: Fix Neutropenia (FIXNET): focusing on neutrophil proteases defects which serve as novel diagnostic and therapeutic options.

Viral infections and immunity; Innate immunity

Human rhinovirus HRV16 and IL-33 may up-regulate inhibitory checkpoint molecule genes in the human lung microvascular endothelium

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Introduction: Human lung microvascular endothelium (ECs) is a target of multiple viruses and are the key regulators of the immune response and virion diffusion during virus infection. Inhibitory checkpoint molecules are related with inhibitory signalling pathways that maintain the balance between the immune response and immune tolerance, and its overactivation in cancer and viral infections inhibits T cell function.

Aim: The aim was to investigate whether the HRV16 infection, and in milieu of IL-33 or after IFN-b blocking, can upregulate inhibitory checkpoint molecules gene expression in ECs.

Methodology: ECs monolayers were treated with HRV16 and after incubation virus was removed and replaced by medium. Also, we performed experiments in milieu of IL-33 or after IFN-b blocking. Cell lysates were harvested in hour: 5, 24, 48, and 72 after infection, and RNA was isolated. Then mRNA expression was assessed using primers for PD-1, PD-L1, CTLA-4, TIGIT, LAG-3, HAVCR2, but also IFN-b, IFN-g, CCL5, TNF-a, CD80, CD86, HLA-DRA, IL-10.

Results: We observed that HRV16 increased the mRNA expression of PD-L1 and LAG-3 by the ECs. Furthermore, it caused a strong expression of inflammatory factors as IFN-b, CCL5, TNF-a, but no IFN-g. Moreover, the highest gene expression of HLA-DRA and CD80 was observed at 24 hours but no CD86 was noticed. We observed that IL-33 increase expression of PD-L1 and LAG-3 in HRV16 infected cells. Additionally, after IFN-b blocking and HRV16 infection we shown decrease of PD-L1 and LAG-3 expression in ECs, in comparison to infected cells.

Conclusions: Taken together, the results suggest that the HRV16 can upregulate some inhibitory receptors expression in ECs, especially in milieu of IL-33. However, it is still unclear whether ECs infected by the HRV16 affect the T cell immune response, especially in terms of the production of antiviral and cytotoxic proteins.

Case report

One patient – different diagnoses

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Introduction: Thrombocytopenia may be a symptom of many diseases, including as inborn errors of immiunity as well coagulation disorders.

Aim: Presentation of the case with overlapping two diagnoses and emphasizing that the diagnosis of one disease does not exclude the other diseases.

Methodology: Immunodiagnostics—tests including immunoglobulins' main class levels, IgG subclasses, specific antibody response to vaccines, and lymphocytes immunophenotyping, were performed between the 1st and 16th years of age in a child with familiar thrombocytopenia due to VWF gene mutation. In order to determine the diagnosis, a molecular genetic tests with Sanger method and whole exome sequencing were used.

Results: We present the case of a boy with congenital thrombocytopenia and recurrent infections, in whom no immune system disorders were initially detected. Subsequent tests revealed hypogammaglobulinemia, impaired antibody response to vaccines, IgG subclass deficiency, and low percentage and number of memory B cells. Molecular genetic analysis revealed a heterozygous variant of the VWF gene initially in the patient mother, grandmother and finally in the patient, indicating the diagnosis of von Willebrand disease type 2B. Due to recurrent infections and gradually occuring hypogammaglobulinemia and impaired

maturation of B lymphocytes additionally panel NGS was done, showing a heterozygous variant in the TNFRSF13B gene. The diagnosis of common variable immunodeficiency (CVID phenotype – TACI deficiency) was established in a child with von Willebrand disease type 2B.

Conclusions: Unexplained symptoms in a patient/patients with established diagnosis should inspire doctors to find out the other explanation.

Veterinary immunology and comparative immunology

Expression of genes related to autophagy in *Lagovirus europaeus*/GI.2 infection in rabbits

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Introduction: Autophagy is an important process for maintaining the homeostasis of organisms, and the role of this process has also been widely documented in viral infections. The links between autophagic flux and viruses are ambiguous, as the role of this process may constitute an antiviral mechanism of the host, but also it may promote viral replication and pathogenesis. Rabbit Haemorrhagic Disease (RHD) is a highly lethal disease caused by *Lagovirus (L.) europaeus*/GI.1 and GI.2. Activation of autophagy has been characterized during infection with GI.1 strains, but in RHD induced by GI.2 strains has not been studied so far.

Aim: The study aimed to determine the expression of genes (*Beclin-1*, *UVRAG*, *Atg12*, *Atg5*, *Atg16L*, *MAP1LC3B*) encoding key proteins involved in autophagic flux in the liver, spleen and kidneys of rabbits experimentally infected with *L. europaeus*/GI.2.

Methodology: The animal experiment included 20 rabbits divided into a control group (n = 10) and an experimental group infected with *L. europaeus*/GI.2 strain (n = 10). Relative gene expression was determined using quantitative real-time PCR and calculated using the Pfaffl method.

Results: We show that in the organs of rabbits from the experimental group, the expression levels of autophagic genes: *Beclin-1* (liver, spleen), *UVRAG* (liver, kidney), *Atg12* (liver, spleen, kidney), *Atg5* (liver, spleen, kidney), *Atg16L* (liver, kidney), *MAP1LC3B* (spleen, kidney) were increased compared to the control group.

Conclusions: Our results indicate that during *L. euopaeus*/GI.2 infection, there is increased expression of key genes for autophagy in rabbit organs. These results may suggest that autophagy is an important process in the context of Rabbit Haemorrhagic Disease induced by the GI.2 strain, but the activation and role of this process remain for further consideration.

Immunogenetics; Immunopathology of pregnancy

Association of genes polymorphisms encoding HLA class I antigen processing machinery proteins with susceptibility and severity of endometriosis

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Introduction: Endometriosis is a severe gynecological condition characterized by the presence of endometrial cells in an abnormal or ectopic locations outside the uterine cavity. Sampson's theory of retrograde menstruation suggests that endometriosis results from the backflow of endometrial cells through the fallopian tubes during menstruation. This process occurs in 90% of women, yet only 10% develop endometriosis, indicating the existence of a mechanism that prevents the immune system from clearing endometrial cells, disrupting its function, and leading to their implantation outside the uterus, forming lesions. Inability to form proper HLA class I – antigen complexes may influence disease development. Proteins associated with antigen processing machinery (APM) could hinder the immune response of CD8+ and NK cells, contributing to endometriosis.

Aim: Evaluation of the association of single nucleotide polymorphisms (SNPs) in APM genes (*ERAP1*, *ERAP2*, *LMP2*, *LMP7*, *LMP10*, *TAP1*, *TAP2*, *TAPBPR*, *LNPEP*) with endometriosis.

Methodology: 329 DNA samples from endometriosis patients and 380 controls were analyzed *via* Real-Time PCR for APM genes using TaqMan probes (30 SNPs).

Statistical analysis was performed using GraphPad InStat, R, and Python.

Results: Of the studied polymorphisms, 6 were associated with endometriosis: in *ERAP1* gene rs10050860 CC ($p = 0.05$), rs17482078 CC ($p = 0.035$), rs2287987 TT ($p = 0.044$), rs7063 AA ($p = 0.010$) and this last SNP correlated with III ($p = 0.0399$) and IV stage ($p = 0.0017$) of disease. In *TAP2* gene rs1800454 CC and rs241447 CC genotypes increased risk ($p = 0.019$, $p = 0.033$, respectively). Rs241447 CC correlated with III ($p = 0.0258$) and IV stage ($p = 0.0073$) of disease.

Conclusions: Studied polymorphisms showed associations with both disease susceptibility and severity.

This study was supported by a grant from the National Science Centre, Poland No. 2021/43/B/NZ5/00328

Autoimmunity and Autoinflammation; Immune tolerance

Extracellular vesicle-carried miR-150 suppresses delayed-type hypersensitivity reaction involved in TNBSA-induced colitis and collagen-induced arthritis in mice

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Introduction: Mouse models of trinitrobenzenesulfonic acid (TNBSA)-induced colitis and collagen-induced arthritis (CIA) are linked by the involvement of Th1 lymphocytes mediating delayed-type hypersensitivity (DTH). Our previous studies uncovered the immunoregulatory role of suppressor CD8+ T cells releasing extracellular vesicle (EV)-carried miR-150 against effector Th1 lymphocytes in DTH induced by haptens or protein antigens.

Aim: Therefore, we investigated the immunoregulatory activity of EV-carried miR-150 in both diseases.

Methodology: Colitis was induced in CBA mice by rectal administration of TNBSA in 50% ethanol and monitored daily. Some mice received intraperitoneal injection of EV-carried miR-150. To induce DTH in CIA, DBA mice were injected twice with collagen in physiological saline into abdominal skin. Five days later, collected DTH effector cells were treated with EV-carried miR-150 and transferred to naive DBA recipients that were intradermally injected with collagen in physiological saline into hind paws, and their swelling was measured 24-72 hours later.

Results: EV-carried miR-150 significantly alleviated active DTH reaction in TNBSA-induced colitis and adoptively transferred DTH in CIA. In both cases these effects were selectively blocked with anti-miR-150.

Conclusions: Our current findings suggest the possibility of therapeutic use of miR-150 in ameliorating the DTH response in the pathogenesis of Crohn's disease and rheumatoid arthritis. Therefore, our observations contribute to a deeper understanding of the pathogenesis of various autoimmune diseases and the development of new therapeutic strategies allowing precise downregulation of self-reactive Th1 lymphocytes.

Supported by Polish Ministry of Education and Science (N41/DBS/000781).

Autoimmunity and Autoinflammation; Immunodermatology

Th1 lymphocytes predominate over their Th17 counterparts in ear skin infiltrates in imiquimod- or IL-23-induced psoriatic inflammation in mice

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Introduction: In experimental studies on the still poorly understood pathogenesis of psoriasis, psoriatic dermatitis could be induced in mice by topically administered imiquimod (IMQ) or intradermally injected IL-23 activating autoimmune/autoinflammatory responses.

Aim: Current studies aimed to compare locally and systemically activated effector T cell subpopulations.

Methodology: IMQ was administered on mouse ear and shaved back skin for 7 consecutive days, while ten doses of IL-23 were injected into ear skin of C57BL/6 mice every other day. Immune cells infiltrating ear skin and draining lymph nodes were analyzed cytometrically, while cytokine production was measured by ELISA.

Results: While serum levels of IL-12/IL-23p40 were similar in IL-23- and IMQ-administered mice, the latter treatment resulted in higher IL-17A and IFN-γ concentrations in serum and in lymph node cell culture supernatants. In the ear skin, IFN-γ+ cells predominated among T lymphocytes in both models, while IL-17A+ T cells were more abundant in lymph nodes of IL-23-injected mice.

Conclusions: The currently observed local predominance of Th1 cells in the ear skin of IMQ-treated mice, and especially IL-23-injected mice, suggests their more im-

portant role in the early stages of psoriatic inflammation, leading to an IFN-γ-dependent activation of Th17 cells in draining lymph nodes. Consequently, both Th cell subpopulations drive each other's effector functions, which exacerbates the systemic autoimmune response in psoriasis.

Supported by Polish Ministry of Education and Science (N41/DBS/001026).

Immunomodulation and Immunotoxicology; Immunotherapy in cardiology; Innate immunity

Calcium channel blockers modulate mouse macrophage activity in a diet-dependent manner

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Introduction: Antihypertensive calcium channel blockers act either on the cardiac conduction system (verapamil) or inhibit the entry of Ca^{2+} ions into smooth muscle cells of blood vessels (amlodipine). High-sodium diet drives the hypertension-related inflammation and macrophages may be modulated both by excessive sodium and drugs due to the numerous ion channels on their surface.

Aim: Current studies evaluated the effects of verapamil and amlodipine on the modulation of macrophage-mediated immune responses and estimated if these processes are diet-dependent.

Methodology: Mice kept on standard (STD) or high-sodium (HSD) diet since weaning, were i.p. administered, for 8 days, with verapamil (5 mg/kg) or amlodipine (3 mg/kg). Mineral oil-induced peritoneal exudate macrophages (PEMs) were examined for surface marker's expression, secretory and phagocytic activities as well as their roles in humoral and contact hypersensitivity responses were also assessed. Two-way ANOVA was used for statistical analyses.

Results: Both drugs reversed HSD-induced increase in ROI secretion. Additionally, verapamil reduced the secretion of NO by LPS-stimulated PEMs, regardless of the type of diet. While the secretion of some cytokines (IL-6, IL-12p40) was enhanced by amlodipine in STD mice, verapamil had the opposite effect in HSD mice. A clear opposite effect was also observed in phagocytosis assay, in which amlodipine impaired phagocytosis in STD mice and enhanced it in HSD mice, in opposition to verapamil. Type of diet modulated drug effects in the induction of humoral and cellular immune responses.

Conclusions: Both, type of diet and antihypertensive drugs modulated macrophage-dependent immune responses. Interestingly, drugs with similar mechanisms of action affected the immune system in opposite way.

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Viral infections and immunity

Nanoconjugates of lactoferrin for treatment of viral infection

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Introduction: Lactoferrin plays an important role in immune regulation and defence mechanisms against bacteria, fungi and viruses. Recently, much effort has been devoted to the development of biomedical applications of nanoparticles.

Aim: The aim of this work was to test whether lactoferrin conjugates can protect the olfactory route from *in vitro* and *in vivo* corona virus infection.

Methodology: Lactoferrin modified AgNPs or AuNPs sized 5 and 30 nm were synthesized and characterised using in-house methods. Antiviral potential was tested *in vitro* using HCoV-229e infected human epithelial MRC-5 cells or MHV- infected NCTC cell line and a mouse model of corona infection with MHV (Murine hepatitis coronavirus). MHV-infected mice were treated with nanoconjugates by repeated instillations and further analysed for clinical signs of infection, cellular, cytokine and chemokine response in respiratory tract as well as within the olfactory tract.

Results: Tests performed in HCoV-229e- and MHV-JHM-infected cell lines demonstrated that inhibition of HCoV-229e/MHV infection was metal and size-dependent with LF-modified 30 nm AgNPs showing the most promising anti-viral activity. During intranasal infection with MHV-JHM – only lactoferrin conjugates of 5 nm Ag/AuNPs showed significant decrease in total brain/lung

titers at 7 days post infection. Lactoferrin-functionalised Au/AgNPs showed stimulation of monocyte early response withing the epithelial mucosa, thus leading to induction of local antiviral response.

Conclusions: Therefore, lactoferrin functionalized gold and silver nanoparticles can consist good candidates for effective anti-viral microbicide to be used *in vivo* due to their effectiveness at lower concentrations and induction of an anti-viral response.

Innate Immunity

Innate immunity of the barrier organ: The skin, in the context of *Staphylococci* infection

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Aim: The interaction between bacterial microbiota and epithelial cells is a crucial factor underlying the pathophysiology of barrier organs. Keratinocytes, the primary constituents of the epidermis, play a pivotal role in skin barrier function, yet their response to different bacteria remains poorly understood.

Methodology: In this study, we present a comprehensive analysis of the transcriptome of human epidermal-like organoids exposed to two distinct bacterial skin colonizers: *Staphylococcus epidermidis* and *Staphylococcus aureus*. The former is commonly associated with positive interactions with the host, while the latter is associated with negative interactions. Additionally, using RT-qPCR, we examined the impact of different strains and mutants of *S. aureus* on selected gene expression levels in stratified keratinocytes. Finally, we assessed the predominant transcriptomic changes induced by *S. aureus* in keratinocytes in clinical samples from patients with atopic dermatitis, a condition commonly colonized by *S. aureus*.

Results: We demonstrate a partly shared but overall highly divergent transcriptomic profile of human epidermal-like tissue in response to *S. epidermidis* and *S. aureus*. The differences observed, along with their potential implications for epidermis, will be discussed within the context of skin innate immunity programs and atopic dermatitis.

Conclusions: By deciphering the gene expression patterns induced by *Staphylococci* on the epidermis at both species and strain levels, we have unveiled the global and specific impact of these bacteria on human keratinocytes, with relevance to *Staphylococci* skin colonization strategies and cutaneous chronic inflammatory diseases associated with *S. aureus*-induced skin alterations.

Autoimmunity and Autoinflammation

Multi-omics analysis of liquid biopsies in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by pain and joint inflammation. Although substantial progress was made in RA diagnosis, current clinical or laboratory criteria are not sensitive enough to identify early RA.

Aim: Finding precise non-invasive biomarkers based on liquid biopsies is essential in achieving long-term clinical and functional outcomes.

Methodology: High-throughput analyses were performed including Whole genome bisulfite sequencing (WGBS) and Protein Mass spectrometry of 20 healthy control (HC) and 31 early (eRA) and 48 advance RA (aRA) sera. In addition, proinflammatory proteins such as the S100A family, were validated by ELISA and correlated with clinical parameters (DAS28-CRP, DAS28-ESR) in RA.

Results: Based on the proteomic analysis we identified significantly different proteins: 89 proteins in eRA vs. HC, 26 proteins in aRA vs. HC and 88 in aRA vs. eRA. Using PCA analysis we were able to distinguish HC from eRA and aRA. Also, ELISA analysis showed that S100A8 ($p = 0.01$, $p = 0.02$) and S100A12 ($p = 0.007$, $p = 0.003$) positively correlated with clinical parameters DAS28-CRP, DAS28-ESR, respectively so they could be used as a biomarker of high disease activity.

Conclusions: Using proteomic analysis we have demonstrated that S100-proteins are increased in eRA sera (proteomics and ELISA). Further WGBS analysis will confirm if the secretion of selected proteins can be sup-

ported by changes in the methylation status of the same genes. Therefore, such an approach allowed us to decipher predictive biomarkers of early diagnosis and gain insight into the different pathological mechanisms underlying the heterogeneity of RA for better patient stratification.

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Adaptive Cell Therapy

The influence of *Nigella sativa* seed essential oil on T lymphocytes and cytokine patterns in females diagnosed with Hashimoto's thyroiditis

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Introduction: Hashimoto's thyroiditis (HT) is a disease characterized by autoimmune activity. Triggered by a combination of genetic predisposition and environmental factors, thyroid antigens are presented by antigen-presenting cells (APC) and thyrocytes, expressing MHC II. This breach in self-antigen tolerance prompts the activation of CD4+ (helper) T cells, CD8+ (cytotoxic) T cells, and B cells, leading to antibody production. The progression of HT can vary significantly and often correlates with a decline in patients' overall health.

Patients typically require chronic treatment with levothyroxine. However, despite this hormone therapy, the autoimmune response targeting thyrocytes persists, increasing the risk of developing additional autoimmune disorders. Moreover, patients frequently experience ongoing symptoms of the disease despite hormonal intervention.

Aim: To verify the effects of selected ethanolic dilutions of essential oil (NSEO) on T lymphocytes in women with Hashimoto's thyroiditis, their ability to proliferate, susceptibility to apoptosis, and cytokine production.

Methodology: Eighteen women were included in the study. The direct material was peripheral blood mononuclear cells (PBMC). Using flow cytometry, the proliferative activity and susceptibility to apoptosis of T cells were determined in the presence of serial dilutions of NSEO. The ability to produce Th1/Th2/Th17 cytokines was also assessed.

Results: Results show that NSEO at the highest concentrations exhibited proapoptotic and antiproliferative effects against T cells. In the presence of NSEO, there was a decrease in the production of IL-17A, IL-10, TNF, and IFN-γ. NSEO exhibits strong immunomodulatory effects.

Conclusions: Supplementation with black cumin oil may be beneficial for HT patients as a support for pharmaceutical therapy.

Immunomodulation and Immunotoxicology

The effect of methylparaben on proliferation and apoptosis of oral squamous cell carcinoma (OSCC) experimental line cells

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Introduction: Oral squamous cell carcinoma (OSCC) is one of the top 10 most common cancers worldwide and represents a significant public health problem globally. The specific location of OSCC lesions means that they can be continuously exposed to carcinogenic factors, such as xenoestrogens. Methylparaben (MeP) is a preservative commonly found in medical devices, cosmetic and hygiene products with proven estrogenic properties.

Aim: The aim of the study was to assess the degree of proliferation and the intensity of the apoptosis process through the mitochondrial pathway in OSCC cells of the SCC-9 line (ATCC CRL-1629) exposed to MeP. The effect of flavonoids: luteolin (Lut) and quercetin (Q) on the proliferation and apoptosis of SCC-9 cells exposed to MeP was also examined.

Methodology: SCC-9 cells were incubated with MeP and Lut or Q. Measurements of proliferation and apoptosis were conducted using flow cytometry. The expression of ER-β receptor, Bax, cBID, Bcl-2, caspase-3 and PI3K, STAT-1 proteins was examined by Western blot method.

Results: The results revealed that MeP, through ER-β receptor, intensifies the proliferation and impairs the apoptosis in SCC-9 cells via a pathway dependent on intrinsic pathway proteins. Furthermore, the presence of Lut or Q reduced proliferation and increased apoptosis in MeP-treated cancer cells.

Conclusions: This study clearly demonstrates the highly negative impact of MeP on SCC-9 cells and may provide a basis for developing strategies to reduce the widespread use of parabens. The beneficial effects of flavonoids can be used in the prevention and treatment of OSCC.

Immunomodulation and Immunotoxicology

Immunomodulatory role of methyl derivatives of flavanone

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Introduction: Inflammation is crucial for the immune system's response to injury and the presence of harmful pathogens within the host. Chronic inflammation contributes to the pathophysiology of many chronic diseases, such as atherosclerosis, diabetes, inflammatory bowel diseases, neurodegenerative diseases, and is also related to an increased risk of cancer. Flavanones (2-arylchroman-4-ones) widely common in citrus fruits possess a broad spectrum of biological activity, including anti-oxidative and anti-inflammatory properties. The beneficial effects of flavanones have been found based on *in vitro* cell cultures and animal studies.

Aim: The aim of this study was to evaluate immunomodulatory role of selected methyl derivatives of flavanone 2'-methylflavanone (5B), 3'-methylflavanone (6B), 4'-methylflavanone (7B), and 6-methylflavanone (8B) on lipopolysaccharide-induced RAW264.7 macrophages.

Methodology: In this experiment we used a mouse peritoneal macrophage RAW 264.7 cell line from ATCC (American Type Culture Collection, Manassas, VA, USA). The potential toxicity of flavanone methyl-derivatives was evaluated using the MTT assay. The concentration of nitric oxide released by macrophages was determined by measuring the accumulation of nitrite, the stable end product, in the culture supernatant according to the Griess reaction. The chemiluminescence was determined using a LB 960 CentroXS3 microplate luminometer. Determination of selected interleukins was carried out by xMAP technology (Bio-Plex) using magnetic microspheres of different shades of red coated with antibodies targeting the cytokines. After incubation and washing, the combined cytokines were labeled using a mixture of biotinylated antibodies, then combined with phycoerythrin-conjugated streptavidin.

Results: Methyl derivatives of flavanone inhibit NO and chemiluminescence generated *via* LPS-stimulated macrophages. Moreover, the tested compounds at 1-20 μM dose-dependently modulate proinflammatory cytokine production as IL-1β, IL-6, IL-12p40, IL-12p70, and TNF-α in stimulated RAW264.7 cells. The strongest anti-inflammatory activity among all the tested flavanone derivatives possess 2′-methylflavanone (5B) and 3′-methylflavanone (6B).

Conclusions: Consuming a significant amount of flavonoids, including flavanones, could provide defense against oxidative stress, inflammation, and chronic illnesses. The 2'-methylflavanone (5B) and 3'-methylflavanone (6B) compounds appear to be potentially useful in the prevention of diseases associated with the inflammatory process.

Viral infections and immunity

Involvement of VCP during replication and release of SDAV virions from neurons – *in vitro* studies

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Introduction: Valosin-containing protein (VCP) is a highly conserved member of ATPases Associated with diverse cellular Activities (AAA+). It is expressed in a variety of cells and localised in the cytosol and on the membranes of organelles. VCP is involved in many processes, e.g. the ubiquitin-proteasome system (UPS) involved in endoplasmic reticulum-associated protein degradation (ERAD).

Given the numerous functions and the widespread occurrence of VCP, the involvement of this protein in viral infections has begun to be investigated. VCP is utilised in the replication cycle of various viral families, including the *Coronaviridae*, which may indicate involvement of VCP in Sialodacryoadenitis Virus (SDAV) infection.

Aim: SDAV, a rat coronavirus with probable zoonotic potential, is a poorly understood infectious agent. There is scarce data on its replication, particularly in neurons. We decided to investigate the involvement of the VCP in SDAV replication cycle in primary cultured murine neurons.

Methodology: SDAV-infected primary murine neuronal cultures were pre- and post-treated with Eeyarestatin I

(EerI) – an inhibitor of VCP in ERAD. Cell cultures were fluorescently labelled and visualised using a confocal microscope and a high-content analysis system. SDAV nucleocapsid (N) protein and VCP were detected with Western Blot.

Results: EerI treatment resulted in statistically significant changes in the fluorescence intensity and protein quantity. Significant decreases in fluorescence intensity and protein quantity were detected in post-treated neurons.

Conclusions: 1. VCP is utilised during SDAV replication in neurons. 2. Post-treatment with 5 and 25 μM/ml EerI significantly inhibits SDAV replication in neurons, confirming that SDAV uses the ERAD pathway.

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Primary Immunodeficiencies

Clinical and laboratory symptoms in SCID – experience of one center

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Introduction: Severe combined immunodeficiency (SCID) is a disease of the immune system that, if left untreated, leads to death. It is important to diagnose it as soon as possible. Depending on clinical symptoms and test results, a typical SCID (TS), atypical SCID (AS) and Omenn syndrome (OS) are distinguished.

Aim: Identification of the most common clinical and laboratory symptoms in various forms of SCID in the Polish population.

Methodology: Retrospective assessment of clinical and laboratory symptoms in 42 patients diagnosed with SCID, referred to Department of Immunology, the CMHI, between years 2010-2023, mostly from the Masovian and Wielkopolska voivodeships (9 children each), Silesia (7 children) and Swietokrzyskie (6 children).

Results: The first symptoms of SCID were infections in 69% of patients (92% with TS, 74% with AS, 0% with OS) and rashes in 22% of patients with SCID (80% with OS). Among all infections, BCG infection occurred in 56% of patients: 83% TS, 44% AS, 25% OS ($p = 0.04997$), bacterial etiology was found in 52% of children with SCID (17%, 62%, 100%, respectively in TS, AS and OS ($p < 0.05$), fungal infections in 43% of children, 80% TS, 33% AS, 0% OS ($p = 0.008316$), cytomegalovirus infection was detected in 18% of children: 17% TS, 18% AS, 20% OS ($p = 0.987$).

Lymphopenia occurred in 80% of patients, 88% in patients with typical and atypical forms, 33% in patients with Omenn syndrome ($p = 0.03346$).

Hypogammaglobulinaemia G was present in 47% of patients with SCID, 64% with TS, 38% AS, 40% OS.

Conclusions: Lymphopenia is the most common laboratory sign and should be an urgent indication for the diagnosis of SCID in infants. The most common infections were: BCG and fungal infections in the typical form, and bacterial infections in the atypical form and Omenn syndrome.

Immunogenetics

Telomere length and telomerase reverse transcriptase gene polymorphism as markers of allogeneic haematopoietic stem cell transplantation outcome

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Introduction: Telomerase reverse transcriptase (*TERT*) is a catalytic subunit of telomerase that maintains genome integrity. Genetic variability within the *TERT* gene affects telomere length (TL) and telomerase activity, and was

shown to influence of haematopoietic stem cell transplantation (HSCT) outcome.

Aim: The present study aimed to analyse the effect of recipient and donor TL and *TERT* single nucleotide polymorphism (SNP) on the occurrence of post-HSCT complications.

Methodology: *TERT* promoter (*TERT*p) SNP (rs2853669) and TL were assessed in 120 recipient-donor pairs employing real-time PCR.

Results: The presence of *TERT*p rs2853669 *T* allele in the recipient was associated with a higher risk for acute graft-versus-host-disease (aGvHD) manifestation ($p = 0.046$) and a significantly shorter aGvHD-free survival ($p = 0.036$). Analysis of TL in patients with non-late onset aGvHD showed that shorter telomeres prevailed in those with longer aGvHD-free survival ($p = 0.011$). TL was confirmed as an independent marker of aGvHD-free survival in a Cox proportional hazards model analysis also including conditioning regimen, donor-recipient settings, HLA compatibility, recipient age ($p = 0.004$). Furthermore, we found that shorter TL characterized donors of patients who developed viral infections post-HSCT ($p = 0.060$) and those with late complete chimerism at 180 day after HSCT ($p = 0.011$).

Conclusions: Our results suggest that recipient allele *TERT*p rs2853669 *T* is a marker of unfavourable outcome in the context of aGvHD while patients with longer TL are predisposed to faster aGvHD development. Shorter TL in donors could be associated with higher frequency of viral infections and later achievement of complete chimerism.

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Transfusion Immunology

Syphilis diagnostics in blood donors and patients – cross-reactivity, challenges and issues

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Introduction: Syphilis remains a significant public health concern globally, with implications for blood transfusion safety. Despite advancements in screening techniques, cross-reactivity continues to pose challenges in accurately identifying syphilis in blood donors. This study addresses the complexities surrounding syphilis screening, focusing on cross-reactivity issues and associated challenges within the blood donor and patient population.

Aim: This research aims to investigate the prevalence of cross-reactivity in syphilis screening, elucidate the underlying causes, and explore potential strategies for mitigation.

Methodology: A review of current knowledge regarding cross-reactivity in the context of diagnosis of treponemal diseases has been conducted. The current algorithm for diagnosing syphilis in blood donation is highly systematic and comprehensive. Screening tests exhibit high sensitivity and specificity, while confirmatory tests belong to the group of specific methods (treponemal) utilizing Western blot-based techniques.

Results: After analysing the available literature, it is evident that there is a challenge in establishing an accurate diagnosis of treponemal diseases. Cross-reactivity and false positive results occur due to morphological and phylogenetic antigenic similarities, despite the utilization of different antigens specific to each disease in treponemal disease tests (such as syphilis and Lyme disease). Frequently encountered nonspecific, indeterminate test results for syphilis necessitate broadening the diagnostic scope to other treponemal diseases. However, there are reports indicating that patients incorrectly diagnosed for Lyme disease may actually have *Treponema pallidum* infection as the primary condition. This is mainly attributed to cross-reactivity in Borrelia IgM tests. Based on current knowledge, it appears that *Treponema pallidum* infection is able to induce cross-reactivity in Lyme disease tests, whereas active or prior Lyme disease does not significantly influence false positive *Treponema pallidum* results.

Conclusions: The precise diagnosis of treponemal diseases is difficult and complex due to the relatively frequent cross-reactivity of the tested samples in nonspecific and specific tests currently available on the market. The suggested solution of the problem would be the implementation of molecular biology methods. It should be noted that with the correct identification of these disease entities, screening tests alone are insufficient – a more specific verification is required, at least such as Western Blot technique.

Immunomodulation and Immunotoxicology

The immunomodulatory effects of hypotensive drugs on the antibacterial response mediated by macrophages

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Introduction: Hypertension remains the most significant preventable risk factor for stroke and coronary artery disease, substantially contributing to global mortality. Because of the prevalent use of hypotensive drugs, global mean blood pressure has remained unchanged or even slightly decreased over the past decades. However, considering the role of the immune system in the pathogenesis of hypertension and the broad spectrum of mechanisms involved in the action of antihypertensive drugs, their possible immunomodulatory effects should be studied.

Aim: To investigate the influence of selected hypotensive drugs on the J774A.1 mouse macrophage cell line response against *Staphylococcus aureus*.

Methodology: J774A.1 cells were cultured (1×10^6/well) in the presence of captopril, propranolol, carvedilol, verapamil, amlodipine, or olmesartan for 24 hours. Subsequently, *S. aureus* at MOI = 10 was added to the selected wells for the next 24 hours. Culture supernatants were tested for NO and cytokine concentrations. In addition, a western blot analysis of cell lysates was performed.

Results: As a result of bacterial stimulation, J774A.1 macrophages secrete only limited amounts of IL-6 and don't initiate the expression of iNOS. However, we identified increased excretion of NO and ROIs as a drug-induced effect. The strongest stimulatory effect was observed after amlodipine and olmesartan administration and the most significant alleviation of the antimicrobial response occurred in the case of verapamil. Furthermore, propranolol decreased macrophage viability.

Conclusions: We demonstrated that hypotensive medications modulate the antimicrobial response mediated by macrophages in different ways, which may be of great importance especially for elderly multimorbid patients struggling with severe bacterial infections.

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Immunooncology

Vitamin D3 analogs treatment regulate the OPN receptors expression and Th17/Treg cells subsets in various mammary gland cancer models

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The development of cancer is a complicated process that affects the whole organism: the immune system and also the patient's calcium and hormonal balance. Vitamin D is one of the important factors for all the above areas. However, still its impact on cancer processes has not yet been clearly classified as positive or negative. Vitamin D modulates, among others: the level of osteopontin (OPN) – a protein associated mainly with the regulation of calcium metabolism. Osteopontin, through interactions with integrins on CD4+ lymphocytes, influences their differentiation into Th17 helper lymphocytes, characterized by the production of interleukin 17 (IL17+). Th17 cells are an important element of the body's immune response during the development of cancer. The aim of our studies was to analyze the direct effect of vitamin D analogues (calcitriol and tacalcitol) on its nuclear receptors in Th17 cells and the consequent changes in IL-17 production. Additionally, it's interactions with osteopontin (OPN) was examined as the example of indirect influence on Th17 cells population in mouse mammary gland cancer models.

We analysed the effect of selected integrins blocking, i.e. CD29, CD51 and CD61, on the process of differentiation of CD4+ cells into Th17 helper lymphocytes. Previous studies have shown that the presentation of the above-mentioned integrins changes most significantly in CD4+ cells after administration of vitamin D or its analogue to animals suffering from cancer. Therefore, the results of these studies will be important for determining the role of vitamin D in the development of the Th17 population in the context of cancer.

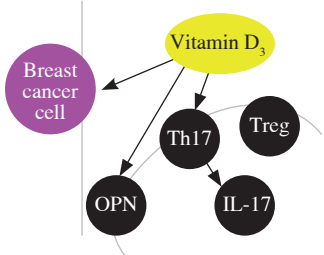

Fig. 1. Simplified diagram of direct and indirect effects of vitamin D on Th17 cells

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Autoimmunity and Autoinflammation

'Krakow Smog' enhances NETs formation in patients with RA

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Introduction: Air pollution, especially particulate matter (PM) PM10 and PM2.5 are considered the most harmful to human health. This is because small PM can penetrate the lower respiratory tract and may directly translocate from the lung to blood stream, where interact with blood cells including neutrophiles. These interactions may affect the neutrophils' function, e.g. causing dysregulation of neutrophil extracellular traps (NETs) formation, and leading to various pathological processes, including initiation and exacerbation of autoimmune disorders.

Aim: Investigating the effect of Krakow air pollution (PM_{KRK}) on the NETs formation in patients with rheumatoid arthritis (RA).

Methodology: Polymorphonuclear neutrophils (PMNs) were obtained from peripheral blood samples of patients with RA and healthy donors (HD) by sedimentation, using 1% polyvinyl alcohol. Next, the cells were exposed to PM_{KRK} (10 and 100 μg/ml) *in vitro* for 3 hours. NETs formation was assessed in supernatants from cell culture by fluorometric detection of free circulating DNA, using PicoGreen dye. Additionally, cell morphology and type of NETosis was assessed microscopically by indirect immunofluorescence using anti-human antibodies directed against the NETs components (myeloperoxidase or neutrophil elastase) and DNA staining with DAPI.

Results: Our preliminary data indicate that *in vitro* exposure of PMNs to PM_{KRK} stimulates NETs formation both in patients with RA and HD, being more intensive in patients with RA. This observation was confirmed by increased concentration of dsDNA in supernatants from cell culture and the higher number of cells releasing NETs after exposure to PM_{KRK}.

Conclusions: 'Krakow's Smog' induces NETs formation, which may suggest the role of air pollution in the pathogenesis and exacerbation of autoimmune disorders.

Immunooncology

NETs in OSCC: key gene expression and involvement of HIF-1

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Introduction: Our research has shown an equal involvement of neutrophil extracellular traps (NETs) in the inflammatory process and oral squamous cell carcinoma (OSCC). The presence of NETs in the neutrophil coculture with squamous cell carcinoma (SCC) cells and the trap location in the tumor tissue prove direct interactions between neutrophils and cancer cells *in vivo*.

Aim: The aim of the study was to understand the mechanisms underlying the NET formation at the level of key gene expression. Since local conditions of hypoxia in the tumor microenvironment favor the neutrophil recruitment, the role of hypoxia-inducible transcription factor 1 (HIF-1) in the NET formation process was assessed.

Methodology: Blood was collected from healthy volunteers and OSCC patients. Neutrophil's RNA was isolated using the TRIzol/chloroform technique. The expression of mRNA for MPO, PADI4, NCF-1, β-actin was assessed by qRT-PCR. Neutrophils were incubated with LPS. NETs biomarkers (MPO, H3) and apoptosis were assessed by flow cytometry. The amount of MPO, HIF-1α, HIF-1β in neutrophil supernatants were measured using ELISA kits.

Results: Decreased MPO mRNA expression was demonstrated in patient neutrophils and no changes in NCF1 mRNA. In contrast, PADI4 mRNA expression in patients was higher than in healthy people. An increase in HIF-1α and HIF-1β concentrations was obtained in the supernatants of LPS-stimulated neutrophils. Lack of changes in apoptosis of LPS-stimulated neutrophils was accompanied by an increase in MPO concentration, the percentage of MPO+/caspase 3- and cells expressing H3.

Conclusions: Excessive PADI4 activity may cause increased NET formation in cancer patients and constitute an poor prognostic factor. High HIF-1 concentrations with an increase in NETs biomerkers indicate the involvement of the hypoxia in the trap generation.

Viral infections and immunity

Rhinovirus HRV16 may limit the replication of coronavirus HCoV229E during infection of the lung vascular endothelium

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Introduction: Human respiratory viruses: rhinoviruses (HRVs) and coronaviruses (HCoVs) cause 50-60% and 10-15% airway infections in healthy individuals. We showed that the lung vascular endothelium infected by HRV16 secretes proinflammatory cytokines and develops a strong antiviral response based on the release of interferons and the activation of IFN-dependent antiviral proteins, including OAS-1, PKR and MX-1 that limit viral replication.

Aim: The main aim of the study was to assess whether HRV16 may limit the replication of HCoV229E in the lung vascular endothelium.

Methodology: Human lung microvascular endothelial cells (HMVEC-L) were incubated with human rhinovirus HRV16 (MOI 3.0). Subsequently, 24 hours after the HRV16 infection HMVEC-Ls were infected with low pathogenic human coronavirus 229E (MOI 1.0). mRNA gene expression and viral copy number were assessed by real-time PCR. Protein levels were measured in flow cytometry and ELISA assay.

Results: Single infection of HRV16 or HCoV229E and co-infection with both viruses cause a decrease in HMVEC-Ls viability. HRV16 activated a strong antiviral response, especially induced mRNA expression and secretion of IFN-β, as well as up-regulation of IFN-β-dependent enzymes – OAS-1, PKR, and MX-1. HCoV229E caused much less activation of antiviral response, notably in the early hours of infection. Endothelial cells pre-treated with HRV16 and then infected with HCoV-229E demonstrated a similar antiviral response compared to cells infected only with RV. Interestingly, the number of HCoV229E copies was significantly lower in endothelium primarily infected with HRV16 compared to HCoV229E alone.

Conclusions: HRV16 may limit the replication of HCoV229E through strong and rapid activation of the antiviral response through the IFN-β-dependent intracellular mechanisms. NCN 2021/41/N/NZ5/02464.

Primary Immunodeficiencies

Novel mutation of complement factor I in a patient with invasive meningococcal disease

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Introduction: Inherited complement deficiencies are rare diseases of the immune system that, if undiagnosed, may have significant health consequences. Complement Factor I (CFI) deficiency is a serine protease with an important role in regulation of complement pathway.

Aim: We aim to present a case report of a 17 year-old patient with invasive meningococcal disease in whom we established diagnosis of CFI deficiency.

Methodology: The patient was diagnosed as a part internal grant project "Correlation of the activity of selected components of complement system with the clinical and genetic phenotype in children with inborn errors of complement system". Genetic analysis was carried out with NGS sequencing of selected genes.

Results: The patient history included invasive meningococcal disease (serogroup Y *Neisseria meningitidis*), bilateral pneumonia complicated with pleural empyema and generalized catheter-related *Candida* infection. Her complement studies showed low C3 (0.22g/l, reference range: 0.83-1.77) and normal C4 concentration as well as low CH50 activity of 14.00 uEq/ml (reference range: 70-180). NGS analysis revealed novel homozygous pathogenic missense mutation c.775T>A p.(Cys259Ser) in CFI gene.

Conclusions: Invasive meningococcal disease should always raise concern about possible inborn errors of immunity including inherited complement disease. Recognizing the deficit and implementing antibacterial prophylaxis along with vaccinations, can protect against severe and life-threatening infections.

Immunology in personalized medicine

Expression level of *NOD1* and *NOD2* in peripheral blood leukocytes in children with primary hypertension

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Introduction: Primary hypertension (PH) is the main form of arterial hypertension in school-age children. However, it is more often diagnosed in adolescents. The regulation of arterial blood pressure is affected by genetic, environmental, endocrine, nervous and immune system factors. Nucleotide-binding and oligomerization domain NOD-like receptors (NLRs) are highly conserved cytosolic pattern recognition receptors engaged in natural immunity. They recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). NOD1 and NOD2 receptors belong to the NLRs group. They are engaged in the development of metabolic diseases (e.g. obesity, diabetes, non-alcoholic fatty liver disease). Their role in the development of PH in children/adolescents is largely unknown.

Aim: The aim of this study was to determine the mRNA expression profile of *NOD1, NOD2* receptors and inflammatory cytokines (*IL-1*β, *IL-6*, *IL-18*, *TGF*-β) in peripheral blood leukocytes in children with PH. The correlations of their expression profiles with clinical parameters were assessed.

Methodology: A sample of 74 children was recruited for the study and was divided into 2 groups: a) study group: 39 pediatric patients with diagnosed PM (29 boys and 10 girls) from the Department of Nephrology, Kidney Transplantation and Hypertension, The Children's Memorial Health Institute in Warsaw; b) control group: 35 normotensive children (19 boys and 16 girls). The following clinical tests were performed: left ventricular mass index (LVMi), cartoid intima-media thickness (cIMT), RWT (relative wall thickness), wall cross-sectional area (WCSA), pulse wave velocity (PWV), pulse wave analysis (PWA) by standard techniques (ultrasound, echocardiography, the oscillometric method). The tests involved measurements of biochemical parameters (glucose, insulin, lipid profile) and evaluation of leukocyte gene expression profiles of *IGF-1*, *IGF-1R*, and *NOD1*, *NOD2* receptors as well as *TGF*-β, *IL-6*, *IL-18*, *IL-1*β by quantitative real-time PCR.

Results: The results were analyzed by means of the Statistica 13.3 software package (StatSoft Polska Sp. z o.o., TIBCO Software Inc.). The differences and correlation indexes were considered significant at $p < 0.05$.

The PH children/adolescents had reduced leukocyte *NOD1* ($p = 0.0002$), *NOD2* ($p = 0.00004$) expression as compared to the control group. The expression level of *NOD1* correlated negatively with PWV ($r = –0.45$), and that of *NOD2* with SBP ($r = –0.46$).

Leukocyte expression of *IL-1*β ($p = 0.0250$) was upregulated but that of *TGF*-β ($p = 0.0022$) was decreased in the PH children, with no changes in the expression of *IL-18* and *IL-6*. Multivariate regression analysis showed that the expression of *IL-1*β positively correlated with RWT (b = 0.43, R = 0.48, R = 0.23) but that of *TGF*-β showed negative correlation with RWT (b = –1,40, R = 0.48, R = 0.23).

Conclusions: Decreased leukocyte *NOD1, NOD2* expression and negative correlations of *NOD1* expression with arterial stiffness parameter (PWV) and *NOD2* with SBP indicate that early subclinical hypertensive arterial injury is associated with decreased pro-inflammatory action of NLRs that may delay PH development. Similarly, low leukocyte *TGF*-β expression and its negative correlation with RWT suggest protection against early organ damage, in contrast to the action of *IL-1b*. Proper balance of protective (compensatory) and non-protective mechanisms is possibly responsible for PH development.

Immunodermatology

Efficacy of novel abdominoplasty skin-derived acellular dermal matrices in chronic diabetic wound treatment

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Chronic wounds remain a significant global health issue. Recently, members of our group developed a novel human acellular dermal matrix (hADM) dressing sourced from abdominoplasty skin, tailored specifically for deep and non-healing diabetic wounds.

In this study, we aimed to evaluate the effectiveness of our novel hADM in facilitating the healing process of deep and diabetic wounds.

Skin samples from post-bariatric patients were decellularized using three methods, namely hADM1) using 1M NaCl and sodium dodecyl sulfate (SDS); hADM2) employing 2M NaCl and SDS; and hADM3) trypsin and Triton X-100. Immunohistochemical and histochemical stainings were used to assess the preservation of extracellular matrix structure and cellular components. Immunogenicity was analyzed by T-cell proliferation assay. Subsequently, WT mice and leptinR knockout mice (db/db) were subjected to evaluate the effects of optimized hADM.

All decellularization protocols preserved the original extracellular matrix traits. Daily tracking of wound closure, coupled with gene expression analyses, provided insights into the therapeutic potential of the novel dressings. Notably, hADM1 showed lower immunogenicity compared to other dressings. During the inflammation phase of wound closure, all examined hADMs exhibited similar results. In the proliferation phase, hADM1 dressing expedited wound healing. Furthermore, hADM1 enhanced wound closure and upregulated the expression of key components such as collagens (Col5a3, Col5a2), integrins (Itga6, Itgb3), and matrix metalloproteinases (Mmp9, Mmp1a).

Our study presents a promising approach for treating chronic diabetic wounds using hADM1 dressing. Nevertheless, further research is warranted to thoroughly assess the safety and efficacy of hADM1 in clinical trials.

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Biological treatment in immune-related diseases

Key aspects of the impact of immunosuppressive drugs on male fertility

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Introduction: To date, the frequency of autoimmune rheumatoid-related diseases (ARRDs) in men has greatly increased. Both its activity and pharmacotherapy affect subsequent po-creative attempts.

Aim: Emphasize the problem of fertility of men with rheumatic diseases.

Methodology: Literature data analysis from database MEDLINE/PubMed - articles focusing on Pregnancy and men with rheumatic diseases

Results: The direct effect of these diseases on pathophysiology of testis is explained by the dysregulation of immune response and increase in production of MCP1, TNF-α, IL-1, IL-6, NOs, disturbed steroidogenesis and formation of antisperm antibodies.

Disease-modifying antirheumatoid drugs (DMARDs) is a group of medications commonly used in men with rheumatoid arthritis, spondylarthritis, psoriatic arthritis and systemic lupus erythematosus. To the DMARDs group belong methotrexate, sulfasalazine, hydroxychloroquine, leflunomide. Other anti-inflammatory drugs – nonsteroidal antiinflammatory (ibuprofen or naproxen) and corticosteroids (prednisone) alleviate clinical symptoms and are often used in combination with DMARDs.

The glucocorticoids disrupt the hypothalamic-pituitary-gonadal axis and can diminish testosterone production. Cyclophosphamide can induce irreversible gonadal damage or extended oligospermia. Methotrexate treatment can provide the direct cytotoxic effect on testicular germ cells. Sulfasalazine decreases sperm motility and concentration, and provides high rates of sperm with abnormal morphology. TNF-inhibitors (Infliximab, Adalimumab, Etanercept) also disrupt the sperm morphology and concentration. Hovever, they are not risk factors for miscarriage, preterm delivery and low birth weight of the offspring.

Conclusions: It is necessary to plan a pro-creation at least 3-4 months after treatment with methotrexate, azathioprine/mercaptopurine, cyclophosphamide, sulfasalazine for the spontaneous recovery of spermatogenesis or use ICSI with spermatozoa extracted directly from the testis taking into account their DNA status.

Veterinary immunology and comparative immunology

Selected potential biomarkers of inflammation in rabbit haemorrhagic disease – preliminary studies

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Introduction: In recent years, there has been a noticeably growing interest in new markers of inflammation in the context of viral diseases, which may yield important

diagnostic and prognostic information. The ability to rapidly identify these markers is crucial for the treatment and management of diseases such as viral hepatitis.

Aim: The aim of this study was to identify and characterise selected potential biomarkers of body inflammation in rabbits experimentally infected with *Lagovirus europaeus*/GI.2.

Methodology: On the basis of the morphology results obtained with an automated veterinary haematology analyser, the neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocyte ratio (dNLR), monocyte-to-lymphocyte ratio (MLR) were calculated, platelet-to-lymphocyte ratio (PLR), mean platelet volume to platelet ratio (MPR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI) and aggregate index of systemic inflammation (AISI). In addition, all results were subjected to statistical analysis.

Results: Significant changes in immunological parameters such as NLR, dNLR, PLR and MPR were observed in rabbits infected with *Lagovirus europaeus*/GI.2. Analysis of the SII, SIRI and AISI indices showed significant differences between the infected and control groups, suggesting a significant impact of the infection on the overall immune status of the organism and may be relevant for diagnosis.

Conclusions: Analysis of changes in immunological indices in rabbits infected with *Lagovirus europaeus*/GI.2 suggests their potential use as monitoring of the immune response to infection. The NLR, dNLR, PLR, MLR and MPR indices, as well as SII, SIRI and AISI, may be useful in the diagnosis and therapy of infection, highlighting their value in understanding and monitoring the course of the disease.

Immunooncology

Potential anticancer effect of INF-λ1 – on head and neck squamous cell carcinoma cells – preliminary study

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer in the world. Recently discovered and described type III interferons (IFNs), including IFNλ1, have anticancer properties comparable to type I IFNs. It has been shown that IFNs-λ exerts a therapeutic effect at lower concentrations and therefore causes fewer side effects. Their pro-apoptotic effect has been demonstrated by sensitizing death receptors of the extracellular apoptosis pathway and participating in Fas-related signaling in many types of cancer. The aim of the study was to investigate the role of INF-λ1 in the regulation of HNSCC proliferation and apoptosis, with particular emphasis on the expression of proteins of the apoptosis receptor pathway.

Methodology: Human HNSCC cell lines (cal-27 – tongue cancer, Detroit 562 – pharynx carcinoma) were treated with INF-λ1 at two concentrations. After incubation with INF-λ1, the percentage of apoptotic cells and their proliferation were assessed by flow cytometry. The expression of apoptotic proteins of the receptor pathway, i.e. Fas receptor, FADD adapter protein and caspases-8 and -3, was assessed by Western blot.

Results: Inhibition of Cal-27 proliferation by INF-λ1 has been proven. Moreover, in both SCC lines, a dose- and time-dependent effect of INF-λ1 on the expression of the tested apoptotic proteins was observed. Increased expression of the Fas receptor, FADD adapter protein, caspase-8 and changes in the expression of caspase-3 were demonstrated. Additionally, studies have shown an increase in the percentage of Cal-27 cells after exposure to INF-λ1.

Conclusions: An antiproliferative effect of INF-λ1 was observed on tongue squamous cell carcinoma (Cal-27) cells, which was not demonstrated for Detroit 562 cells, indicating a different antitumor effect of INF-λ1 depending on the cell type. The obtained results also suggest a pro-apoptotic effect of the tested cytokine on both SCC cell lines in a manner dependent on the receptor apoptosis pathway.

Immunogenetics

Assessing the impact of IGHV and TP53 mutations on clinical outcomes in chronic lymphocytic leukemia

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Introduction: CLL involves the clonal growth of B lymphocytes, marked by CD5+, CD23+, and CD19+

phenotypes, and is made heterogeneous by various genetic abnormalities. Key historical prognostic factors include cytogenetic changes (e.g., del(13q), trisomy 12, del(11q), del(17p)). Targeted treatment with BTK and BCL-2 inhibitors have shifted focus to the significance of IGHV status and TP53 mutations in CLL outcomes.

Aim: We examined the relationship between IGHV status, TP53 mutations, and clinical outcomes in CLL patients at the Lower Silesian Oncology Center in Wroclaw.

Methodology: Study was conducted on 116 patients (59 F/57 M, median age: 68 (37-88) years) diagnosed with CLL. NGS techniques were used to determine the mutation status of IGHV and to analyze the mutations in TP53 gene.

Results: The cohort analysis revealed: 1. 21% of patients had deletion 11q, 8% had trisomy 12, 64% had deletion 13q, and 9% had deletion 17p. 2. 20% of patients had *TP53* mutations (VAF > 5%). 3. 56% had an unmutated IGHV status (U-CLL), with *IGHV1-69*01* and *IGHV3-21*01* being the most prevalent IGHV genes. 4. U-CLL patients experienced a significantly shorter time to first (median: 516 days vs. 2028 days, HR = 2.321, 95% CI: 1.466-3.676) and second treatment (2179 days vs. 4549 days, HR = 2.405, 95% CI: 1.235-4.685) compared to those with mutated IGHV (M-CLL). This was not observed in patients with *TP53* aberrations. 5. 70% with del 17p ($p = 0.009$) and 54% with *TP53* mutations ($p = 0.012$) required multiple therapies compared to those without *TP53* aberrations.

Conclusions: This study highlights the critical role of BCR signalling and IGHV mutations in CLL's progression, with BCR pathways possibly outweighing but not undermine the importance TP53 aberrations' impact. Further research on BCR pathway and IGHV is essential.

Veterinary and comparative immunology

New arthropod-borne viruses of the 21st century – their impact on defence mechanisms and disease resistance

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Introduction: Climate-change driven changes in the ecosystems but also induced a new epizootic and epidemiological implications. The negative effects of climate stressors (e.g. temperature, nutrients) on the nonspecific defence mechanisms and protection against new pathogens were observed. Viruses are endowed with a great ability to adapts to different environments. It means altering their cellular tropism and crossing host animal species barriers.

Aim: Therefore, in order to expand the knowledge of new viruses and their pathogenicity, it is crucial to critically analyse the literature in terms of their transmission, tropism to cells, and effects on cellular and humoral defence mechanisms.

Methodology: All available databases were searched to provide the most accurate picture of the real situation regarding new mosquito-borne viruses and their threat to animal and human health.

Results: Since the beginning of the 21st century, we have seen the emergence and outbreaks of new viral diseases transmitted by different vectors. Some of them are new to humans, and hence we do not have a good protection or immunity against them. Arthropod – boned viruses have continued to emerge in recent years, posing a significant health threat to many of people worldwide. Mosquitoes and also ticks transmit wide range of viruses to humans and animals worldwide. Many new viruses are replicate in two distant hosts – insects and humans or insects and plants. Ticks are the primary vectors for pathogens of animals and the secondary vectors for pathogens of humans. During the last 10 years, it has been noticed that the number of reports on ecoepidemiology of arthropod-borne diseases in humans and animals has increased. Discovery of new viruses Zika, Powassan, Bourbon, Oropouche or Heartland, has raised many questions in the virologists as it is not clear where from these viruses come, either they are already existing viruses, or a novel species evolved from viral pathogens.

Conclusions: Summing up, a new viral pathogens induce a changes in cell mediated immunity and specific immune response. Monocytes/macrophages differentiated into dendritic cells play an important role in innate and adaptive immunity, due to their antiviral potential, capacity to stimulate CD4+ and CD8+ T-cell responses and ability to regulate the specific Ig production by B cells. Study on pathological complications regarding to these infections are still investigable.

Immunology in personalized medicine

BTLA gene variation rs1982809 as cancer risk factor

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Introduction: B and T cell attenuator (BTLA) is one of the immune check -points molecules which negatively regulates immune response. Recent data showed that in similar to blockade of PD-1 or CTLA-4 also BTLA inhibition might be effective immunotherapy in wide spectra of cancers. Taking mentioned above into account, we hypothesized that variation in *BTLA* gene may be associated with susceptibility to cancer. In fact, we showed that polymorphism rs1982809 in *BTLA* gene concerned susceptibility several cancers.

Aim: The aim of present study is to analyse the influence of rs1982809 on general risk of cancer development. For this purpose we included in our study previously published and unpublished yet data for patients (pts) and additional healthy controls.

Methodology: Altogether 1640 adult cancer pts (chronic lymphocytic leukemia (CLL) – 321; non- small cell lung cancer (NSCLC) – 382; clear cell renal cell carcinoma (ccRCC) – 179; multiple myeloma – 203; bladder cancer – 314; prostate cancer – 268) and 791 unrelated healthy controls were typing for rs1982809 using the TaqMan^{N}SNP Genotyping Assays.

Results: In our published previously results we found out that possessing of G allele for rs1982809 can be considered as risk factor for CLL, NSCLC, ccRCC. However for prostate cancer, bladder cancer and multiple myeloma we observed similar trend which do not reach statistical significance.

Here we combined genotyping data for all investigated cancers pts together and find out that possessing of G allele for rs1982809 increased the risk of cancer by about 25% (OR = 1.2440, 95% CI: 1.0481 to 1.4766, $p = 0.0125$).

Conclusions: Our results indicated that polymorphism rs1982809 located in 3' UTR of *BTLA* gene is associated with general carcinogenesis. This fact can be taken into consideration when discussing a personalized approach in immunotherapy based on blocking BTLA.

Microbiom and immunity

Health-promoting properties of fermented products

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Introduction: Fermentation is a natural food modification process that is gaining popularity and has become the subject of scientific research on many levels. Despite the enormous benefits of consuming fermented foods, many people are still unaware of positive benefits they can bring to human health. Fermentation enriches diet with beneficial biologically active substances, such as organic acids, vitamins and bioactive peptides. These in turn have a immunostimulant effect that can support the immune system in preventing and fighting various infections and diseases.

The lactic fermentation process contributes to the production of substances with beneficial health effects. Probiotic bacteria not only improve digestion but also influence the balance of intestinal microflora, which is important for overall health and immunity.

Aim: This work aims to present various health-promoting effects that can be achieved using ingredients produced during the fermentation of products.

Methodology: Review and analysis of specialized literature based on Scopus and PubMed databases.

Keywords used: fermented foods; microbiome, eubiosis, dysbiosis; immunity.

Results: Consuming fermented foods in a daily diet brings various, confirmed health benefits. It includes improvement of immune responses and reduced risk of in-

fection. Moreover, there is a huge health potential for fermented food in the context of reducing inflammation and supporting natural protective barriers.

Conclusions: Fermented foods are an effective way to support immunity and improve overall health. Regular consumption of such products may have far-reaching consequences for the promotion of a healthy lifestyle and disease prevention including cancer.

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Microbiome and immunity

Assessment of the antioxidant and protective potential of fermented kale extract

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Introduction: Kale (*Brassica oleracea* var. *sabellica*) is considered a highly nutritious vegetable, containing numerous vitamins and minerals, as well as flavonoids, and polyphenols. Recent studies have shown that the fermentation process of kale leads to metabolic transformations of the phenolic compounds, and thus changes the biological activity of the product.

Aim: The study evaluates the potential cytoprotective and immunomodulatory effects of fermented extract obtained from kale on the Caco-2 cell line.

Methodology: Caco-2 cell line of human colorectal cells were preincubated for 24 hours in the presence of different concentrations of fermented extracts and then exposed to the oxidative stress inducing agent (H_2O_2). Malondialdehyde (MDA), lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and ATP assays were conducted. To assess the anti-inflammatory activity of the extract, levels of the proinflammatory interleukins IL-1β and tumor necrosis factor (TNF-α) were monitored in LPS-treated cells.

Results: Incubation of Caco 2 cells with the fermented extract resulted in protection from oxidative stress induced by H_2O_2 which was evidenced by a decrease of MDA and LDH levels as well as an increase of viability and antioxidant activities (CAT, SOD and GSH) as compared to cells treated with H_2O_2 alone. No significant impact on proinflammatory cytokine levels: IL-1β and TNF-α were detected.

Conclusions: The results of the analyses indicate that the fermented kale extract has significant antioxidant and cytoprotective properties.

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Vaccination and vaccines

Assessment of levels specific antibodies after vaccination against tetanus and hepatitis type B in workers of X-ray departments

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Introduction: In workers of X-ray departments observed lower levels of immunoglobulins, especially immunoglobulin class G (lgG). IgG is associated with humoral immune memory, which protects against infections. IgG production is a T'-cell dependent process and depends on both T and B cells and their cooperation. Vaccination against tetanus and viral hepatitis type B (HBV) is used to protect population from these infections.

Aim: The aim of the study was to assess the level of tetanus and HBV specific IgG after vaccination in workers of X-ray departments.

Methodology: Levels of specific IgG against HBV (anti-HBsIgG) measured in 119 workers (mean age 41 years and mean period of employment 10 years) and 80 subject of control group (mean age 39 years). Anti-tetanus IgG (ATIgG) assessed in 59 employees (mean age 39 years and mean period of employment 11 years) and 33 persons of control group (mean age 40 years). In workers the average time after tetanus and HBV vaccination was 19 and 8 years respectively. In the control group these periods after vaccination were similar. In all employees an annual effective dose of X-ray radiation was below 1 mSv.

ATIgG and anti-HBsIgG concentrations < 0.1 IU/ml and < 10 mIU/ml were considered as not protective respectively. Levels of antibodies measured with ELISA method. Additionally, workers divided in to subgroups below and over 40 years old and below and over 10 years of employment.

Results: Lack of protective ATIgG determinate in 11 (18.6%) of 59 workers, whereas in the control group everybody showed protective level of ATIgG ($p = 0.021$).

Concentrations of ATIgG were significantly lower in employees than in control group ($p = 0.01$). In 19 (16%) of 119 workers and 6 (7.5%) of 80 persons of control group protective level anti-HBsIgG was not found ($p = 0.12$), but concentrations of anti-HBsIgG in both groups were not significant.

No protective levels of ATIgG and anti-HBsIgG in subgroups divided in relation to age below and over 40 years old and period of employment below and over 10 years did not show statistical significance with exception subgroup below 40 years (lack of antibodies anti-HBsIgG in 5 of 61 workers and in subgroup over 40 years old (14 of 58) ($p = 0. 03$).

Conclusions: Results showed that exists association between occupational exposure to low level of ionizing radiation and immune response connected with vaccination against tetanus toxin and HBs antigen of HBV. Workers of X-ray departments should be routinely examined in relation to their post-vaccine response and time of its duration.

Oral presentation session

Surface immune checkpoints as a potential biomarker in pregnancy and idiopathic recurrent pregnancy loss

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Introduction: Due to the genetic diversity between the mother and the fetus heightened control over the immune system is crucial. Immunological parameters determined by clinicians in women with idiopathic RSA include quantity and activity of NK and NKT cells, quantity of regulatory T lymphocytes, and ratio of pro - inflammatory cytokines which indicate imbalance in Th1 and Th2 cells response. The processes are controlled by immune checkpoint proteins (ICPs) expressed on the surface of immune cells.

Aim: We aim to investigate differences in the expression of ICPs on T, Treg lymphocytes, NK cells, and NKT cells in peripheral blood samples collected from RSA women, pregnant women, and healthy multiparous women. We aim to discover new insights into the role of the ICPs involved in recurrent pregnancy loss.

Methodology: Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation from blood samples obtained from 10 multiparous women, 20 pregnant women (11-14 week of pregnancy), and 20 RSA women, maximum 72 h after miscarriage. The PBMCs were stained for flow cytometry analysis. Standard flow cytometry immunophenotyping of PBMCs was performed using antibodies against classical lymphocyte markers, including CD3, CD4, CD8, CD56, CD25, and CD127. Additionally, ICPs were investigated using antibodies against PD-1 (CD279), TIM-3 (CD366), VISTA, TIGIT, and LAG-3.

Results: We observed differences in the surface expression ICPs in analyzed subpopulations of lymphocytes between pregnant and RSA women. We noted diminished expression of PD-1 and LAG-3 on T (Th and Tc), Treg, NK and NKT cells on RSA women lymphocytes, and impaired expression of TIM-3 and VISTA on CD8 cytotoxic T and NK cells with accompanying increased expression of TIGIT and TIM-3 on NKT cells.

Conclusions: The changes in the expression of surface immune checkpoints indicate their involvement in the regulation of pregnancy. The data might be utilized to develop specific therapies for RSA women based on the modulation of ICPs expression.

Innate Immunity

Shedding light on interactions between neutrophils, neutrophil extracellular traps (NETs) and extracellular vesicles (EVs) during inflammation

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Introduction: Neutrophils utilize various weaponry to combat infection and it includes neutrophil extracellular traps (NETs). NET interactions with other biological entities such as extracellular vesicles (EVs) are rather obscure. EVs released by numerous body cells, including neutrophils, are microstructures that carry a bioactive cargo facilitating cell-to-cell communication. Both NETs and EVs can be released into blood where they function but not much is known about their interrelation.

Aim: Therefore interactions and co-dependency of EVs and NETs formed during murine endotoxemia were studied in situ directly in the vasculature of live mice.

Methodology: Endotoxemia served as a model of systemic inflammation and NETs and EVs were studied by

means of intravital microscopy, flow cytometry, immunocytochemistry, TEM, Nanoparticle Tracking Analysis (NTA).

Results: NETs and EV release was captured to occur in real time *in vivo* as well as profound interactions between neutrophils, EVs and NETs. Additionally, some EVs were deposited by neutrophils directly in NETs. Whereas platelet and monocyte/macrophage-derived EVs dominated in blood and other body fluids, these were mostly neutrophil EVs that interacted with NETs. Despite the interaction, NETs did not affect EV release, however, EVs inhibited NET formation. Specifically, erythrocyte-derived EVs (eEVs) downregulated NET release via Siglec-E-dependent interactions with neutrophils.

Conclusions: NET and EV interplay does occur *in vivo* but the process in not bidirectional. By impacting release of eEVs it is potentially feasible to modulate formation of NETs. The latter is of importance as NET deposition in vasculature is connected to adverse side effects.

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Viral infections and immunity

Differences in viral loads in saliva between patients with rheumatoid arthritis and patients with other rheumatic diseases and healthy controls

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Introduction: Rheumatoid arthritis (RA) is a disease with a complex pathogenesis involving genetic factors and environmental factors, among others, viruses. Ebstein-Barr virus (EBV), Cytomegalovirus (CMV), and Parvovirus B19 (B19) are most commonly cited in the context of RA pathogenesis. The work presented here addresses the association of selected viruses with the pathogenesis of RA.

Aim: The project aimed to establish the diagnostic potential of saliva. The viral load of the saliva of patients with RA, other rheumatic diseases (ORD) and healthy controls (HC) was assessed.

Methodology: Viral DNA was isolated from saliva collected from patients diagnosed with RA (n = 15), ORD (n = 20) and HC (n = 20). Subsequently, quantitative real-time polymerase chain reaction was performed using GeneProof diagnostic tests to check the exact DNA levels of EBV, CMV, and B19. The results obtained were compared between the study groups using the Delta Delta Ct method.

Results: The amount of EBV DNA was found to be significantly higher in patients diagnosed with RA, while no significant differences were found in saliva DNA levels of this virus between healthy subjects and patients with other rheumatic diseases. DNA belonging to CMV and B19 was not detected in the saliva of any of the patients and HC for the study.

Conclusions: The results indicate the diagnostic potential of saliva, which, as a non-invasively collected material, could provide an alternative to other materials used in diagnosis, such as blood. The higher concentration of DNA belonging to EBV in the saliva of RA patients could be a signal that this virus is involved in the pathogenesis of RA. However, this hypothesis would require further, more extensive research.

Clinical Immunology

Assessing monocyte subsets expressing innate immune checkpoints in CLL patients

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Introduction: Immune dysfunction in chronic lymphocytic leukemia (CLL) affects not only B lymphocytes but also classical ($CD14^{++}CD16^{-}SLAN^{-}$), intermediate ($CD14^{+}CD16^{+}SLAN^{-}$) and non-classical ($CD14^{dim}CD16^{+}SLAN^{+}$) monocytes. The antitumor properties of monocyte subsets may be also related by the SIRPα-dependent pathway. SIRPα is an inhibitory receptor that sends a 'don't eat me' signal when bound to the CD47. CD47 is often overexpressed on cancer cells. However, the inhibition of the phagocytic activity of a subpopulation of monocytes in CLL, determined by the expression of negative innate immune checkpoints, is still unclear.

Aim: This study aimed to compare the expression of SIRPα in monocyte subtypes between CLL patients and healthy controls.

Methodology: PBMCs were isolated from the whole blood of 40 CLL patients and 10 healthy volunteers using gradient centrifugation. The PBMCs were then labelled with the following antibodies: anti-CD14 V450, anti-CD16 FITC, anti-SLAN APC, anti-SIRPα PerCP-eFluor710. All samples were acquired using a CytoFLEX LX Flow Cytometer (Beckman Coulter), and the data were analysed using Kaluza.

Results: In this study, it was found that the percentage of intermediate and nonclassical monocytes was significantly higher in CLL patients compared to the control group ($p < 0.05$). Furthermore, the percentage of CD16+ monocytes with up-regulated expression of SIRPα was significantly higher in CLL patients compared to the control group ($p < 0.05$). Additionally, the percentage of nonclassical monocytes with SIRPα was significantly higher compared to the percentage of classical monocytes ($p < 0.01$).

Conclusions: Monocytes with higher expression of SIRPα, both among SLAN+ or SLAN-, are more susceptible to inhibition and may exhibit reduced phagocytic activity against leukemic cells.

Microbiom and immunity

Effects of halophilic archaea on human immune cells

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Introduction: Halophilic microorganisms are known to constitute the natural microbial communities of hypersaline ecosystems. Underground salt mines combine low nutrient availability, darkness and hypersaline conditions, where halotherapy sessions can be performed. The presence of halophilic archaea in the salt mines may thus have an impact on human health during halotherapy sessions.

Aim: In our study we investigated whether the halophilic archaea *Halorhabdus rudnickae*, isolated from the Wieliczka Salt Mine, can induce immune responses in human cells.

Methodology: Monocyte-derived dendritic cells (MoDCs) were obtained from the buffy coats of healthy donors and stimulated with *H. rudnickae*. The uptake of halophilic archaea was determined by fluorescence microscopy. The responses of MoDC to *H. rudnickae* were determined by virtue of the expression of surface markers, apoptosis, cell cycle and cytokine production by the MoDCs and by MoDC-T cell co-cultures. The anticancer activity using cancer cell lines was assessed by cytotoxic assays.

Results: We observed that *H. rudnickae* was taken up by MoDCs and induced the expression of the surface markers CD86, CD80 and CD83, as well as the production of IL-10, IL-12 and TNF-α. Moreover, a strong production of IFN-γ by naďve, but not memory T cells, was seen in MoDC-T cell co-cultures. Halophilic archaea neither interfered with the MoDC cell cycle and did not induce apoptosis. Interestingly, *H. rudnickae* secreted metabolites that were active against the ovarian cancer cell lines A2780 and its cisplatin-resistant derivative A2780cis.

Conclusions: Halophilic archaea are recognized by human MoDCs and activate human T cells. The anti-cancer effects against ovarian cancer cells open potential new possibilities for the treatment of frequent and difficult to treat cancers.

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Viral infections and immunity

Neuroinflammation and neurodegeneration in latent HSV-1 brain infection

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Introduction: Many studies have showed that there is a correlation between herpes simplex virus type 1 (HSV-1) infection and development of neurodegeneration processes later in life, such as Alzheimer's disease. Receptor-dependent Fas/FasL apoptotic pathway can participate both in direct elimination of HSV infection, but also in a complex regulation of the local neuroinflammatory response and mounting of the specific anti-viral response.

Aim: The aim of this work was to elucidate the role of Fas/FasL apoptotic and non-apoptotic pathway in HSV-1 induced neuroinflammation and its possible role in neurodegeneration.

Methodology: We used the mice without Fas or FasL expression to test how latent, long term infection with the neuropathogenic clinical strain of herpes simplex virus type 1 (HSV-1) influences development of neuroinflammation and neurodegeneration. Behavioural tests (labirynth test and NOR) were used to access behavioural changes.

Results: Early during infection (up to 7 day), Fas- and FasL-deficient mice (lpr and gld) were partially protected from encephalitis compared to WT mice and Fas/FasL de-

ficiency promoted both HSV-1-specific cytotoxic T cells as well as monocyte response within the CNS. In contrary, latently infected Fas- and FasL-deficient mice (over 90 days) showed increased viral titers, higher levels of proinflammatory responses, and worse results of memory tests in comparison to WT mice.

Conclusions: Our data indicate that the Fas/FasL pathway leads to excessive neuroinflammation during HSV-1 infection, which is associated with a diminished anti-viral response and an excessive neuroinflammation. However, Fas/FasL pathway is important in protection from long-term neuroinflammation and neurodegeneration.

Immunology in personalized medicine

Association of *ERAP1* and *ERAP2* gene polymorphisms and ERAP2 protein with the susceptibility and severity of rheumatoid arthritis in the Ukrainian population

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Introduction: Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints. CD8+ T cells recognize antigenic pepides presented by HLA class I molecules. Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 contribute to peptide preparation.

Aim: A possible association of ERAP1 and ERAP2 polymorphisms with RA, and also possible association of the serum level of soluble ERAP2 on severity of disease.

Methodology: DNA isolation, single nucleotide polymorphism genotyping by real-time PCR (TaqMan SNP Genotyping Assay). Serum ERAP2 concentrations measured by enzyme-linked immunosorbent assay (ELISA). Statistics: the two-tailed Fisher's exact test; the D'Agostino-Pearson K2 normality test; the receiver operating characteristic (ROC) analysis, including the area under the curve (AUC) determination.

Results: We found significant associations of ERAP1 rs30187, rs27044, and rs26618, as well as ERAP2 rs2248374, with susceptibility to RA. ERAP1 rs26653 and ERAP2 rs2248374 were also associated with disease active score (DAS28), and some polymorphisms were also associated with anti-citrullinated protein or anti-mutated citrullinated vimentin antibodies.

RA patients secreted higher concentrations of ERAP2 than controls. Patients with mild activity (DAS < 3.2) secreted a four times lower concentrations of ERAP2 than patients with severe disease (DAS28 > 5.1). We detected a higher level of ERAP2 in RF-positive patients than in RF-negative patients. ERAP2 concentration above 3.47 ng/ml indicated middle RA, whereas concentration above 5.85 ng/ml indicated severe phase of RA.

Conclusions: Some ERAP1 and ERAP2 polymorphisms seem to be associated with susceptibility to RA or the severity of the disease, and may have a diagnostic value. The ERAP2 protein tested in serum could be a valuable biomarker of RA severity.

Immunooncology

Comparison of microRNA expression profiles of colorectal cancer and normal colon epithelium cell lines and extracellular vesicles derived from them

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Introduction: An increasing prevalence of colorectal cancer prompts a need for development of new methods for early tumor detection. MicroRNAs (miRs) are short non-coding RNA molecules that play a pivotal role in gene regulation. miRs are effectively transferred to extracellular vesicles (EVs) – membrane structures commonly released by cells.

Aim: Our study examined the expression of miRs in four colon cancer cell lines and EVs derived from them (TEVs) in comparison to a normal colon epithelium cell line and its EVs.

Methodology: TEVs and EVs were isolated by ultracentrifugation from culture supernatant of SW480, SW620, SW1116, HCT116 and normal CCD841CoN cell lines and characterized according to MISEV2023. MiRs were analyzed by small RNA sequencing and validated by quantitative PCR.

Results: The analysis revealed 20 miRs highly expressed in colon cancer cell lines and efficiently transferred to TEVs, including miR-96, miR-182, miR-196b, miR-200a, miR-22b, miR-200c, miR-425. KEGG and GO analysis of these miRs showed their involvement in development, proliferation, invasion and migration of colon

cancer, as well as in vesicle maturation and transport-associated pathways. In parallel, normal cells expressed miRs engaged in proinflammatory response and tumor suppression, such as miR-369 and miR-143. A large group of miRs, including miR-193-3p and miR-199a, associated with i.e. tumor suppression, were downregulated in TEVs in comparison to EVs. Moreover, we identified a number of miRs (i.e. miR-1290, miR-423, miR-320c, miR-143, miR-125) to be more effectively transferred to TEVs/EVs than other ones.

Conclusions: Our results suggest a difference in the expression of a number of miRs between EVs derived from colon cancer and normal colon epithelium which might suggest their clinical relevance as potential cancer biomarkers.

Veterinary immunology and comparative immunology

White and red blood cell picture in rabbits experimentally infected with *Lagovirus europaeus*/GI.2.

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Introduction: Haematological factors in infections play a major role in assessing the body's health. As previous research on the *Lagovirus europaeus* (previously rabbit haemorrhagic disease virus) indicates, the analysis of haematological factors, including the immunological profile, is an important element in the assessment of the physiological condition of animals infected with this virus.

Aim: The aim of the study was to conduct a detailed characterization of the white blood cell and red blood cell count in rabbits infected with the *Lagovirus europaeus*/GI.2 virus.

Methodology: Haematological tests were performed in accordance with routine methods used in laboratory diagnostics. A blood count was conducted using a Mindray veterinary haematology analyser (model BC-30-Vet). Additionally, all results were subjected to statistical analysis.

Results: Haematological tests in rabbits infected with the *L. europaeus*/GI.2 virus showed that most blood parameters were within the normal range. Although slight differences were noted, they were not clinically significant. There was a decreasing trend in the levels of RBC, HGB, and HCT in infected rabbits. Similar changes were also noted in the case of PLT and WBC, but the analysis of the proportion of different types of leukocytes did not reveal any significant deviations from the norm.

Conclusions: The analysis of haematological results in rabbits infected with *L. europaeus*/GI.2 showed that the blood count is not suitable for monitoring the course of rabbit haemorrhagic disease (RHD) in its hyperacute form. Despite statistically significant changes in the level of some blood parameters, such as the number of erythrocytes, haemoglobin concentration, and the number of leukocytes, the results still remained within the reference norm. This suggests that morphology results alone do not provide a complete picture of the disease.

Immunogenetics

HLA eplet mismatch affects acute graft-versus-disease-free survival after HLA non-identical allogeneic haematopoietic stem cell transplantation

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Introduction: Haploidentical haematopoietic stem cell transplantation (HSCT) is increasingly used as an option for patients without an HLA-matched donor. However, HLA incompatibility constitutes a known risk factor for development of acute graft-versus-host-disease (aGvHD), which is still common despite the use of prophylaxis. Recent studies point to the importance of mismatches in eplets – small amino-acid sequences on the surface of HLA molecules that could be relevant for HSCT outcome.

Aim: The study aimed to analyse the prognostic value of eplet mismatches in predicting aGvHD onset in patients after HLA-mismatched unrelated and haploidenti-

cal HSCT. Eplet mismatch data were also compared with the presence of HLA antibodies after HSCT.

Methodology: High-resolution HLA genotyping data (A, B, C, DRB1 and DQB1) from 40 donor-recipient pairs were used for analysis of eplet mismatches and relationships with anti-HLA antibodies 30 and 90 days after transplantation.

Results: HLA class I and II high eplet mismatch load was associated with shorter aGvHD-free survival ($p = 0.019$). This relationship was especially visible for HLA class I ($p = 0.017$) and when HLA-A ($p = 0.038$), HLA-B ($p = 0.018$), and HLA-C ($p = 0.048$) loci were analysed separately. As expected, most patients, almost 70%, presented with anti-HLA class I and II antibodies 30 days after HSCT. The numbers slightly dropped at day +90. However, no association between the presence of anti-HLA antibodies and eplet mismatch load was observed.

Conclusions: HLA eplet mismatch load seems to be indicative of aGvHD-free survival in patients after mismatched and haploidentical HSCT.

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Immune disorders in pediatric; Biological treatment in immune-related diseases

Who sows the cytokine storm, reaps MAS – a clinical case of a 3-years-old girl with systemic juvenile idiopathic arthritis complicated by macrophage activation syndrome

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Introduction: Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease occurring in the pediatric population. There are a few subtypes of this disease, but one of the most interesting is systemic JIA (sJIA). The most severe complications of sJIA is macrophage activation syndrome (MAS). This condition is characterized by fever, very high levels of proinflammatory cytokines (IL-6), hiperferritinemia, elevated level of transaminases, triglycerides, D-dimers, and decreased fibrinogen. Many studies have shown that interleukin 6 (IL-6), IL-10, IL-12, IL-18, and interferon gamma are involved in the pathogenesis of MAS. The treatment involves pulses of systemic glucocorticoids and cyclosporine, but lately, more attention has been given to biological therapy. It is considered that anakinra should be included in the first line of treatment.

Aim: The aim of our study is a case of a 3-year-old girl with sever MAS as an onset of sJIA complicated by disseminated intravascular coagulation. The initial symptoms of sJIA were urticaria and high fever persisted for a few weeks. During hospitalization, many infections were ruled out. However, the results indicated significantly elevated inflammation markers (ferritin, CRP, IL-6). Following the Ravelli criteria, we diagnosed MAS. Treatment was initiated with steroid pulses, followed by cyclosporine; however, the clinical condition did not improve. Despite intensive therapy, skin petechiae were observed, and laboratory tests revealed a very high INR along with an extremely low level of fibrinogen. The patient required multiple plasma transfusions and clotting factor administrations. Due to the severity of the girl's condition, we initiated biological treatment with anakinra, resulting in a gradual improvement in her condition.

Viral infections and immunity

Immune checkpoint molecules as predictive marker of COVID-19 severity

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Introduction: Despite the development of specific vaccines and drugs, the progression to severe COVID-19 remains considerable concern. Immune exhaustion may be linked to the disease severity and measured by expression of selected immune checkpoint proteins.

Aim: This study aims to outline the relationship between the baseline expression of immune exhaustion lymphocyte markers and the severity of COVID-19.

Methodology: Immunophenotypes from the first day of hospitalization were analysed in the 525 COVID-19 patients. In this group 61 (11.6%) progressed and required mechanical ventilation (MV) and 113 (21.5%) died. Clinical, laboratory and radiologic data were associated with baseline immunophenotypes. Extent of inflammatory lung infiltrations were automatically analysed with a trained artificial neural network (ANN).

Results: The risk of MV was higher in younger patients and positively correlated with the expression of PD-1 on CD19+ B cells and CD3-CD16+CD56+ NK cells;

PD-L1 on CD3+ T cells and CD19+ B cells; CD200R on CD3+ T cells, CD3+CD4+ T cells, CD19+ B cells and CD3+CD16+CD56+ NKT-like cells.

The risk of death was higher in older patients and positively correlated with the expression of PD-1 on CD19+ B cells, CD3-CD16+CD56+ NK cells and CD3+CD16+CD56+ NKT-like cells, PD-L1 on CD3+ T cells and CD19+ B cells; CD200R on CD3+ T cells, CD19+ B cells, CD3-CD16+CD56+ NK cells and CD3+CD16+CD56+ NKT-like cells.

Conclusions: Baseline immunophenotype markers are predictive of outcomes and severity of COVID-19 and may identify patients at risk of progression. Combining the severity of immune exhaustion with biochemical findings, clinical variables and ANN-based lung tissue involvement may be a useful tool for clinical practice.

Innate Immunity

Acquired innate immunity: Crosstalk between β-glucan trained macrophages and biofilm forming *P. aeruginosa* strains

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Introduction: Interaction of M1 macrophages with biofilm forming bacteria might result in a toxic, hyperinflammatory response. Such activated macrophages, termed BAMs (biofilm associated macrophages), along with microbial pathogens, contribute to exacerbation of various chronic inflammatory infections (e.g. cystic fibrosis) and cause tissue injury.

Aim: *In vitro*: To compare the phenotype of trained macrophages with that of naďve macrophages stimulated with *P. aeruginosa* (PA) or biofilm matrix exopolysaccharide (PA-EPS). *In vivo*: To test the defence abilities of trained macrophages against severe PA infections.

Methodology: C57BL/6 mice sensitive to *P. aeruginosa* infections were used. Peritoneal macrophages were trained with *Saccharomyces cerevisiae* β-glucan (BG) and exposed to the biofilm forming PA strain isolated from the patient with severe lung cystic fibrosis (CF). The complex investigation of proteome, secretory properties, expression of phenotypic markers and antimicrobial properties of BG-trained macrophages exposed to PA was performed. Moreover, the effect of trained macrophages in the air pouch model of PA infection was investigated.

Results: PA bacteria and PA-EPS stimulated the detrimental, hyperinflammatory response of naďve macrophages. Oppositely, trained macrophages acquired a beneficial phenotype with mixed pro-inflammatory and pro-resolution characteristics without losing anti-bacterial properties. Most importantly, transfer of trained macrophages into infected air pouches markedly ameliorated the course of infection. PA bacterial growth and formation of biofilm were profoundly suppressed.

Conclusions: Our results suggest that training of macrophages with β-glucan might be a new therapeutic strategy in *P. aeruginosa* biofilm infections, including CF.

Primary immunodeficiencies; Secondary immunodeficiencies

Could the reactivation of EBV and immune checkpoint malfunctions unveil the secrets behind some primary and secondary immunodeficiencies?

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Introduction: Immunodeficiencies (ID) weaken the immune system, leading to infections, higher cancer risk, and autoimmune disease susceptibility. They are categorized into primary (PID) due to genetics, and secondary (SID) from external factors or diseases. Recent findings emphasize the importance of immune checkpoint imbalance and Epstein-Barr virus (EBV) reactivation in PID and SID development and progression.

Aim: The study aimed to compare the immune system parameters in patients with Chronic Lymphocytic Leukemia (CLL) and Common Variable Immunodeficiency (CVID). The study focused on immune checkpoint molecules such as PD-1/PD-L1, CTLA-4/CD86, and CD200R/CD200, as well as EBV reactivation.

Methodology: The study included patients with newly diagnosed CLL, CVID, and healthy individuals (HV), for whom EBV reactivation was assessed using specific

antibody serology and quantitative determination of viral load, as well as immunophenotypic analysis of peripheral blood with particular emphasis on T and B cell expressing immune checkpoints and their concentrations in serum.

Results: EBV reactivation markers were higher in ID patients than in HV, indicating increased viral activity in the immunodeficient. Immune checkpoint analysis showed significant changes in T and B cell populations in CLL and CVID patients, suggesting a link between immune checkpoint dysfunction and EBV reactivation in PID and SID.

Conclusions: Our research highlights the complex relationship between immune system dysfunctions, EBV reactivation, and immune checkpoint modulation in the development of PID and SID. The abnormalities in immune checkpoint signaling, combined with EBV reactivation, point towards a possible mechanism of tumor progression and immune evasion.

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Immunonephrology

Are the toll-like receptors "Key" to understanding the pathogenesis of primary glomerulonephritis?

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Introduction: Primary glomerulonephritis (GN), a key cause of end-stage renal disease, is influenced by genetic, environmental, and lifestyle factors. Research suggests the Epstein-Barr virus (EBV) and deregulation of innate immunity, especially Toll-like receptors (TLRs), might contribute to GN's pathogenesis.

Aim: The study investigated the correlation between T, B lymphocytes, and NK cells expressing TLRs in correlation with EBV reactivation markers in GN's immunopathogenesis.

Methodology: The study involved 100 GN patients and 25 healthy controls. Diagnoses were confirmed via kidney biopsy, with comprehensive blood and pathogen testing. The research focused on lymphocyte immunophenotyping, soluble TLR levels, and EBV detection through serological tests and PCR. Statistical analysis examined the relationships between TLR expression, EBV reactivation, and GN.

Results: Patients with glomerulonephritis (GN) exhibited heightened expression of TLRs (TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9) on T and B lymphocytes, as well as NK cells, compared to control subjects. Furthermore, elevated levels of soluble TLRs in the plasma of GN patients suggest an involvement of TLRs in the pathogenesis of GN. Also, the reactivation of EBV was notably apparent in GN patients, especially those with proliferative forms of the disease, highlighting the pivotal role of EBV in the progression of GN.

Conclusions: This study emphasizes the impact of TLR deregulation on glomerulonephritis (GN) and suggests that TLRs have the potential to serve as biomarker molecules. While the study verifies the presence of EBV reactivation in GN patients, further research on TLR receptors is necessary to explore new potential therapeutic strategies that can enhance the efficacy of GN treatment.

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Immune disorders in pediatric

Protective shield: Assessing the safety of COVID-19 vaccination in children who previously battled MIS-C

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Introduction: COVID-19 vaccinations are effective and safe method of preventing infection and the spread of the pandemic. Multisystem Inflammatory Syndrome in Children associated with SARS-CoV-2 infection (MIS-C) remains an enigmatic disease that has not been fully explored. Therefore, questions arise regarding the safety of vaccinations in this group of patients.

Aim: The aim of the study was to analyze existing literature and summarize the single-center experience of the Department of Pediatric Pulmonology and Rheumatology at the Medical University of Lublin on the topic.

Methodology: Literature data analyses were conducted using databases: PubMed, Scopus, and Google Scholar. An in-depth analysis was performed on cases of MIS-C patients who had been vaccinated against COVID-19. Essential clinical and laboratory data were gathered from the patients before vaccination and at specific time points after vaccination.

Results: Literature analysis indicates that COVID-19 vaccinations are safe and effective in this patient group,

offering tangible benefits. The limited cases of MIS-C development in vaccinated children are likely attributed to incomplete vaccination schedules and inadequate immune responses, rather than direct cause-and-effect correlations. Our findings align with existing literature, showing statistically significant increase in the level of protective anti-SARS-CoV-2 antibodies with absence of moderate to severe reactions post-administration, and no recurrence of MIS-C.

Conclusions: Vaccination against SARS-CoV-2 in patients with MIS-C is well-tolerated and effective, supported by both literature and experiences from our and other medical centers. Scientific societies recommend maintaining a 3- or 6-month interval between diagnosis and vaccination. Although there are no reports of recurrent MIS-C to our knowledge, the ongoing discovery of various aspects of the SARS-CoV-2 virus pandemic emphasizes the necessity of assuming that the benefits of COVID-19 vaccination in this patient cohort outweigh potential adverse effects.

The study was funded by the Medical University of Lublin, DS No. 410.

Primary immunodeficiencies

γδT cells are impaired in patients with primary antibody deficiencies

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Introduction: Inborn errors of immunity (IEI) often involve defects in antibody synthesis, manifested, among others, as specific antibody deficiency (SAD) and common variable immunodeficiency disease (CVID). γδT cells, constituting a small subpopulation (2-5%) of T cells, play a crucial role as a link between adaptive and innate immune responses, contributing to anti-microbial and anti-tumor surveillance.

Aim: Our study aimed to assess the quantitative and functional status of γδT lymphocytes in adult patients diagnosed with CVID and SAD.

Methodology: The study involved 28 patients (18 with CVID, 10 with SAD) undergoing IgG replacement therapy and 13 healthy volunteers (HV's). After obtaining written consent, we collected 20 ml of whole blood, isolated peripheral blood mononuclear cells, and stained them for flow cytometry analysis. On the 7th and 14th day of cell culturing, we determined γδT cells numbers and assessed cell cycle.

Results: Statistical analysis revealed significantly higher γδT Vδ1 subpopulation percentages in CVID patients in comparison with γδT Vδ2 and an inverted Vδ1/Vδ2 ratio. In SAD, Vδ2 lymphocytes predominated with noteworthy overexpression of PD-1, CTLA-4, and NKp30 on γδT, and TIGIT on Vδ2 subpopulation. γδT proliferation ability was markedly impaired in both groups of patients compared to HV's, with no observed differences in cell cycle phases.

Conclusions: These findings suggest that patients with antibody deficiencies may exhibit γδT pathologies, both quantitative (CVID) and functional (SAD), potentially contributing to their symptoms. Despite proper IgG substitution, the observed lack of correction highlights the need for further investigation into the underlying mechanisms.

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Biological treatment in immune-related diseases

Successful treatment hypereosinophilic syndrome with dupilumab in a patient resistant to anti-IL5 and anti-IL5r therapies – a case report

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Introduction: A 31-year-old woman with a lifelong history of severe asthma and hypereosinophilic syndrome (HES) history presented with uncontrolled symptoms and frequent exacerbations despite being treated with high-dose inhaled corticosteroids and two biologics: mepolizumab (escalating doses from 300 to 700 mg every 4 weeks) and benralizumab (30 mg every 4 weeks). These treatments were discontinued due to diminishing efficacy and lack of responsiveness, respectively. After an excessive evaluation, dupilumab was introduced.

Aim: Within the first two weeks of dupilumab treatment, the patient showed remarkable clinical improvement. At the six-month follow-up, there was a substantial increase in her Asthma Control Questionnaire (ACQ) score from 3.5 to 0.9 and Asthma Quality of Life Questionnaire

(AQLQ) score from 2.3 to 5.9. Additionally, she experienced no exacerbations and was able to reduce her daily oral corticosteroid dosage from 25 mg to 10 mg of prednisone. No significant side effects or increases in eosinophil count were observed.

Conclusions: HES is a diverse group of conditions marked by persistent eosinophilia and related organ damage. This case underscores dupilumab's potential as a viable treatment alternative for patients with HES and concurrent asthma, particularly when other biologics targeting IL-5 such as mepolizumab and benralizumab are ineffective. Dupilumab targets the IL-4/IL-13 pathway, which may confer a unique therapeutic benefit in managing these complex cases.

Immunomodulation and Immunotoxicology

The influence of algae and their toxins on the immune system – current state of knowledge

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Introduction: Algae, mainly marine algae, are widely used as sources of food, cosmetics, drugs, and recently also potential source for novel therapeutic compounds. Climate-change driven changes in the algae may also change HAB toxicity. The toxicity of HAB is already known to vary significantly among isolated of some algal species. The effects of climate stressors (e.g. temperature, nutrients, salinity turbidity, anthropogenic) on the toxin-producing microalga are very important for aquatic animal health and human consumer. The dramatic effects observed on fish are thought to be due to algal polyether toxins, known as the prymnesins, but their lack of environmental detection has resulted in an uncertainty about the true immunotoxic agents. However, their biological and immunological effects on immune cells have not been fully elucidated.

Aim: Therefore, to broaden the knowledge for potential of algae in biomedical and pharmaceutical fields, it is crucial to critically analyse the literature in terms of the use of algae and their toxins as immunostimulators and targets for therapy in the treatment of many diseases.

Methodology: All available databases have been searched through to provide the most accurate picture of the data available in the subject. Detailed studies of HABs o eukaryotic microalgae in the natural environment are limited and warrant investigation.

Results: It has been noticed that algae and their toxins might cause several positive effects on the immune system, including anti-inflammatory, antiviral, anticancer and metabolic effect. As far as anti-inflammatory effect is concerned, it has been proved that algae contain a significant amount of carotenoids, namely beta carotene, lycopene, and lutein, providing it with good antioxidant properties. Some of the species of algae can trigger the migration of circulating natural killer cells. Algae are also able to stimulate the mobilization of T and B lymphocytes. Moreover, by the high level of omega-3 fatty acids, algae show the ability to inhibit the formation of inflammatory prostaglandins and arachidonate metabolites.

As far as the antiviral effect of algae is concerned, there are studies drawing the attention to compounds like cyanovirin-N, with the potential to inactivate strains of the HIV virus and to inhibit cell-to-cell and virus-to-cell fusion. Other, like calcium spirulan (Ca-SP) selectively inhibit the penetration of enveloped viruses, like *Herpes simplex*, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1. Ca-SP is also known to inhibit tumor invasion and metastasis. Often referred to as "golden alge", *Premnesium parvum* has caused particular issues for aquatic animal health in the Europe and North America in the last decade. As a result, the biotic factors that impact *P. parvum* growth and toxicity have been studied intensively in many laboratories.

Conclusions: Summing up, algae and algal metabolites appears to have a huge potential and may provide new paths for treatment – such an important concern in the face of antibiotic resistance. But the climate-change induces a new toxic effect of microalge and HABs on the animal and human health, especially on defense mechanisms and protection against diseases.

Veterinary and comparative immunology

Rabbit as a pivotal model animal for studying diseases of various etiologies

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Introduction: The usage of the experimental animals is currently decreased according to the 3R rule, nevertheless there are still many cases where it is impossible to rule out such a model. Rabbits are one of the most frequently used experimental animals for research, particularly as

a bioreactor to produce antibodies, but also as a very good model for several human viral diseases such as rabies, syphilis, HIV, papillomavirus and most importantly human fever viruses (HFV), but also other diseases like acute liver failure (ALF) that can be caused by paracetamol toxicity, drug-induced liver injury associated with prescription drugs, herbs and dietary supplements, hepatic ischemia, and autoimmune or viral liver diseases.

Aim: The aim of the work is to show the diversity and utility of rabbits as a model animal based on others and self-studies.

Methodology: All available databases have been searched through to provide the most accurate picture of the data together with the own studies performed on the rabbit haemorrhagic disease virus model for the HVF and AFL.

Results: Rabbit as an experimental model provides a wide possibility to study – from the immune system evolution to its genetic features, including the antibody rearrangement or different resistances against bacterial proteases activity. It is also a good model to understand the co-evolution between vertebrate hosts and viral pathogens. Moreover, it is used to study new therapies and treatments for cancer, including innovative drugs. Our own studies show that rabbit model is perfect to study the host-virus interactions on the molecular level, including the significance of apoptosis and autophagy in the course of viral infections causing liver damage. It is worth mentioning that obtaining the reference values for immunological parameters in European rabbits contribute to expanding the knowledge of the model.

Also, there are several studies showing the use of rabbit model for metabolic issues and digestive tract diseases due to its sensitivity and lability.

Conclusions: The data show that rabbit is an excellent, yet a challenging model to study.

Recommendation

Recommendations from the Statistical Editor of Allergy, the official journal of the European Academy of Allergy and Clinical Immunology (EAACI)

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Introduction: In recent years, there has been a decline in the quality of statistical reporting, contributing to the prevalent misinformation and disinformation about vaccines. Therefore, it is recommended to not only publish statistical recommendations but also to implement them into the daily operations of biomedical journals, such as Allergy, the official journal of the European Academy of Allergy and Clinical Immunology (EAACI).

Aim: The aim of the conducted research is to summarize the previous work of the statistical editor in the official journal of EAACI, which is targeted towards allergists and immunologists.

Methodology: For analysis, 35 selected statistical reviews from Allergy were included, reflecting common errors in diverse statistical analyses.

Results: Among the most commonly made errors by authors regarding statistical analysis are the omission of several assumptions necessary for the application of appropriate statistical tests. Improper selection of measures of heterogeneity and their interpretation in meta-analysis also deserve attention. One recommendation for conducting statistical reviews is to calculate the effect size, as results are often overinterpreted. Additionally, it is advised to provide a more thorough description of the applied statistical tests, as superficial descriptions are frequently observed in this regard. Other aspects to consider include addressing the impact of missing data, mentioning the individual responsible for conducting the analysis in the cover letter, selecting appropriate descriptive statistics, and various other statistical aspects.

Conclusions: There is a recommendation to place greater emphasis on implementing statistical recommendations, including in journals aimed at allergists and immunologists.

Autoimmunity and Autoinflammation; Immunity of aging and aging-related disease

A study of sirtuin 1 (SIRT1) expression in patients with rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the joints and various organs. Multiple epigenetic changes have been identified and correlated with the aggressive phenotype of RA, including the involvement of sirtuins (SIRT1-7) – NAD+-dependent deacetylase proteins, regulating important metabolic pathways which can affect immune cells responses (T and

B cells, neutrophils, macrophages and chondrocytes) which are directly involved in the development of inflammation in RA. It has been shown that SIRT1 activity has the potential for treating many age-relating diseases, such as type II diabetes, cardiovascular disease, cancer, as well as RA.

Aim: The aim of our study was to examine the expression of the SIRT1 gene and protein level in healthy individuals and patients diagnosed with RA.

Methodology: For our research, we used 30 samples of blood plasma and tissue fragments (synovial membranes) collected from individuals without RA (control group) and 46 samples from patients diagnosed with RA. We assessed SIRT1 gene expression by real-time PCR and SIRT1 protein level by ELISA (in plasma) and IHC (in synovial membranes). We also examined the correlation of SIRT1 expression with patients' clinical parameters.

Results: Statistical analysis showed that SIRT1 expression at the gene and protein level is significantly lower in the group of RA patients compared to healthy individuals, and interestingly, it does not correlate with clinical parameters of patients.

Conclusions: SIRT1 influence a number of cellular processes involved in the development of RA. Our data show that SIRT1 may be a real target for RA therapy in the future, and by activation of SIRT1 we can probable limit the development of RA and provide a more precise and effective treatment tool, limiting the adverse effects of therapy.

Transfusion Immunology

What is the best blood type?

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The valuation of blood began with the appearance of the term "blue blood", whose owners were associated with the aristocracy. As we know, people belonging to higher social classes did not have sun-tanned skin during work as well as tired hands, which resulted in their blue veins being more clearly visible against the background of pale skin. Historians indicate that another reason for the "blue blood" of the rich may have been the widespread use of silver tableware for meals and silver utensils for hygiene, which facilitated the absorption of larger amounts of silver, leading to silversmithing. One of its symptoms was a lifelong blue skin color.

The discovery of blood groups by K. Landsteiner initiated the scientific exploration of the secrets of blood groups. The current names ABO were given to blood groups by L. Hirszfeld, who was also the first to start extensive population research.

The eugenic definition of the best race, the white Nordic, was a dead end. It reached its apogee during Hitler's rule. According to his ideology, one of the characteristics of the best pure blood of the human race, the master race, was to be blood type A.

Blood group is a set of antigens that are present on the surface of red blood cells. There are currently over 345 different antigens classified on red blood cells. These antigens have different structures: polypeptide proteins, polysaccharides. Some of them may detach from the blood cell surface and circulate in the plasma or settle on the vascular endothelium. These antigens can perform specific functions and therefore influence our health, well-being and even character.

Currently, scientists are interested in how blood type determines a person's risk of developing specific diseases. Many theses have already been presented in this regard. The current conclusions are mainly based on statistical data. The dependence of the risk of disease on the blood type has been confirmed in the case of dementia, a number of cancers, bacterial infections (including streptococci, staphylococci, *Helicobacter pylori*), malaria, cholera, coronary heart disease, venous thromboembolism, diabetes, or greater susceptibility to stress, anxiety, cognitive impairment, memory disorders, and even reduced reproductive abilities.

Although the above-mentioned relationships have been confirmed by many scientific studies, the mechanisms that determine them are still being sought. Due to the different risks of pathologies associated with a specific blood group, it is difficult today to determine which group would be best for us. Transfusiologists say that the best blood type is the one that best serves the recipient after transfusion and does not cause adverse reactions.

Viral infections and immunity

The potential protective effect of eosinophils in the infection of human lung vascular endothelium by the human coronavirus 229E

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Introduction: Respiratory viruses may cause exacerbations of chronic respiratory diseases. Recent studies have shown a possible role of eosinophils in eliminating viruses. Eosinophils are equipped with toolkits for antiviral response. The role of eosinophils in modulating the antiviral response of the lung vascular endothelium during viral infections is unknown.

Aim: Assess if eosinophils may support the vascular endothelium in immune responses against viral infection.

Methodology: Eosinophils isolated from the peripheral blood of healthy individuals by negative immunomagnetic selection. For the antiviral activation of eosinophils, an *in vitro* model of 24h incubation with TLR agonists: poly I:C and R848 was used. To analyse eosinophils activation and mRNA expression real time PCR was used. Protein concentrations were assessed by ELISA assay. In a second step, human lung microvascular endothelial cells (HMVEC-L) were incubated with supernatants from activated eosinophils, and then anti-inflammatory and antiviral responses were assessed by mRNA expression in real time PCR and protein release by ELISA assay.

Results: In the first step was observed mRNA up-regulation of interferons, and proteins of intracellular mechanisms of antiviral immunity – 2'-5'-oligoadenylate synthetase 1 (OAS-1), protein kinase R (PKR) and interferon-induced GTP-binding protein Mx-1 (MX-1). Eosinophils after stimulation produced RANTES, IL-6, and IFN-β. In the second step, endothelial cells incubated with eosinophil-derived supernatants had higher mRNA expression of IFN- β, OAS-1, and PKR, and produced IFN-β, IL-6, and RANTES.

Conclusions: Activated eosinophils display the potential the support the lung vascular endothelium during viral infection and therefore they may play a significant role in the antiviral response of the lung endothelium.

Immunotoxicology

Harmful effects of imazalil on human neutrophil function – *in vitro* studies

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Introduction: Due to its high efficacy, imazalil (IMZ) has been a widely used pesticide for many years, and is therefore a food and environmental contaminant. IMZ, as an androgen receptor blocking compound, interferes with testosterone activity, and can be classified as an EDCs (endocrine-disrupting chemicals). Due to its widespread occurrence, it is detected in human plasma. Numerous studies show that EDCs present in the environment can modulate immune system function by, among others, affecting neutrophil activity

Aim: The aim of the study was to evaluate the effects of imazalil and testosterone on the expression of CD molecules associated with various neutrophil functions, such as adhesion, chemotaxis and phagocytosis.

Methodology: The material for the study was neutrophils isolated from whole blood of men (voluntary blood donors) from the Regional Centre for Transfusion Medicine in Bialystok. Cells were incubated in the presence of IMZ or testosterone. Flow cytometry was used to evaluate the expression of selected CD antigens. Statistical analysis of the results was performed using Statistica version 13.3 program (StatSoft, Inc., Tulsa, OK).

Results: The results of cytometric analysis showed that exposure of cells to imazalil led to an increased percentage of neutrophils with CD14 and CD62L expression compared to cells not treated with the xenobiotic, as well as to cells exposed to testosterone. A lower percentage of neutrophils with CD11c and CD15 expression was also observed in the presence of IMZ. There was no effect of testosterone on the expression of the tested CDs in neutrophils.

Conclusions: Imazalil induces significant changes in the phenotype of neutrophils, which, consequently, in people exposed to the compound, may predispose to impaired innate immunity through a pathway dependent on key neutrophil functions.

Immunomodulation and Immunotoxicology

Immunotoxic effects of cyanotoxins

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Introduction: Cyanotoxins are secondary metabolites of cyanobacteria that can cause water blooms observed around the world. Humans can be exposed to cyanotoxins mainly through the oral route, but inhalation and transdermal routes can also cause toxins to enter the body. According to the target organ, cyanotoxins can be classified as hepatotoxins, cytotoxins, neurotoxins, dermatoxins, and irritant toxins. However, recent studies report the ability of cyanotoxins to affect also immune system cells.

Aim: The aim of the study was to present the current state of knowledge about the possible effects of exposure to various groups of cyanotoxins in the context of their impact on the immune system.

Discussion: The analysis of available data shows that cyanotoxins have a very broad spectrum of activity, including both stimulating and immunosuppressive effects. The most widely studied group of cyanotoxins are microcystins, with the most frequently detected and most toxic form microcystin-LR. Their main effect is hepatotoxicity resulting from blocking the activity of protein phosphatase type 1 and type 2A (PP1 and PP2A) in the cytoplasm of liver cells. Most information about immunotoxic effects concerns this group of toxins. However, data on other cyanotoxins, including cylindrospermopsin, are also present.

Conclusions: In the absence of comprehensive solutions to the problem of excessive growth of cyanobacteria and various negative effects of exposure to their metabolites, further research is needed to understand the mechanisms of action of cyanotoxins and determine concentrations that are safe for humans, especially in drinking water.

Vaccination and vaccines

Unveiling the role of secretory leukocyte protease inhibitor (SLPI) in modulating the immune response elicited by pertussis vaccines

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Introduction: The re-emergence of pertussis in high-income countries poses a challenge. Many factors contribute to this resurgence, with the transition from whole-cell (wP) to acellular vaccine (aP) being significant. SLPI, secreted mainly by mucosal and innate immune cells, is proposed to regulate the quality of antigen-specific immune responses.

Aim: Delineate differences in cellular response induced by aP and wP vaccines in mice lacking SLPI expression.

Methodology: C57BL/6-SLPI WT and KO mice received two doses of each vaccine. Immune cells were analysed by flow cytometry. OVA presentation by bone marrow-derived dendritic cells (BMDCs) was evaluated using OVA-specific CD4 T cells, and pertussis antigen recall experiments were conducted to assess type of immune response. ELISA was used to determine cytokine levels.

Results: Results revealed increased CD4 T cell infiltration at wP vaccination sites compared to aP. CD44+ and CD69+ markers were highly upregulated in wP-immunised mice, with no significant difference between WT and KO mice. Peritoneal exudate cells (PEC) from wP-vaccinated mice induced Th1 and Th17 responses, with KO mice showing lower IFN-γ and IL-17 levels. Although wP induced higher SLPI levels than aP in peritoneal lavage fluid, PEC cells from wP-immunised mice secreted the lowest SLPI levels upon pertussis antigen re-exposure. Moreover, we examined OVA presentation by BMDCs from aP and wP vaccinated WT and KO mice. No differences in OVA-specific CD4 T cell proliferation were observed regarding the SLPI levels or type of vaccine. Lastly, we showed greater infiltration of neutrophils to the site of immunisation in wP-immunised KO mice in comparison to WTs.

Conclusions: High SLPI levels at wP immunization sites suggest no direct correlation between SLPI and IFN-γ secretion, however, reduced Th1 and Th17 responses in wP-immunized KO mice may be linked to increased neutrophil infiltration, necessitating further investigation.

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Primary Immunodeficiences

Secret behind Kimura disease

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Introduction: Kimura disease is a rare form of chronic inflammatory condition of unknown origin involving

subcutaneous tissue and lymphatic nodes that can mimic other lymphoproliferative disorders including inborn errors of immunity (IEI).

Aim: We would like to present a complicated diagnostic case of a child with histopathological suspicion of eosinophilic lymphogranuloma who was finally diagnosed with Activated Phosphoinositide 3-kinase δ syndrome (APDS).

Methodology: Retrospective analysis of patient medical records, flow cytometry studies, trio whole exome sequencing.

Results: 3-year-old boy was admitted to Department of Immunology with suspicion of Kimura disease. The child had complicated history of hydrocephalus, drug-resistant epilepsy, subfebrile episodes of unknown origin, lymphadenopathy, splenomegaly, decreased muscle tone, short stature, dysmorphic features and delayed psychomotor development. His immune studies showed decreased IgG concentration, elevated IgM, low complement concentrations and CH50 activity. Lymphocyte immunophenotyping revealed decreased number of CD3+CD8+ lymphocytes, low recent thymic emigrants and significantly increased translational B cells. 18FD-FDG PET/CT imaging revealed generalized lymphadenopathy. Infectious and malignant causes were excluded. Genetic studies confirmed clinical suspicion of APDS revealing heterozygous PIK3CD de novo missense mutation. Patient currently receives immunoglobulin replacement therapy and is scheduled for treatment with mTOR inhibitor.

Conclusions: Patients with lymphoproliferation, especially when accompanied by other symptoms, should always raise suspicion not only of malignancy, but of IEI as well. Initial diagnosis of Kimura syndrome, which is a benign condition, could postpone proper treatment and further worsen patient prognosis.

Autoimmunity and Autoinflammation

Role of extracellular vesicles in neutrophil and endothelial cell activation – possible link to the pathophysiology of granulomatosis with polyangiitis

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Introduction: In granulomatosis with polyangiitis (GPA), inflammation affects and destroys blood vessels.

Aim: In this study, we focused on the role of extracellular vesicles in the pathophysiology of GPA.

Methodology: We analyzed the response of HUVEC cells to neutrophil-derived extracellular vesicles (EVs) and the response of neutrophils to circulating EVs isolated from GPA patients. HUVECs/neutrophils were stimulated with EVs and miRNA/mRNA/protein expression was measured. In addition, selected cytokines were measured in serum samples from GPA patients.

Results: Comparison of differentially expressed miRNAs/mRNAs between non-stimulated/EV-stimulated cells revealed two patterns: HUVEC – up-regulation of 11 mRNA transcripts (including: CXCL8, DKK1) and downregulation of 3 miRNAs (including: miR-661, miR-664a-3p); downregulation of 9 mRNA transcripts (including FASLG, CASP8) and upregulation of 9 miRNAs (including miR-223-3p, miR-142-3p); Neutrophils – up-regulation of 14 mRNA transcripts (including: IL-1b, MMP9) and downregulation of 2 miRNAs (miR-548c-3p, miR-6221-5p); downregulation of 5 mRNA transcripts (including CXCL4, SART3) and upregulation of 3 miRNAs (miR-223-3p, miR-629-3p, miR-573). Stimulated HUVECs released IL-8, DKK-1, ST2, GDF-15, while stimulated neutrophils IL-8, IFN-α, PR3, MPO and MMP-9. Cytokines released by both cell types were also elevated in the serum of GPA patients. Comparison of the miRNA profile in neutrophil-derived EVs and EVs isolated from plasma of GPA patients showed that 8 of the 10 most abundant miRNAs were similarly expressed (including miR-223-3p, miR-142-3p). Moreover, HUVECs transfected with mimic miRNA (miR-223-3p, miR-142-3p) produced elevated levels of IL-8 and ST2.

Conclusions: In conclusion, our data suggest that EVs may play an important role in the pathophysiology of GPA.

Viral infections and immunity

Human lung microvascular endothelium from certain human individuals may present the constitutive resilience to HCoV-229E infection

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Introduction: Epidemiological and clinical observations during COVID-19 pandemic suggest that there may exist several possible manners of individual responses to coronaviruses - from asymptomatic to severe, often fatal,

pneumonia. We proved that the human lung microvascular endothelium (HMVEC-L) expresses surface entry receptors for high- and low-pathogenic HCoVs and possesses a wide range of effective innate mechanisms to limit viral replication.

Aim: To compare two different models of response of HMVEC-L to infection with human coronawirus 229E (HCoV-229E).

Methodology: HMVEC-L from 2 healthy patients (Patient 1 and Patient 2) were incubated with HCoV-229E for 3 h, washed out and cultured for 120 h. In relevant time points mRNA expression of anti-viral and inflammatory genes were assessed. HCoV-229E copies, AP-N (entry receptor) presence and apoptotic cells rate were also measured in flow cytometry.

Results: In HMVEC-L from both patients viral copies were present right after 3 hour exposition to HCoV-229E. (2000 and 4000 copies/µl in 0 hpi, respectively). However, in cells form Patient 2, neither replication (2100 copies/µl), nor proinflammatory (RANTES 2,4, IL-6 1,2-fold) and antiviral (IFN-β 0,6-fold) response at 72 hpi were observed. No cytopathic effect was shown. By contrast, in HMVEC-L of Patient 1 extensive virus replication was noted, which was accompanied by the high inflammatory (RANTES 1200-, IL-6 76-), antiviral response (IFN-β 47-fold) (1,057 × 10^6 viral copies/µl) and cytopathic effect.

Conclusions: HCoV-229E may infect HMVEC-L from both patients that shows a surface expression of AP-N. However, HMVEC-L from certain human individuals may present the constitutive resilience to HCoV-229E infection. Thus, the lung vascular endothelium may play an important role in anti-viral responses.

Transfusion immunology

Alloimmunization with platelet antigens: is it worth considering when making a diagnosis?

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Introduction: Class I human leukocyte antigens (HLA) and human platelet antigens (HPA) are expressed on platelets. In cases of the donor-recipient or mother-fetus serological incompatibility of these antigens, there is a risk of triggering a specific immune response, resulting in the production of alloantibodies. The presence of anti-HLA and/or anti-HPA antibodies may contribute to the occurrence of post-transfusion or post-transplant reactions, platelet transfusion refractoriness (PTR), as well as fetal neonatal alloimmune thrombocytopenia (FNAIT). The number of patients with antibodies against platelet antigens constantly increases.

Aim: The assessment of the incidence of antibodies against platelet antigens in patients who experienced symptoms of a post-transfusion reaction (A), who developed PTR (B), and with suspected FNAIT (C).

Methodology: Blood samples from 447 patients (A: 205, B: 169, and C: 73) were tested, using lymphocytotoxicity test (LCT) (Inno-train, Germany) or enzyme-linked immunosorbent assay PakPlus (Immucor, Spain).

Results: The presence of anti-HLA and/or anti-HPA antibodies was detected in 134 (29.38%) patients. These antibodies were detected in 34 (16.59%), 82 (48.52%), and 18 (24.66%) patients from groups A, B, and C, respectively. The detected antibodies showed the following specificity: anti-HLA (121, 90.3%), anti-GPIIb/IIIa (24, 17.91%), anti-GPIa/IIa (8, 5.97%), and anti-GPIb/IX (3, 2.24%).

Conclusions: The screening for antibodies against platelet antigens is a powerful diagnostic tool that can dispel many doubts and help with providing patients with blood and its components compatible with their HLA class I antigens or HPA antigens.

Immunology in personalized medicine

Expression level of *NLRP3* and *IPAF* in peripheral blood leukocytes in children with primary hypertension

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Introduction: Primary hypertension (PH) is characterized by systemic, low-grade chronic inflammation. Both innate and adaptive components of immunity are engaged in PH development. Important components of innate immunity are inflammasomes which are multi-protein complexes that assemble in the cytosol in response to infectious agents or changes associated with cell injury. They generate active forms of pro-inflammatory cytokines IL-1β and IL-18 from their inactive precursors by the ac-

tion of caspase 1. Inflammasomes are composed of oligomers (sensors), caspase 1 and adaptor protein that links the two. There are many types of inflammasomes which use 1 of 10 different NLR – family proteins (Nucleotide binding/Leucin rich repeats) as sensors. The NLRP3 inflammasome uses NLRP3 (NOD-like receptor family, pyrin domain containing 3) as a sensor. The NLRP3 inflammasome is expressed in innate immune cells including macrophages and neutrophils and other cells. The NLRP3 inflammasome is activated by uric acid, cholesterol crystals, extracellular ATP, reduced intracellular potassium ion concentration (plasma membrane damage) and reactive oxygen species (ROS). IPAF (ICE – protease Activating Factor) or NLRC4 (NLR – Caspase recruitment domain containing protein 4) inflammasome is expressed in many cell types including immune cells like monocytes, macrophages, lymphocytes. IPAF activation depends on action of NAIPs (NLR family apoptosis inhibitory proteins) which serve as upstream sensors for NLRC4 (IPAF) inflammasome assembly. Both IPAF and NLRP3 are engaged in cardiovascular diseases such as obesity, atherosclerosis, diabetes and hypertension. However, their role in PH development in children adolescents is not known.

Aim: The aim of this study was to determine the mRNA expression profile of *NLRP3* and *IPAF* in peripheral blood leukocytes of PH children and age matched controls and to evaluate its correlation with clinical parameters, including LVMi (left ventricular mass index), cIMT (carotid intima media thickness), PWV (pulse wave velocity), WCSA (wall cross sectional area).

Methodology: The group of seventy four children was recruited for the study and was divided into 2 two parts: a) 39 pediatric patients with diagnosed PH (29 boys and 10 girls) from the Department of Nephrology, Kidney Transplantation and Arterial Hypertension, Children's Memorial Health Institute in Warsaw; b) control group consisting of 35 normotensive children (19 boys and 16 girls). The clinical tests (ultrasound, Echocardiography, the oscillometric method) and laboratory tests (biochemical, molecular methods) were performed on each patient and control participant.

Results: The PH children/adolescents had reduced expression of leukocyte *NLRP3* ($p = 0.0007$) and *IPAF* ($p = 0.0054$) as compared to the control group. The *NLRP3* expression correlated with *Caspase 1* ($r = 0.68$), and *IL-1*β ($r = 0.46$) levels. The *IPAF* expression also showed positive correlations with that of *Caspase 1* ($r = 0.66$), *IL-1*β ($r = 0.58$) and *IL-6* ($r = 0.36$).

Multivariate regression analysis revealed correlations between *IPAF* expression and LVMi and *NLRP3* and cIMT.

Conclusions: Decreased expression of leukocyte *NLRP3* and *IPAF* in the PH children/adolescents suggests compensatory mechanism that may protect against more vigorous inflammatory reactions at the early stages of PH. However, still positive correlations of the expression of *IPAF* and of *NLRP3* vs. *IL-1*β and *Caspase 1* and of *IPAF* vs. LVMi and of *NLRP3* vs. cIMT , may indicate on induction of leukocyte inflammatory activities reflected here by their associations with LVMi and cIMT values.

Immunity and stem cells

The mesenchymal stem cells (MSC) as active contributors to enhanced MMP-9 expression following TNF stimulation

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Introduction: The lung resident Mesenchymal Stem Cells (lrMSC) role in the context of inflammation processes concurrent with pulmonary remodeling remains elusive.

Aim: We aimed to assess the role of MSC in the response to inflammation induced by house dust mite (HDM) extract.

Methodology: Neutrophilic asthma inflammation was induced by intranasal application of HDM extract. The single cell suspension was obtained by lung dissociation and lrMSC were analysed on FACS Canto II. Isolated human adipose derived MSC (hAD-MSC) were utilized to investigate the cell responses under *in vitro* condition. The hAD-MSC were incubated with media derived from human peripheral blood mononuclear cells (PBMC) stimulated with HDM, LPS or NaCl for 24 h-120 h or directly incubated with stimulants. Additionally hAD-MSC were incubated with individual cytokines (IL-1β, TNF, IFN-g). The viability and phenotype were confirmed by flow cytometry, gene expression was assessed by qPCR, protein level was determined by Western blot and cytokine content was analysed by ELISA.

Results: We observed statistically significant decrease of lrMSC number in experimental asthma model. Additionally, isolated lrMSC possessed reduced ability to differentiate. Similarly, to hAD-MSC after 24 h stimulation with HDM/LPS. However, there was no differences in hAD-MSCs marker characteristic. Interestingly, MMP-9 gene expression after priming and TNF stimulation was elevated which was also observed on protein level.

Conclusions: Study reveals the influence of inflammation, induced by HDM extract, on MSC. We found a significant decrease in lrMSC numbers and reduce differentiation capacity in an experimental asthma model, similar in hAD-MSC following HDM/LPS stimulation. However, prior stimulation of hAD-MSCs enhanced MMP-9 release, indicating a potential role in tissue maintenance.

Immunomodulation and Immunotoxicology

Immunomodulation of T cell response by peptides targeting the BTLA-HVEM complex in melanoma

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Introduction: Immune checkpoint inhibitors have revolutionized cancer treatment by boosting the immune response against tumors. The BTLA-HVEM complex plays a critical role in regulating immune cell activation and proliferation, making it an appealing target for immune-modulating therapies. This is the first research describing the immunomodulatory properties of peptides based on HVEM protein2 targeting the BTLA-HVEM inhibitory checkpoint.

Aim: The main aim of my presented study was to analyze the effect of synthetic ligands of the BTLA-HVEM complex on T-lymphocyte activity.

Methodology: The ligands studied were designed and synthesized at the Department of Biomedical Chemistry, University of Gdansk. This is the peptide that binds specifically to BTLA, named HVEM(14-39). The study material consisted of peripheral blood samples from 10 patients diagnosed with melanoma from the Department of Oncology and Radiotherapy. The first step was the isolation of PBMC cells, followed by magnetic isolation of CD4+ and CD8+ cells. The isolated T cells were stimulated with the examined compounds for 3 to 5 days. Then, activation markers (CD25 and CD69) apoptosis and proliferation rate were analyzed cytometrically using DCT method.

Results: The addition of HVEM(14-39) reduced the percentage of BTLA+ CD8+ T cells and increased the subpopulation of HVEM+ CD8+ T cells. Additionally, HVEM(14-39) enhanced T cell activation, proliferation, and the shift toward effector memory T cell subpopulations. Finally, it disrupted the inhibitory interactions between the BTLA–HVEM complex and immune and melanoma cells.

Conclusions: Peptide-based immunotherapy targeting the BTLA–HVEM complex offers a promising alternative to monoclonal antibody-based therapies, with the potential for fewer side effects and higher treatment efficacy.

Immunomodulation and Immunotoxicology

Infectious fever as an ally of the immune system

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Fever is the most easily noticeable response to infection. Unfortunately, for quite some time, fever has been perceived solely as an unpleasant symptom, swiftly alleviated with non-steroidal anti-inflammatory drugs.

During this lecture, I will discuss various aspects of fever. I will present scientific evidence suggesting that inhibiting infectious fever may be a mistake. It has been repeatedly demonstrated that fever is an adaptive mechanism and can play a significant role as an ally of the immune system in the fight against pathogens. Its effects can be observed at the level of direct interaction with the pathogen as well as at the level of modulating the immune response itself.

Interestingly, a growing body of evidence suggests that the ability to induce infectious fever can also be considered as a marker of how responsive or sensitive the immune system is. In line with this hypothesis, it has long been shown that the absence of fever during infection may cause patients to be more susceptible to cancer. As fever arises from innate immunity, it implies that the immunosuppression, often seen in cancer patients, extends beyond the acquired immune system. Therefore, it is necessary to re-examine this mechanism in the hope that its appreciation will open up new perspectives in the treatment of both infections and oncological diseases.

Modern diagnostics of immune related diseases

Immune markers of hypertension in pregnancy

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Hypertensive disorders of pregnancy (HDPs) are common complications of pregnancy affecting both mother and fetus, with clinical manifestations such as chronic hypertension, gestational hypertension (GH), and preeclampsia (PE). The disease is genome-wide associated, but the immune system's role is postulated. Pregnancy resembles organ transplantation, where the fetus, despite paternal antigens, persists without immunosuppression. This phenomenon is called "Medawar's Paradox". Despite advances in this field, the mechanism that governs fetomaternal tolerance is still poorly understood but may provide critical insight into achieving immune tolerance in organ transplantation.

We have tested a bunch of immune effectors, to select promising biomarkers of HDPs, that refer to NK cells phenotype, killer immunoglobulin-like receptors (KIRs), their ligands, missing KIR ligands (MSLs) and cytokine milieu. We have noted that MSLs in the healthy group were balanced by various receptors, such as CD94 or inhibitory CD279, expressed on NK cells. Conversely, in HDP patients the number of MSLs was associated with the activation detected as the increased level of CD69+ NK cells. Another was that IL-22, MDC/CCL22 and IL-2/IL-4 ratios have been identified and cut-off values have been proposed to diagnose preeclampsia.

In conclusion, the coexistence of the fetus and mother during pregnancy may be linked to the unresponsiveness of the mother's immune system to the fetal antigens controlled by NK cells with particular suppressive immunophenotype. The match between fetal HLA and the mother's KIR receptors seems to play an important role in the regulation of NK cells. We have also demonstrated that a set of cytokines, IL-22, MDC, and IL-2/IL-4 can be used to diagnose preeclampsia, and what's more to discriminate from gestational hypertension. We believe that our findings will result in better patient management and risk stratification, the aimed characteristics of personalized medicine.

Transfusion immunology

Effect of immunotherapy with monoclonal antibodies on the interpretation of immunohaematologic test results

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Introduction: Monoclonal antibodies are increasingly used in the treatment of haematological malignancies. For example, an anti-CD38 antibody is used in the treatment of multiple myeloma (MM), which unfortunately significantly falsifies the results of laboratory tests, especially immunohaematological ones.

Aim: Analysis of the results of immunohaematological tests in patients diagnosed with MM – before and during treatment with an anti-CD38 antibody.

Methodology: In 2022-2023, at the Regional Centre for Transfusion Medicine in Bialystok, blood samples from 11 patients treated with an anti-CD38 antibody (group 1) and 75 patients who had not yet been treated with this antibody (group 2) were tested. Each patient was tested for ABO and Rh blood type, Duffy phenotype, direct antiglobulin test and presence of immune antibodies (if detected, their specificity was determined), using the BIO-RAD microcolumn method. Additionally, all patients had their Rh, Kell, Kidd and MNS phenotypes determined, using the test tube method.

Results: In patients undergoing the anti-CD38 antibody therapy (group 1), positive reactions between their serum and all panel cells and donor cells were observed. Such reactions were not observed in patients from group 2. Moreover, it was noticed that the anti-CD38 antibody therapy did not affect the determination of ABO, Rh, Kell, Duffy, Kidd and MNS phenotypes.

Conclusions: Taking into account patient's safety due to the significant impact of anti-CD38 therapy on the results of immunohaematologic tests it is highly important to test blood group, phenotype and perform antibody screening before the first application of anti-CD38 to the patient and inform the immunhaematology lab about the planned and ongoing monoclonal antibody therapy.

Immunomodulation and Immunotoxicology

A comprehensive review of current data on the use of therapeutic apheresis in the treatment of complications of monoclonal antibody therapy

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Introduction: The use of monoclonal antibodies (mAbs) has undoubtedly revolutionized therapy in almost every field of medicine, and specific classes of mAbs, such as immune checkpoint inhibitors (ICIs), are currently the subject of intensive research. However, the use of this advanced form of immunotherapy is associated with the development of various adverse immune reactions. The prevention and treatment of these complications is therefore becoming a significant challenge, and therapeutic apheresis (TA) seems to play an important role here.

Aim: Lecture will introduce the classification, pathophysiology and epidemiology of most common side effects/ adverse events of mAbs therapy. The lecture will further present role of TA procedure in management of mAbs therapy complications. The provided examples of TA applications in specific mAb therapies may result in a better understanding of the mechanism of action of TA, and may also contribute to a wider use of mAbs in the future, also in the group of patients at increased risk of developing complications of this form of immunotherapy.

Methodology: A scoping review was conducted to identify and synthesize an existing literature on therapeutic apheresis application in mAbs therapy complications.

Results: The identified mechanisms of action of TA, enabling the use of this therapy in the management of complications of mAbs therapy, are: i) removal of circulating mAbs, ii) removal of mAbs' targets, iii) restoring immune balance and iv) removal of antibodies generated as a result of mAbs therapy.

Conclusions: TA is an important form of therapy in the event of the development of complications of mAbs therapy, but further research is required to develop precise TA treatment regimens.