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RESEARCH REPORT 2021

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DEPARTMENT OF EXPERIMENTAL ONCOLOGY
Head: Professor Leon Strządała, Ph.D.

Laboratory of Experimental Anticancer Therapy
Head: Professor Joanna Wietrzyk, Ph.D.

Determination of the role of the vitamin D receptor in the development of resistance of colorectal cancer cells to 5-fulorouracil

The aim of the research was to determine whether the level of the vitamin D receptor (VDR) influences the effectiveness of the anti-cancer effect of 5-FU on colon cancer cells. It has been shown that VDR silencing in LS180 cells significantly increases their clonogenic abilities, while VDR overexpression gives the opposite effect. LS180/leVDR cells overexpressing the VDR are characterized by a slow migration rate. Both high and low VDR levels do not affect the distribution of LS180 cells in particular phases of the cell cycle. LS180/shVDR cells with silenced expression of the VDR are characterized by a significantly reduced adhesion ability to type IV collagen and fibronectin; on the other hand, overexpression significantly increases the adhesion. Caspase-3/7 activity in LS180/leVDR cells is significantly increased after exposure to 5-FU (8 μM, 72 h). The cells of the LS180 cell line with a silenced VDR are characterized by greater malignancy than cells with its overexpression. The obtained results of this research task constituted the starting material for the SONATA-16 research project, which received funding.

Research on the application of new biologically active compounds in anti-cancer therapy

The implementation of a the new task aimed at assessing the anti-cancer activity of thiosemicarbazones, which are obtained in cooperation with the Wrocław University of Technology, has been started. The mechanism of action of thiosemicarbazones is very complex and not fully understood, but it is assumed to be related to their affinity for Fe (II) and Fe (III) iron ions. This makes it possible to bind the iron that is part of the so-called R2 subunit of ribonucleotide reductase (RNR), the level of which is elevated in neoplastic cells. The aim of the research was to obtain new heterocyclic thiosemicarbazone derivatives and an extensive library of simple, non-heterocyclic thiosemicarbazones, which will allow for systematic research on the structure – activity relationships. So far, about 100 thiosemicarbazones have been obtained. The antiproliferative activity towards 3 human tumor cell lines and one normal line was determined. The most active were derivatives of salicylaldehyde and 2-hydroxyacetophenone, and 2- (diphenylphosphino) benzaldehyde. Moreover, some of the tested compounds showed a significant selectivity of action against cancer cells with selectivity index (SI) values between 10 and 40. For some of the compounds, these values exceeded 100. In the next stage of the research, N-substituted analogs of selected compounds will be synthesized. The obtained results are the basis for the Opus grant project prepared for the next NCN competition.

Determination of the effect of pro-inflammatory cytokines produced by dendritic cell transductants on the inhibition of tumor cell growth in 3D culture

The planned topic is an extension of the issue of the interaction of genetically modified dendritic cells with overproduction of pro-inflammatory cytokines (BM-DCs) with neoplastic cells. In this reporting period, the focus was on optimizing the 3D culture conditions for cancer cells. For this purpose, cells isolated from subcutaneously growing mouse MC38

tumor were grown at different densities in plates coated with commercially available type I collagen, Matrigel or without the coating substance. Additionally, the influence of the presence of serum in the culture medium on the stability of the formed spheroids was investigated. As a result of the conducted *in vitro* experiments, it was observed that the resulting 3D structures were more compact and stable in cultures with coating substances and with serum-free culture medium, however, the spheroids were not stable enough to be used for further experiments. Therefore, further stages of research will focus on creating a procedure that will enable the obtaining of stable neoplastic spheroids.

Laboratory of Tumor Molecular Immunobiology
Head: Professor Wojciech Kałas, Ph.D.

In our current studies we asked about the relation of cellular senescence, cancer therapeutics and immune surveillance, namely natural killer cell activity. There are a few experimental systems suitable for measuring NK cell activity. All experimental set-ups for measuring NK cell activity require vast quantities of NK cells, which is the limiting factor of accuracy and practicality. Thus, we decided to design and work out an improved system for measuring the anticancer activity of NK cells, based on direct interaction of cells. In our preliminary studies, we used NK92 cells with a variety of cancer cells (HTC116, HT29, BxPC3, Caco2, Caki). By using fluorescent labels, we can detect the interaction of cancer cells and NK92 in 5:1, 1:1 and 1:5 target to effector ratios (vs. 1:10 up to 1:50 in standard procedures) during as fast as 15-minute incubation. By extending co-incubation of cancer and NK92 cells up to 2h, we can detect cell death of vulnerable cancer cells. Additionally, we studied the activity of NK62 cells against cancer cells in an overnight protocol, indicating overall activity of NK92 cells against the cancer cells.

To study the activity of NK92 cells against senescent cells, we optimized a procedure for long-term drug-induced senescence of cells. We found that the activity of NK92 cells against senescent cells is highly disturbed. Interestingly, the influence of senescence induction on NK92 activity was different in various effectors: target ratios. Using our newly developed experimental system, we can directly address the question of NK cell activity against senescent cells.

Such a new method will allow us to study NK cells activity against cancer cells in various conditions, especially senescence, without many practical obstacles found in standard methods.

Laboratory of Biomedical Chemistry
Head: Professor Tomasz Goszczyński, Ph.D.

Metallacarborane-based strategies to overcome antibiotic resistance of pathogens

The rapid spread of antibiotic-resistant pathogens is a global problem forcing governments, health organizations, and the scientific community to increase the efforts to develop novel classes of antibiotics. An effective solution to this problem requires the use of all chemical space for a search of novel chemical leads for antimicrobial therapy. Boron clusters are inorganic compounds with versatility and ease of modification similar to organic compounds providing new opportunities to develop unprecedented antimicrobial agents. Boron clusters are abiotic structures, resistant to metabolism; nevertheless, they interact with components of biological systems, such as proteins, lipid membranes, and nucleic acids, but through different mechanisms. Boron cluster-containing compounds showed antimicrobial and anti-biofilm activity against Gram-positive and Gram-negative bacteria, as well as fungi.

Importantly, their activity remains unchanged when used against multi-drug resistant (MDR) pathogens.

In our studies, we synthesized metallacarborane-containing antimicrobial agents active against *Pseudomonas aeruginosa* and *Yersinia spp.* Metallacarborane I-COSAN was O-linked or N-linked to short aliphatic chains (four to five C atoms) or a cyclohexyl ring. For *Yersinia spp.* IC_{50} values were under 10 μM for all tested compounds, whereas the activity against *P. aeruginosa* was approximately an order of magnitude worse. Tested metallacarborane derivatives are the best growth inhibitors of *Yersinia spp.* reported in the literature. Scanning electron microscopy (SEM) revealed no cellular damage or changes in morphology in the drug-resistant *Y. enterocolitica* strain after exposure to the metallacarborane derivatives. This suggests that the mechanism of action of the compounds is related to the blockade of bacterial cell division. The O-linked metallacarboranes showed lower toxicity than the N-linked ones. Additionally, the compounds were safe for zebrafish larvae development, causing no visible developmental deformities, except for unmodified metallacarborane, which was toxic at a concentration of 10 μM. Interestingly, we showed that bacterial resistance can be generated to the unmodified metallacarborane and O-linked derivatives but not to the N-linked derivatives. This finding suggests that N-linked metallacarboranes may be a basis for the development of resistance-proof antibiotics.

DEPARTMENT OF CLINICAL IMMUNOLOGY

Laboratory of Clinical Immunogenetics and Pharmacogenetics
Head: Professor Katarzyna Bogunia-Kubik, Ph.D.

Factors associated with development and progression of multiple myeloma

Multiple myeloma (MM) is a haematologic disorder characterized by the presence of abnormal plasma cells. Our research objective was to analyse single nucleotide polymorphisms (SNPs) in the genes coding for IL-17A and IL-17F, as well as IL-23R receptor, in patients with multiple myeloma. Three SNPs (rs2275913 in IL17A, rs763780 in IL17F, and rs7530511 in IL23R) were investigated in a group of 135 patients and 281 healthy individuals. Genotyping results were analysed in the context of the available clinical data. Allele IL23R rs7530511 C was found to be more common in patients than in healthy individuals ($p<0.001$). Furthermore, a higher percentage of IL17A rs2275913 A carriers among men with multiple myeloma than healthy men was observed ($p=0.076$). Progression-free survival also tended to be more common in patients with rs2275913 A ($p=0.104$), especially in men ($p=0.074$), but not in women ($p=0.545$). Additionally, allele rs2275913 A was more frequently detected among men than women in the group of multiple myeloma patients ($p=0.054$).

Immunomodulatory effect of genetic variation and expression of selected proteins on the predisposition to disease and the effectiveness of biological therapy in patients with rheumatic diseases

The main aim of the study was to assess the potential relationships between allelic variability of the IL13 gene and the development of rheumatoid arthritis (RA), as well as the effectiveness of biological treatment with TNF-α inhibitors. Two SNPs (rs20541 and rs2066960) occurring in the gene encoding IL-13 were analysed. Genotyping was performed using LightSNiP assays and melting curve analysis on DNA samples of 466 patients of Polish origin and 113 patients of Greek origin diagnosed with RA. The control group consisted of

222 Poles and 100 Greeks. For the Polish patients, genotyping results were related with anti-TNF treatment outcome.

There was no difference in the distribution of alleles and genotypes between RA patients and healthy individuals in both the Polish and Greek population. Similarly, no significant correlation with clinical parameters for the rs2066960 polymorphism was found in the Polish and Greek patients. However, in the Polish group of RA patients, the rs20541 GG genotype was found to be associated with a lower disease activity before the biological treatment initiation (p=0.023), while the G allele was associated with better outcome of anti-TNF therapy after 12 and 24 weeks (p=0.033 and p=0.026, respectively). Moreover, in rs20541 AA homozygous patients the ineffectiveness of anti-TNF therapy with TNF inhibitors was observed more frequently than in patients with other genotypes (p=0.019). These results suggest that IL13 rs20541 SNP may affect the response to treatment and be a significant indicator of the effectiveness of the anti-TNF therapy.

Laboratory of Immunogenetics and Tissue Immunology
Head: Professor Izabela Nowak, Ph.D.

ERAP, KIR, and HLA-C profile in recurrent implantation failure

The mother's uterine immune system is dominated by uterine natural killer (NK) cells during the first trimester of pregnancy. These cells express killer cell immunoglobulin-like receptors (KIRs) of inhibitory or activating function. Invading extravillous trophoblast cells express HLA-C molecules, and both maternal and paternal HLA-C allotypes are presented to KIRs. Endoplasmic reticulum aminopeptidase 1 (ERAP1) and 2 (ERAP2) shape the repertoire of HLA class I-presented peptides (immunopeptidome). The ERAPs remove N-terminal residues from antigenic precursor peptides and generate optimal-length peptides to fit into the HLA class I groove. The inability to form the correct HLA class I complexes with the appropriate peptides may result in a lack of immune response by NK cells. The aim of this study was to investigate the role of *ERAP1* and *ERAP2* polymorphisms in the context of *KIR* and *HLA-C* genes in recurrent implantation failure (RIF). In addition, for the first time, we showed the results of ERAP1 and ERAP2 secretion into the peripheral blood of patients and fertile women. We tested a total of 881 women. Four hundred ninety-six female patients, together with their partners, participated in *in vitro* fertilization (IVF). A group of 385 fertile women constituted the control group. Women positive for *KIR* genes in the Tel AA region and *HLA-C2C2* were more prevalent in the RIF group than in fertile women ($p/p_{corr.}$ = 0.004/0.012, OR = 2.321). Of the ERAP polymorphisms studied, two of them (rs26653 and rs26618 in ERAP1) appeared to affect RIF susceptibility in *HLA-C2*-positive patients. Moreover, fertile women who gave birth in the past secreted significantly more ERAP1 than IVF women and control pregnant women ($p < 0.0001$ and $p = 0.0005$, respectively). In the case of ERAP2, the opposite result was observed, i.e., fertile women secreted far less ERAP2 than IVF patients ($p = 0.0098$). Patients who became pregnant after *in vitro* fertilization embryo transfer (IVF-ET) released far less ERAP2 than patients who miscarried ($p = 0.0032$). Receiver operating characteristic (ROC) analyses indicate a value of about 2.9 ng/ml of ERAP2 as a point of differentiation between patients who miscarried and those who gave birth to a healthy child. Our study indicates that both ERAP1 and ERAP2 may be involved in processes related to reproduction and may have a diagnostic value.

Associations of genes for killer cell immunoglobulin-like receptors and their human leukocyte antigen-A/B/C ligands with abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is an immune-mediated disease with a genetic component. The multifactorial pathophysiology is not clear and there is still no pharmacotherapy to slow the growth of aneurysms. The signal integration of cell-surface KIRs (killer cell immunoglobulin-like receptors) with HLA (ligands, human leukocyte class I antigen molecules) modulates the activity of natural killer immune cells. The genetic diversity of the KIR/HLA system is associated with the risk of immune disorders. This study was a multivariate analysis of the associations between genetic variants of KIRs, HLA ligands, clinical data and AAA formation. Genotyping was performed by a single polymerase chain reaction with sequence-specific primers using commercial assays. Patients with *HLA-A-Bw4* have a larger aneurysm by an average of 4 mm ($p = 0.008$). We observed a relationship between the aneurysm diameter and BMI in patients with AAA and co-existing coronary artery disease; its shape was determined by the presence of HLA-A-Bw4. There was also a nearly 10% difference in *KIR3DL1* allele (an inhibitory receptor recognizing Bw4 epitope) frequency between the study and control groups. High expression of the cell surface receptor KIR3DL1 may protect, to some extent, against AAA. The presence of HLA-A-Bw4 may affect the rate of aneurysm growth and represents a potential regional pathogenetic risk of autoimmune injury to the aneurysmal aorta.

Laboratory of Clinical Immunology
Head: Professor Andrzej Lange, M.D., FRCP (London), Dr med Sci

Cytokine storm study in alloHSCT patients throws light on Covid-19 pathomechanism, which becomes critical when adaptive immunity is not effective enough

Covid-19 pandemic surges focused our interest on a study on immune system dysregulation triggering organs damage in infected people. It appeared that the clinical outcome of severe Covid-19 resembles that seen in patients receiving allogeneic hematopoietic stem cell transplantation. In both situations, when the course of the disease is approaching a critical level, thrombocytic microangiopathy and macrophage activation syndrome are seen in both compared clinical entities. The hallmark of severe Covid-19 pathomechanism is the spread of pyroptosis as an effect of hyperactivation of the NFkappaB signaling pathway, which goes effectively downstream when innate immunity receptors recognize pathogen- and damage-associated molecular patterns. Pro-inflammatory cytokines are produced in excess, pyroptosis spreads, and DAMPs are released. To close the vicious circle of self-perpetuation of overwhelming inflammation, adaptive immunity must be dampened. In alloHSCT patients, there are several consecutive events that spark an inflammatory response; it starts from the toxicity of the conditioning regimen, followed by virus reactivation(s) frequent in immune-compromised host, ending with the acute graft vs host disease. In those circumstances, myelopoiesis is stressed, which results in the presence of myeloid-derived monocytic suppressor cells, which characterize the lack of DR membrane receptors. They are highly immunosuppressive and constitute the risk of infections. In this way, overwhelming inflammatory processes continuously spread. The immune system is inefficient in fighting infections, homeostasis breaks, and the clinical situation becomes critical. We have provided the clinicians with some tools to see in advance the malfunction of the immune system. Among the proposed diagnostic tools are several numerical abnormalities in blood cells, but more specifically (i) the presence of CD14+DR- MDMSC, (II) the increase in gamma delta frequently V2 negative cells, (iii) domination of some clones inflicted in the previous infectious combats (iv) the pool of exiguous naïve T cells.

In our studies, we investigated the response to the Covid-19 vaccine to assess (i) the potential of vaccination and (ii) to evaluate the effect of vaccination in individuals with

a previous history of coronaviruses infections. The study documented that the response to vaccination wanes with elapsing time, with a 7-month period from the vaccination marking a decrease in antibodies levels. T cell response assessed by using IGRA test showed an appreciable level of response, which is associated with the presence of active immune system function measured by the proportions of T cells harboring DR epitope.

Booster vaccinations proved to be very effective, resulting in the tremendous increase in SARS CoV2 RCB specific antibodies. The study results published and presented during several webinars provided clinicians with a tool to identify those who experienced Covid-19 before the vaccination and with the facts pertaining to the effect and role of the booster vaccination, which actually has a high degree of life protection potential in confronting the consecutive waves of SARS CoV2 variants and strains.

DEPARTMENT OF PHAGE THERAPY
Head: Professor Andrzej Górski, Ph.D.

Bacteriophage Laboratory
Head: Professor Andrzej Górski, M.D.

Searching, isolation and characterization of new therapeutic phages specific for *Acinetobacter baumannii*

The search for new phages specific to *Acinetobacter* and completing the characterization of isolated phages were continued. Low temperatures (4°C and -70°C), magnesium, and calcium ions increased the efficiency of the amplification process, which is important in view of the difficulties with achieving high titer of these phages.

Searching, isolating, and characterizing new therapeutic phages specific for *Enterobacter* spp.

Three new phages active against *Enterobacter* spp only with lytic spectrum ranging from 40% to 60% were obtained and characterized. The phage lysates retained their titers during six month storage both at -70°C and at 4°C, while storage at 37°C caused a loss of their activity. The standard parameters of the life cycle of the studied phages, such as the adsorption time, the latency period, as well as burst size, were also determined.

Stability studies of Escherichia, Klebsiella and Enterococcus phages isolated from patients with urinary tract infection (UTI)

The stability was tested in 4°C during a 3-month time period. Fifty bacterial host strains isolated from patients with UTI were collected in order to characterize phage host range and lytic activity. Among 16 examined phages, most demonstrated a high level of stability with 8 phages maintaining baseline lytic activity. Only one phage showed a severe decrease in its titer to the point where it was not active. The results should allow us to develop optimal microbiological media for the storage of phage preparations for treating UTI.

Phage inactivation by the sera of patients with bacterial infections subjected to phage therapy (PT)

Analysis was performed of serum antibody responses against phages administered intravesically or intravesically and intravaginally in 22 patients with chronic urinary and

urogenital multidrug resistant bacterial infections. A single course of 3 days of PT did not induce significant serum antibody responses against administered phages. In some patients PT was continued orally and intravaginally up to 30 days and still low level of antibody responses was detected. These data combined with good therapy results achieved in some patients suggest that this mode of PT may be an efficient means of therapy for urogenital infections and a reliable model for a clinical trial of PT.

PT of UTI

The results of the intravesical application of bacteriophage preparations in 15 adult patients with urinary tract infections were retrospectively analysed. Eleven women and 4 men were treated twice daily for 3 days with targeted phages against *E. coli*, *K. pneumoniae*, *E. fecalis* and/or *P. aeruginosa* (4 patients had mixed infections). In the cases of vaginal infection, phage preparations were applied also intravaginally. Good response to treatment (as defined earlier: Międzybrodzki, Ryszard et al. "Clinical aspects of phage therapy." *Adv Virus Res*. 2012;83:73-121. doi:10.1016/B978-0-12-394438-2.00003-7) was observed in 6 patients (40%), whereas pathogen eradication was obtained in two of them (13.3%). These results suggest that this mode of phage application is not superior to our previous results observed in a parallel group of patients in which phages were applied orally and/or intravaginally.

Laboratory of Phage Molecular Biology
Head: Professor Krystyna Dąbrowska, Ph.D.

Immune response to staphylococcal bacteriophages in mammals: kinetics of induction, immunogenic structural proteins, natural and induced antibodies

Bacteriophages are able to affect the human immune system. Phage-specific antibodies are considered as major factors shaping phage pharmacokinetics and bioavailability. So far, general knowledge of phage antigenicity nevertheless remains extremely limited.

A3R and 676Z phage-derived proteins were produced in E. coli expression system and purified. They were used to induce antibodies in the murine model. Next, we applied the immunogold Electron Microscopy technique to locate the studied proteins. We further evaluated the immune response to these proteins and whole A3R and 676Z phages in the murine model and in the human sera in the ELISA test. We assessed the potential of phage-specific and phage protein-specific antibodies to neutralize phages in routine test dilution.

We identified the location and confirmed the structural role of four phage proteins in the virions, with the focus on external capsid head (Mcp, ORF059), tail sheath (TmpH) or baseplate (ORF096). The immune response elicited by these proteins in mice revealed that major capsid protein Mcp was the major inducer of specific antibodies. Antibodies specific to ORF096 were able to neutralize antibacterial activity of the phages. In a part of healthy population (N=55), we observed pre-existing antibodies able to neutralize studied phages: A3R (30%) and 676Z (35%). Importantly, inhibition of phage activity was not markedly pronounced. Antibodies present in patients sera (N=13) did not neutralize phages used in therapy. None of the studied proteins played a particular role as antibody inducer.

Phages can induce phage-specific antibodies, including those neutralizing ones. However, in our experience their impact on therapeutic efficacy appeared negligible, and did not preclude a favorable outcome of phage therapy. The difference between specific immune response that can be elicited by high doses of parenterally applied phages (as in an animal model) and the response observed in patients treated with bacteriophages allow for a 'therapeutic window'.

LABORATORY OF BIOLOGY OF STEM AND NEOPLASTIC CELLS
Head: Professor Aleksandra Klimczak, Ph.D., D.Sc.

Isolation of mesenchymal stem cells from peripheral nerves and analysis of their biological properties

In search of new strategies for the application of peripheral nerve regeneration, we developed a protocol for isolating mesenchymal stem/progenitor cells (MSCs) from human peripheral nerves. Stem/progenitor cells were isolated from fragments of peripheral nerve collected from organ donors (approval of the Bioethics Committee KB-792/2019) using enzymatic digestion. The phenotype of adherent cells, carried out with the use of immunofluorescence staining and flow cytometry techniques, showed the expression of markers characteristic for MSCs, i.e. CD73, CD90, CD105, HLA-ABC antigens. Moreover, peripheral nerve stem/progenitor cells expressed the marker PW1/Peg3 (regulator of stem/progenitor cell differentiation) in co-expression with CD105. Peripheral nerve stem cells expressed PDGFRa (platelet growth factor alpha receptor), Tuj1 (beta III tubulin) characteristic of neuronal cells, and lack of expression of the CD56 marker (NCAM – neuronal cell adhesion molecule). Analysed cells were negative for antigens characteristic for hematopoietic stem cells CD34, CD45, HLA-DR and for the markers SSEA4 and CD146. Stem/progenitor cells isolated from peripheral nerves, cultured in dedicated differentiation media, were differentiated into adipocytes, chondrocytes and osteocytes.

In this study we documented the possibility of isolating adherent cells from the peripheral nerves with the phenotype of tissue-specific stem/progenitor cells (CD73+, CD90+, CD105+, Tuj1+) capable of multilineage differentiation. This new tissue-specific source of stem/progenitor cells can be applied for research in peripheral nerve regeneration.

Characteristics of primary cancer cells and cancer stem cells from ovarian tumor

The populations of neoplastic cells from human postoperative ovarian cancer tissues and from the ascitic fluid from the peritoneal cavity were isolated using CD133 MicroBead Kit Tumor Tissue (Miltenyi Biotec). Isolated cells were characterized with flow cytometry and immunofluorescence staining. Cells isolated from postoperative ovarian cancer tissues expressed the markers CD44, CD73, CD90 and CD105, but were negative for CD133, the marker characteristic of cancer stem cells. Immunofluorescence staining revealed the expression of the CD44 adhesion molecule and the expression of markers characteristic of tumor-associated fibroblasts FAP (fibroblast activation protein), PDGFRα (platelet derived growth factor receptor alpha), and the Snail (epithelial-mesenchymal transition marker) in tumor cells isolated from postoperative tissues. Cells isolated from the ascitic fluid were positive for CD73, CD90, CD105, CD44 and negative for the CD34 antigen. Cells with the phenotype of CD133 (4%–20%) and CD24 (2%–12%) were present in the ascitic fluid. Moreover, tumor cells from the ascitic fluid expressed FAP, PDGFRα and Snail as documented by immunofluorescence staining. This method allows for the acquisition of primary cancer cells from tumors and peritoneal fluid from patients with ovarian cancer. An important achievement was the identification of cells with the CD133+ and CD24+ phenotype characteristic for ovarian cancer stem cells. Isolation of a specific population of ovarian cancer cells will allow us to test the effectiveness of anti-cancer therapies for these cells.

DEPARTMENT OF ANTHROPOLOGY
Head: Professor Sławomir Kozieł, Ph.D.

Maternal genetic origin of the late and final Neolithic human populations from present-day Poland

The aim of the study was to identify maternal genetic affinities between the Middle to Final Neolithic (3850–2300 BC) populations from present-day Poland and possible genetic influences from the Pontic steppe. We conducted studies of ancient DNA from populations associated with Złota, Globular Amphora, Funnel Beaker, and Corded Ware cultures (CWC). Sequenced genomic libraries on Illumina were platformed to generate 86 complete ancient mitochondrial genomes. Some of the samples were enriched for mitochondrial DNA using hybridization capture. The maternal genetic composition found in Złota-associated individuals resembled that found in people associated with the Globular Amphora culture, which indicates that both groups likely originated from the same maternal genetic background. Further, these two groups were closely related to the Funnel Beaker culture-associated population. None of these groups shared a close affinity to CWC-associated people. Haplogroup U4 was present only in the CWC group and absent in the Złota group, Globular Amphora, and Funnel Beaker cultures. The maternal genetic composition found in Złota-associated individuals resembled that found in people associated with the Globular Amphora culture, which indicates that both groups likely originated from the same maternal genetic background. Further, these two groups were closely related to the Funnel Beaker culture-associated population. None of these groups shared a close affinity to CWC-associated people. Haplogroup U4 was present only in the CWC group and absent in Złota group, Globular Amphora, and Funnel Beaker cultures.

Maternal distress and social support are linked to human milk immune properties

Possible alterations of maternal immune function due to psychological stress may reflect immunoactive factor levels in breast milk. This study aimed to assess the association between maternal distress and breast milk levels of secretory IgA (SIgA), IgM, IgG, and lactoferrin (LF). We hypothesized that this association is moderated by maternal social support achieved from others during lactation. The study group included 103 lactating mothers and their healthy five-month-old infants. Maternal distress was determined based on the State Anxiety Inventory and the level of salivary cortisol. Social support was assessed using the Berlin Social Support Scales. Breast milk samples were collected to test for SIgA, IgM, IgG, and LF using the ELISA method. Milk immunoactive factors were regressed against maternal anxiety, social support, salivary cortisol, and infant gestational age using the general regression model. Maternal anxiety was negatively associated with milk levels of LF ($\beta = -0.23$, $p = 0.028$) and SIgA ($\beta = -0.30$, $p = 0.004$), while social support was positively associated with milk IgG ($\beta = 0.25$, $p = 0.017$). Neither anxiety nor social support were related to milk IgM. No association was found between the level of maternal salivary cortisol and immunoactive factors in milk. Our results suggest that maternal psychological wellbeing and social support may affect milk immune properties.

DEPARTMENT OF MICROBIOLOGY

Laboratory of Molecular Biology of Microorganism (LMBM)
Head: Professor Anna Pawlik, Ph.D.

Replication of bacterial chromosomes

We are interested in mechanisms of the initiation of bacterial chromosome replication in Campylobacterota, many of which are human or animal pathogens (e.g. *Helicobacter pylori*, *Campylobacter jejuni*). Chromosome replication is initiated by the initiator protein DnaA that binds a unique chromosomal region called the origin of chromosome replication (*oriC*), unwinds DNA, and helps load proteins that form a replisome. The general architecture of *oriC*s is universal; however, the structure of *oriC* and the mode of orisome assembly differ in distantly related bacteria. *H. pylori oriC* consists of two DnaA box clusters and a DNA unwinding element (DUE); the latter can be subdivided into a GC-rich region, a DnaA-trio and an AT-rich region. We showed that the DnaA-trio submodule is crucial for DNA unwinding, possibly because it enables proper DnaA oligomerization on ssDNA soon after initial DNA unwinding. However, we also observed the reverse effect: DNA unwinding, enabling DnaA-ssDNA oligomer formation – stabilized DnaA binding to dsDnaA boxes. This suggests the interplay between DnaA binding to ssDNA and dsDNA upon DNA unwinding. We also identified that *H. pylori oriC* contains two ATP-DnaA boxes (i.e. boxes which bind only DnaA-ATP but not DnaA-ADP). Thus, our results expand the understanding of *H. pylori* orisome formation, indicating another regulatory pathway of *H. pylori* orisome assembly.

Bacterial response to stress

We are interested in bacterial factors regulating stress response in bacteria belonging to selected species of Campylobacterota. We have recently focused on HP1021 – an atypical response regulator that controls genes' transcription and chromosome replication initiation. We discovered that HP1021 is a sensor of oxidative stress. We focused on the molecular mechanism of HP1021 regulation, and we found that the binding of zinc and the redox state of cysteine residues are crucial for regulating HP1021 DNA binding activity.

Secondary metabolism in *Streptomyces*

Bacteria from the genus *Streptomyces* are potent producers of bioactive compounds with highly diverse structures and functions. Our laboratory focuses on coelimycin synthesis regulation – the earliest coloured specialized metabolite synthesized in the life cycle of *Streptomyces coelicolor* A3. *cpkO* and *cpkN*, which are two SARP activators of the coelimycin synthesis, raised our particular interest. We have recently characterized how CpkO and CpkN affected coelimycin synthesis and stationary-phase antibiotics production. Detailed profiling of promoter activities demonstrated that CpkO is the upper-level cluster activator that induces CpkN, while CpkN activates type II thioesterase ScoT, necessary for coelimycin synthesis. What is more, we show that *cpkN* is regulated by quorum sensing gamma-butyrolactone receptor ScbR. We also characterized a GntR-like DNA binding transcription factor SCO3932, encoded within an actinomycete integrative and conjugative element, which is involved in the secondary metabolite biosynthesis regulation. SCO3932 binds promoters of polyketide metabolite genes, such as *cpkD*, a coelimycin biosynthetic gene, and *actII-orf4*-an activator of actinorhodin biosynthesis. Increased activity of SCO3932 target promoters, resulting from SCO3932 overproduction, indicates an activatory role of this protein in *Streptomyces coelicolor* A3(2) metabolite synthesis pathways.

Antimicrobial therapy

Bacterial resistance to antibiotics is becoming more and more problematic. Thus new antimicrobial compounds and therapies are required to eradicate pathogenic bacteria efficiently. We are primarily interested in the photochemical eradication of bacteria in skin infections using the flexible organic light-emitting diode (OLED) and new photosensitizing chemicals with potential bactericidal activity. New photosensitizing compounds are investigated using *Staphylococcus aureus* as a model species and clinical strains - bacteria isolated from patients' diabetic foot.

Laboratory of Microbiome Immunobiology
Head: Professor Sabina Górska, Ph.D

The ability of selected bacteria of the genus *Bifidobacterium* to regulate the mechanisms of innate immunity

The aim of our research was to investigate the effect of selected *Bifidobacterium* species on the activation of the STAT3 transcription factor and the ERK1/2/NF-κB/iNOS signaling pathway responsible for the regulation of nitric oxide production.

Inductive nitric oxide synthase (iNOS) is a characteristic enzyme of M1 macrophages. The nitric oxide (NO) produced by it is a local mediator that regulates a number of physiological and pathophysiological processes in the human body. It acts as an antimicrobial effector molecule and regulates a number of cellular processes: immune cell differentiation and function by nitration of key molecules involved in transcriptional or signaling pathways. Moreover, the low concentration of NO protects the intestinal mucosa and also influences the regulation of mucus secretion. However, it was observed that inhibition of iNOS activity or deletion of the iNOS gene, resulting in a significant decrease in nitric oxide levels, increased intestinal inflammation. In turn, the administration of exogenous NO precursors produced a therapeutic effect. Activation of the transcription factor NF-κB leads to the expression of iNOS in macrophages, while activation of NF-κB is under the control of the p38 and ERK 1/2 signaling pathways. STAT3, through direct interaction with NF-κB p65, acts as a dominant negative inhibitor of NF-κB activation, inhibiting iNOS expression and NO production.

In our research we worked on mouse myeloid macrophages from the BMDM lineage. Macrophages were incubated with selected strains of *Bifidobacterium* for 24 h at 37°C in the presence or absence of the kinase inhibitor ERK 1/2-U0126. Changes in the level of activation of ERK 1/2 kinases as well as STAT3 and NF-κB p65 transcription factors were determined by Western blotting. The level of released NO was determined in the supernatants by the Griess method. The effect of the strains on the survival/proliferation of BMDM was determined by the MTT test.

The production of NO and expression of iNOS was dependent on the studied *Bifidobacterium* strain. We observed that the increase in iNOS expression in macrophages in response to *Bifidobacterium* is dependent on the activation of MAPK-ERK 1/2 kinases. Pre-incubation of BMDM with a selective MAPK-U0126 inhibitor significantly inhibited NO production by macrophages incubated with *Bifidobacterium*, which confirms that iNOS expression and NO production in macrophages are under the control of ERK 1/2 kinases. The influence of *Bifidobacterium* on the activation of the NF-κB transcription factor, responsible for the control of the expression of the iNOS gene – the enzyme producing NO – has also been demonstrated. We did not observe the activation of the STAT3 transcription factor by studied *Bifidobacterium* strains. We noticed a significant increase in macrophage proliferation (on average by about 50%) in response to selected *Bifidobacterium*: strain, e.g., 218, 219, 366, 368, 369 and 371.

DEPARTMENT OF TUMOR IMMUNOLOGY

Laboratory of Molecular and Cellular Immunology
Head: Professor Małgorzata Cebrat, Ph.D.

Danio rerio model for identifying Nwc protein function

Despite the data collected on the gene expression pattern, protein structure and its potential interaction partners of a mouse knockout, the function of the NWC protein has not yet been precisely determined. The NWC knockout mouse has a phenotype which manifests itself by weak functional impairment of the sperm. At the same time, the protein partners of NWC protein selected in several independent tests suggest that NWC plays a role in transport to or from the cell flagellum/cillia. The NWC protein contains three highly conserved domains. The research task was aimed at extending the existing research by analyzing the function of the Nwc protein in zebrafish, which is frequently used as a research model in the analysis of flagella development and structure, because ciliated hair cells are already present in the lateral line at an early stage of development. It is also important that the zebrafish embryos are transparent, which makes it possible to observe the hair cells in the inner ear. Additionally, a zebrafish mutant (pou4f3: GAP-GFP) with labelled hair cell membranes is available.

The plan was to analyze the expression of *NWC* at different stages of the development of zebrafish larvae. For this purpose, the larvae were collected in several development stages (from the first to the 5th day after fertilization) and were used for Real-Time RT-PCR and confocal microscopy analysis. The choice of the age of the analyzed larvae was dictated by the moment of the appearance of the hair cells and the stages of the formation of the inner ear during the development of zebrafish. It has been shown that on the mRNA level, the *NWC* gene is already expressed on the first day after fertilization. The next step in the attempt to identify the function of the Nwc protein was the transient silencing of the *NWC* gene in zebrafish larvae using morpholino oligonucleotides.

Danio rerio [Tg (pou4f3: GAP-GFP)] embryos with labelled ciliated cells in the ears and side line were microinjected with morpholino oligonucleotides specific for two different UTR fragments of the *NWC* transcript, blocking the translation of the gene. According to standard methodology, tests using different concentrations were performed for both types of morpholino oligonucleotides in order to select those for which no effect on fish survival was observed. The morpholino oligonucleotides used in the project had a fluorescent marker attached to the 3'-end, which allowed for verification whether the particles injected into the yolk are transported to the cells of the developing larvae. *In vivo* observations in fluorescence microscopy of embryos 24 hours after fertilization confirmed the spread of morpholino in all tissues. Until the antibody is obtained, the reduction of the expression level of the Nwc protein in the fish treated with morpholino cannot be confirmed. Nevertheless, a preliminary analysis of the morphant phenotype in light and confocal microscopy was performed. There were no abnormalities in the shape and number of otoliths in the inner ear, which would indicate damage to the sensory epithelium. However, analysis of confocal microscopy images of the sensory epithelium of morpholino oligonucleotide-treated embryos at stages 3, 4, and 5 days post-fertilization showed a reduction of length in inner ear hair cell kinetocilia in morpholino-treated embryos. The strength of the effect was different for the two different morpholino oligonucleotides and was dose dependent. The similar phenotype obtained with the use of morpholino oligonucleotides targeting two different sites in the *NWC* UTR proves the specificity of the observed effect.

Laboratory of Tumor Immunology
Head: Professor Arkadiusz Miążek, Ph.D.

Assessing alpha-enolase 1 as a target for immunotherapy of melanoma

Alpha-enolase (ENO1, EC 4.2.1.11) is an evolutionary conserved, glycolytic metalloenzyme responsible for the reversible dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate. It functions as a homodimer but may also assemble in supramolecular complexes with cytoskeletal, mitochondrial or cell surface proteins displaying catalytic and "moonlighting" activities. Despite being ubiquitous, ENO1 overexpression often reflects pathophysiological and metabolic status of the cell. An increase in ENO1 expression accompanies numerous human diseases (e.g. rheumatoid arthritis, systemic sclerosis, lupus erythematosus, Alzheimer's) including over 18 classes of solid and hematological cancers. Accumulated evidence demonstrates that in various cancers ENO1 overexpression contributes to cancerous cell survival, proliferation and the maintenance of the Warburg effect. Mechanistically, both epigenetic regulators (e.g. DHX33-containing protein complex) and transcription factors (e.g. HIF-1a) operate on ENO1 promoter to increase ENO1 transcription during hypoxia-a predominant growth milieu of many cancers.

The aim of this study was to investigate the prognostic value of ENO1 in surgical resections from 112 melanoma patients and to assess its expression and enzymatic activity in several human melanoma cell lines cultured in normoxia and hypoxia.

We found that overexpression of ENO1 in tumor cells from patients correlated with unfavorable prognosticators such as mitotic activity, Clark level, Breslow thickness, and the presence of ulceration. We also found a positive correlation of elevated ENO1 expression with a greater thickness of the neoplastic infiltrate and a worse long-term prognosis for patients with cutaneous melanoma. Melanoma cell lines derived from lymph node metastases were found to significantly upregulate ENO1 activity during hypoxia. The latter finding suggests that ENO1 may contribute to survival in the hypoxic milieu of lymph nodes. Therefore, overexpression of ENO1 promotes the invasiveness of melanoma cells and positively correlates with aggressive clinical behavior. Our observations point to a potential prognostic and therapeutic value of ENO1 in melanoma.

DEPARTMENT OF EXPERIMENTAL THERAPY
Head: Professor Michał Zimecki, Ph.D.

Laboratory of Immunobiology
Head: Professor Michał Zimecki, Ph.D.

Generation of cytotoxic activity by LF-Fc chimera

Previous studies demonstrated an ability of a chimera composed of lactoferrin (LF) and Fc fragment of IgG1 (LF-Fc) to generate TNF α in human peripheral blood monocytes and K562, natural killer cell line. In addition, LF-Fc, but not LF alone, induced the production of granzyme B in monocyte cultures. Present studies revealed that this process was accompanied by an increased expression of PI3K component upon LF-Fc treatment, suggesting the involvement of PI3K/Akt/mTOR signaling pathway in activated monocytes. LF alone did not affect expression of this molecule. The chimera, in a prolonged (72h) culture, caused a change in the appearance of THP-1 monocytic leukemia cells from suspended to adherent cells, accompanied by increased cell viability. An aggregation of HL-60 cells was also registered

upon contact with LF-Fc. No effect on pinocytosis of HRP in J774E mouse macrophages was registered. Also, no antibacterial and antifungal actions of LF-Fc were found. Both LF and LF-Fc demonstrated identical antiviral actions towards HSV-1 virus.

Implementation of three-dimensional culture for cellular studies

The studies on four types of polymeric carriers were carried out. They included fibrinogen, agarose, chitosan containing tripeptidic RGD sequences, and gel with polypeptidic, synthetic fragments of bone morphogenetic proteins (BMP). For the colonization of the carriers C2C12 myoblasts, HeLa cells, mouse splenocytes and human PBMC were used. The cell condition was evaluated by MTT colorimetric method and ALP test and gene expression analysis. The biocompatibility studies of the polymeric carriers showed that the cells adhered to the fibers already after 24 h, and after 72 h developed a multilayer spatial structure. The microscopic evaluation conducted for 7 days revealed a constant and dynamic growth of the cells. C2C12 and HeLa cells acquired after 72 h a spindle-shaped form and formed a cellular mass closely adhering to the polymer fibers, which in time constituted a compact structure, completely covering fibers of the culture framework. MTT and ALP tests also confirmed the good condition of cells with the exception of PBMC, which survived only for 72 h. The experiments with the gel framework, containing immobilized polypeptidic fragments of BMP, showed that they stimulated the cell growth and activity. Thus, these polypeptides may replace culture media in cases when a flow of cell culture medium finds obstacles within a multi cellular spatial structure. Such an effect was, in fact, demonstrated in the case of C2C12 in ALP test. Initial, dynamic cell growth was also reflected by an increase in expression of MAP kinase pathway and cell marker genes, particularly in the case of splenocytes. A decline in cell cultures, also confirmed in microscopic examinations, was associated with a decrease of cell marker expression and an increase of caspase expression, indicating cell apoptosis.

Laboratory of Immunopathology
Head: Professor Edyta Pawlak, M.D., Ph.D.

Analysis of mutation profile using NGS technology in chronic lymphocytic leukaemia cells from PBL patients in relation to p27KIP1 protein expression levels

Targeted sequencing of exon sequences of 198 genes was performed using a custom-designed unique SeqCap EZ Choice probe set on the Illumina MiSeq FGx platform in PBL patients showing low (n=12, MFI: 1806.58±274.94 [1371.00-2278.00]) and high (n=12, MFI: 5844.42±1614.16 [4225.00-10144.00]) intracellular expression of p27Kip1 (determined by flow cytometry) with an established IgVH hypermutation profile. Genetic variants were identified according to the recommendations of the GATK package. Variants were classified using the following databases: ClinVar, Varsome, COSMIC and IARC TP53 and manually (IGV program) to filter for typical genetic differences, and to avoid sequencing artefacts and presumed germline variation. Gene copy number identification was performed using CNVkit v0.9.5 analysis. Statistical analysis was performed using Statistica 13.1 software.

The genetic mutation profile differed between the studied patient groups: mutations in *TP53*, *NOTCH1*, *RPS15* genes were observed in the group with high p27Kip1 expression, while mutations in *ATM*, *SF3B1*, *MYD88* and *CHD2* were observed in the group of patients with low p27Kip1 expression. An interesting finding is that mutations identified in the group of patients with high p27Kip1 protein expression were only observed in the group of patients with non-mutated IgVH. In the group of patients with low p27Kip1 expression, a higher

number of pathogenic mutations were observed in the group of patients with mutated IgVH, which is consistent with studies on large cohorts of PBL patients.

The impact of the FKBP5 gene polymorphisms on the relationship between traumatic life events and psychotic-like experiences in non-clinical adults

Common variations of the *FKBP5* gene are implicated in psychotic disorders by modulating the hypothalamic–pituitary–adrenal axis reactivity to stress. It has been demonstrated that some of them might moderate the effects of childhood trauma on psychosis proneness. However, these associations have not been investigated with respect to traumatic life events (TLEs). The aim was to explore whether the *FKBP5* polymorphisms moderate the effects of TLEs on the level of psychotic-like experiences (PLEs).

A total of 535 non-clinical adults were included in the study, and genotyping of six *FKBP5* polymorphisms (rs3800373, rs9470080, rs4713902, rs737054, rs1360780 and rs9296158) was performed. The Prodromal Questionnaire-16 (PQ-16) and the Traumatic Events Checklist (TEC) were administered to assess PLEs and TLEs, respectively. Among the rs1360780 CC homozygotes, a history of physical abuse was associated with significantly higher PQ-16 scores. This difference was not significant in the rs1360780 T allele carriers. Similarly, a history of physical abuse was associated with significantly higher PQ-16 scores in the rs9296158 GG homozygotes but not in the rs9296158 A allele carriers. Finally, emotional neglect was related to significantly higher PQ-16 scores in the rs737054 T allele carriers but not in the rs737054 CC homozygotes.

This indicates that variation in the *FKBP5* gene might moderate the effects of lifetime traumatic events on psychosis proneness.

Deregulated expression of immune checkpoints on circulating CD4 T cells may complicate clinical outcome and response to treatment with checkpoint inhibitors in multiple myeloma patients

Unlike solid-tumor patients, a disappointingly small subset of multiple myeloma (MM) patients treated with checkpoint inhibitors derive clinical benefits, suggesting differential participation of inhibitory receptors involved in the development of T-cell-mediated immunosuppression. In fact, T cells in MM patients have recently been shown to display features of immunosenescence and exhaustion involved in immune response inhibition. Therefore, the aim of our research was to identify the dominant inhibitory pathway in MM patients to achieve its effective control by therapeutic interventions.

Flow cytometry was used to examine the peripheral blood (PB) CD4 T cell characteristics assigned to senescence or exhaustion, considering PD-1, CTLA-4, and BTLA checkpoint expression, as well as secretory effector function, i.e., the capacity for IFN-γ and IL-17 secretion. Analyses were performed in a total of 40 active myeloma patients (newly diagnosed and treated) and 20 healthy controls. At the single-cell level, we found a loss of studied checkpoints' expression on MM CD4 T cells (both effector (Teff) and regulatory (Treg) cells) primarily at diagnosis; the checkpoint deficit in MM relapse was not significant. Nonetheless, PD-1 was the only checkpoint distributed on an increased proportion of T cells in all MM patients irrespective of disease phase, and its expression on CD4 Teff cells correlated with adverse clinical courses. Among patients, the relative defect in secretory effector function of CD4 T cells was more pronounced at myeloma relapse (as seen in declined Th1/Treg and Th17/Treg cell rates).

Although the contribution of PD-1 to MM clinical outcomes is suggestive, our study clearly indicated that the inappropriate expression of immune checkpoints (associated with

dysfunctionality of CD4 T cells and disease clinical phase) might be responsible for the sub-optimal clinical response to therapeutic checkpoint inhibitors in MM.

Laboratory of Reproductive Immunology
Head: Professor Anna Chełmońska-Soyta, Ph.D, V.D.

Immunological mechanisms associated with reproductive processes in health and disease

Title: IL-24 expression in women with endometriosis

Endometriosis is a gynecological disease characterized by the presence of endometrial cells outside its natural location. It is accompanied mainly by chronic inflammation, intermenstrual bleeding, and severe pain, with one of its consequences being infertility. In previous years, we demonstrated the presence of IL-24 in a population of human Treg cells. The study was continued in 2021. IL-24 is commonly found in Treg lymphocytes. Its role for the function of these cells is unknown. However, taking into account the dysregulation of immune response in the course of endometriosis consisting in increased activity of tolerogenic cells, the aim of this study was to determine the expression of IL-24 in regulatory B and T cells and to examine the concentration of this cytokine in the blood serum of patients with different stages of endometriosis.

The serum IL-24 levels did not show any differences between the serum levels of IL-24 in patients suffering from endometriosis compared to the control group. However, the expression of IL-24 in lymphocytes was higher in CD4+CD127-CD25hiFOXP3IL24+ Treg lymphocytes in the general population of patients with endometriosis, while no differences were found between the groups of patients with stage III and IV of the disease. On the other hand, the analysis of IL-24 expression in the general population of T helper lymphocytes revealed significant differences in IL-24 expression between patients with different stages of the disease.

The results indicate that helper T cells are characterized by a higher expression of IL-24 in women with endometriosis, indicating the involvement of this cytokine in the regulation of the immune response in this disease.

Laboratory of Genetics and Epigenetics of Human Diseases
Head: Professor Lidia Karabon, Ph.D

Genetic and epigenetic control of immune response regulating molecules in disease development, progression and treatment

Variation in gene encoding the co-inhibitory molecule BTLA is associated with the risk of disease and survival in clear cell renal carcinoma patients.

The successful introduction of immune checkpoint blockade approaches to renal-cell carcinoma (RCC) treatment indicates the importance of receptors regulating the T cell response for RCC risk and progression. B and T lymphocyte attenuator (BTLA) is one of these receptors which negatively regulate T cell activation.

The aim of our study was to investigate the association between BTLA gene polymorphisms with susceptibility to RCC as well as with overall survival (OS) of RCC patients and specifically clear cell RCC (ccRCC) patients. Altogether, 310 RCC patients (232 ccRCC) and 480 healthy subjects were genotyped for the following polymorphisms: rs2705511, rs1982809, rs9288952, rs16859633, rs9288953, rs2705535, rs1844089. Here we found that presence of rs1982809 G allele (genotype GG+GA) is associated with increased

risk of RCC (OR 1.39, 95% CI 1.04-1.85, p=0.03). This association was also observed in the patients with malignant tumors (OR 1.37, 95%CI 1.02-1.86, p=0.04). In the ccRCC patients with high grade tumors the frequency of rs1982809 [GG] genotype was significantly higher as compared to those with low grade or to the controls (0.14 vs. 0.06, p=0.05 and 0.14 vs. 0.06, p=0.03, respectively). Moreover, we noticed the trend for overrepresentation of carriers of rs2705511 C allele in RCC patients, especially in the RCC patients with malignant tumors as compared to the controls. On the other hand, during long-term observation (6.5 years), we discovered that possessing of A allele at BTLA rs1844089 SNP, together with advanced disease (stage >3, tumor grade >3, tumor diameter ≥70mm), is an independent risk factor of death, which increases the HR (hazard ratio) of death by more than twofold (HR=2.21, 95%CI 1.28-3.83). Furthermore, the OS of patients bearing this allele is 6 months shorter than for homozygous [GG] patients (42.5 vs. 48.2 months).

Our results indicate that genetic variation within the gene encoding the BTLA is significantly associated with clear cell renal cell carcinoma risk and overall survival.

MiR155-5p regulation of BTLA expression in chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in western countries and accounts for 25% of all leukemia, with approximately 70% of lymphoid leukemias. CLL is characterized by the gradual accumulation of mature B cells expressing B-lineage-specific markers such as: CD19, CD20, CD23, and additionally CD5 antigen, in lymphoid tissues, bone marrow, and peripheral blood (PB).

CLL is a clinically and molecularly heterogeneous disease, however dysfunction of the immune system both innate and adaptive leading to increases the incidence of secondary malignancies and infections is observed in many patients.

The results of our and others studies indicated aberrant expression of the immune check-points receptors in the T and B cell compartment in CLL patients. BTLA is a member of the immunoglobulin superfamily providing inhibitory signaling via the T cell receptor (TCR) or the B cell receptor (BCR). In our previous study we showed lower protein expression of BTLA despite significantly higher expression of BTLA mRNA in CLL patients as compared to controls. This observation points towards altered post-transcriptional regulation of BTLA in CLL cells. Therefore, we postulate that BTLA expression is regulated by microRNAs.

The aim of our recent study was to verify the hypothesis about miR155-5p regulation of BTLA expression. In line with earlier data, we observed that expression of BTLA mRNA and miR-155-5p is elevated in CLL (p=0.034 and p=0.0006, respectively) as well as in MEC-1 cell line (p=0.009 and 0.016, respectively). We showed that inhibition of miR-155-5p in CLL cells transfected with miR155-5p inhibitor partially restored BTLA protein expression in CLL patients (p=0.01) and in MEC-1 cell line (p=0.058). Our studies suggest that miR-155-5p is involved in BTLA epigenetic regulation.

DEPARTMENT OF IMMUNOLOGY OF INFECTIOUS DISEASES
Head: Professor Andrzej Gamian, Ph.D.

Laboratory of Medical Microbiology
Head: Professor Andrzej Gamian, Ph.D.

Lipidomic analysis of propionic bacteria and preliminary characterization of extracellular vesicles secreted by *Cutibacterium*

The aim of the study was to perform a comparative analysis of lipid profiles of bacteria of the genus *Propionibacterium* and *Cutibacterium*. Propionic acid bacteria include probiotic, commensal, opportunistic, and even human pathogenic strains. One of the stages of the research was the creation of a database of MALDI-TOF mass spectra of lipid extracts for diagnostic purposes. The next stage of the study was the preliminary chemical characterization of extracellular vesicles secreted by *Cutibacterium*. These vesicles secreted into the culture medium, are composed of lipids, proteins, and nucleic acids and can perform various biological functions. As part of the the studies, lipid extracts of seven strains of *Cutibacterium* from PCM collection were obtained and analyzed using 1D and 2D thin-layer chromatography, and a pilot database of MALDI-TOF mass spectra was created. Methods for the isolation of bacterial extracellular vesicles from *Cutibacterium acnes* were optimized. A fraction of extracellular vesicles with dimensions consistent with literature data was obtained. These results will be necessary for the creation of a complete, comparative set of MALDI-TOF lipid profiles of *Cutibacterium*, constituting a specific fingerprint for individual strains of significant diagnostic value. This will allow for the determination of the chemical structures of glycolipids. The final result of the research will be comprehensively characterized various lipid compounds that will be used in diagnostics and for the production of vaccines. Using an optimized vesicle isolation method, it will be possible to obtain these nanostructures from other *Cutibacterium* and *Propionibacterium* taxa. The obtained fractions of extracellular vesicles will be purified by ultracentrifugation in the density gradient and extraction for lipid analysis (TLC and MALDI-TOF MS). Our Laboratory was also involved in the elaboration of vaccines against the SARS-CoV-2 virus responsible for Covid-19 disease and a vaccine against *Clostridioides difficile*, an anaerobic pathogen involved in post-antibiotic acute diarrhea that leads to severe infection. Studies performed in our laboratory have innovative and applied importance because these results aid in the production of antigens for vaccines against SARS-CoV-2 virus and *Clostridioides difficile* bacteria.

Laboratory of Virology
Head: Professor Egbert Piasecki, Ph.D.

The search for new methods of antiviral therapy is primarily focused on the use of substances of natural origin. In this context, a triterpene compound, betulin 1, proved to be a good starting point for derivatization. Thirty-eight betulin acid ester derivatives were synthetized, characterized, and tested against DNA and RNA viruses. Several compounds exhibited 4- to 11-fold better activity against Enterovirus E (compound 5) and 3- to 6-fold better activity against Human alphaherpesvirus 1 (HHV-1; compound 3c). Time-of-addition experiments showed that most of the active compounds acted in the later steps of the virus replication cycle (e.g. nucleic acid/protein synthesis). Further in-silico analysis confirmed *in vitro* data and demonstrated that interactions between HHV-1 DNA polymerase and the most active compound, 3c, were more stable than interactions with the parent non-active betulin 1. The results were published in *Journal of Medicinal Chemistry, 2021; 225: 113738.*

In the ethnomedicine the *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. fruits and roots are used to treat immune-related diseases. Because of the overexploitation of the roots, the species is considered to be endangered and is put on the Red List in some countries (e.g. the Republic of Korea). Therefore, the aerial parts of E. senticosus might be explored as a new sustainable source of compounds with an adaptogenic activity. This study is aimed to evaluate the adaptogenic activity of the *Eleutherococcus senticosus* fruits intractum to support the use of the fruits in folk medicine. The effect on IL-2 and IL-10 release by peripheral blood leukocytes (PBLs) was measured by the ELISA, the CPE on the A549 and PBLs were determined with trypan blue and the MTT. The innate immunity assay was done in the VSV-

PBLs model. Metabolic profiling was done using HPLC-DAD and HPLC-RID. We report for the first time that the intractum (300 μg/mL) and eleutheroside E (100 μg/mL) and B (100 μg/mL) do not act as a virucidal agent (VSV). The intractum and eleutherosides E and B caused the increase of the PBLs proliferation up to 24.61 and 100%, respectively. The decreased viral replication in the VSV-PBLs-Int model might be associated with an increased secretion of IL-10 (328 pg/mL). Eleutheroside E and B did not affect the innate immunity. No eleutherosides were determined in the intractum, the ethyl acetate layer contained caffeic and protocatechuic acids. A large amount of myo-inositol and D-mannitol was found (267.5 and 492.5 mg/g DE). Our observations justify the traditional use of the fruits in Russia in immune-related diseases. The results mean that there are other compounds than eleutherosides responsible for the adaptogenic effect, probably myo-inositol and caffeic acid, for which an immunostimulatory activity has already been confirmed. The results were published in *Journal of Ethnopharmacology, 2021; 268: 113636.*

Transport of proteins, transcription factors, and other signaling molecules between the nucleus and cytoplasm is necessary for signal transduction. The study of these transport phenomena is particularly challenging in neurons because of their highly polarized structure. The bidirectional exchange of molecular cargoes across the nuclear envelope (NE) occurs through nuclear pore complexes (NPCs), which are aqueous channels embedded in the nuclear envelope. The NE and NPCs regulate nuclear transport but are also emerging as relevant regulators of chromatin organization and gene expression. The alterations in nuclear transport are regularly identified in affected neurons associated with human neurodegenerative diseases. This review presents insights into the roles played by nuclear transport defects in neurodegenerative disease, focusing primarily on NE proteins and NPCs. The subcellular mislocalization of proteins might be a very desirable means of therapeutic intervention in neurodegenerative disorders. The review was published in *Molecular Neurobiology, 2021; 58: 983-995.*

Laboratory of Genomics & Bioinformatics
Head: Professor Łukasz Łaczmański, Ph.D.

Molecular characteristics of cancer cells: Analysis of transcriptomes and epigenetic data

In the process of cancer transformation, three groups of genes play a key role: proto-oncogenes, suppressor genes and mutator genes. Identification of genetic changes, modifying individual response to drugs and potential prognostic and predictive molecular markers (response to treatment) is the key to improving the effectiveness of the treatment. This will allow the selection of groups of patients that require the introduction of a different therapeutic standard (individualization of treatment time, doses), thereby improving not only the prognosis, but also the quality of life.

The aim of the project:

1. Finding transcriptome changes that could be markers to predict treatment responses.
2. Finding markers that could be predictors of the cancerogenesis based on small RNA.
3. Finding changes in the methylation profile associated with transcriptome tumor changes.
4. Developing a model by combining transcriptomic and epigenetic data.

An analysis of the transcriptomic profile of 51 samples from patients with breast cancer was performed. The patients were divided into two groups: responding well to treatment (Y) and responding poorly to treatment (Paclitaxel or Doxorubicin), marked with N. First, a classic analysis of DE (differential expression) was performed between patients Y and N. The analysis showed no statistically significant differences. Subsequently, an analysis was

performed on breast cancer cell lines. A group of approximately 220 genes strongly correlated (positively and/or negatively) with the response to Paclitaxel or Doxorubicin was selected. The machine-learning algorithm (sPLS-DA) was then used to select from these 220 genes that best separate responder and non-responder groups. A list of 36 genes was obtained, the expression of which may be correlated with the response to treatment with Paclitaxel or Doxorubicin.

A bioinformatic model of response to treatment with the given preparations will be made, based on the transcription profiles of 51 examined patients, containing the genes selected in the previous stage. Work on this project will be carried out in 2022 and will be completed with publication.

Analysis of the gut microbiome and bacterial transcriptome in relation to the diet and gastrointestinal symptoms in patients with autism

Autism is a neurodevelopmental disorder whose etiology is still unknown, and the pathophysiology of comorbid symptoms is unclear. It is believed that both genetic and environmental factors influence the initiation and development of this disease. Our knowledge indicates major microbial changes among autistic patients and healthy cohorts, but so far no specific changes in the composition and diversity of the gut microbes have been identified. The literature emphasizes the need to standardize such a study in order to obtain high-quality data that will be able to meet the inclusion criteria.

We suppose that there is a correlation between the microbial state of the gut, the bacterial transcriptome in the gut, and the occurrence of gastrointestinal symptoms and the severity of nervous system symptoms in patients with autism. The main goal of the project is to analyze and determine the species and quantitative composition of the intestinal microflora of children with autism and coexisting gastrointestinal symptoms on the example of the Polish population using next-generation sequencing techniques (NGS, RNA-Seq) with a combined analysis of the bacterial transcriptome.

The aim of the project:

1. Determining the composition of the gut microbiota and the differences between the autistic group and the control group.
2. Establishing the transcriptional activity of bacteria colonizing intestines of children with autism.
3. Determining the correlation between gastrointestinal symptoms, diet and intestinal dysbiosis in children with autism.

The preliminary results, based on the analysis of the V3V4 16S rRNA fragment (UniFrac index), show that statistically significant ($p < 0.05$) differences in the bacterial composition of fecal microbiota may be observed between the group of neurotypical siblings and children from the control group (PERMANOVA test, $p = 0.003$) and between neurotypical siblings and children with autism spectrum disorders (PERMANOVA test, $p = 0.001$). There was no statistically significant difference between the groups of children with autism spectrum disorders and the control group (PERMANOVA test, $p = 0.563$).

On the other hand, on the basis of the Bray-Curtis index we show that the diversity of the composition of the microbiota is statistically significant between all groups. Differences are observed between neurotypical siblings and children from the control group (PERMANOVA test, $p = 0.001$) and between neurotypical siblings and children with autism spectrum disorders (PERMANOVA test, $p = 0.001$), as well as between groups of children with autism spectrum disorders and children from the control group (PERMANOVA test, $p = 0.029$).

These results provide an indication that the trait (bacterial sequence) that differentiates ASD children from control children is close to the phylogenetic tree (because the UniFrac weighted index reduces the beta distance for similar traits in the phylogenetic tree).

The next step of the analyses will be to verify the obtained results by analyzing the second fragment (V7V9) and increasing the size of the control group.

DEPARTMENT OF IMMUNOCHEMISTRY
Head: Professor Czesław Ługowski, Ph.D.

Laboratory of Glicobiology
Head: Professor Marcin Czerwiński, Ph.D.

Evaluation of the organization of genes encoding Gb3/CD77 synthase in birds

The aim of the study was to evaluate the number and sequences of genes encoding Gb3/CD77 synthase in birds. While the human Gb3/CD77 synthase (encoded by the *A4GALT* gene) is a glycosyltransferase that synthesizes the Galα1→4Gal moiety mainly on glycosphingolipids, its pigeon homolog prefers glycoproteins as acceptors. Such glycan structures may serve as receptors for Shiga toxins, which are released by Shiga toxin-producing *E. coli* (STEC) and *Shigella dysenteriae* serotype 1. These toxins present a serious threat to the human population because they may cause haemorrhagic colitis and often fatal hemolytic uremic syndrome (HUS). The birds seem to be partially resistant to Stx and could be a spillover host, so we evaluated the genomic organization of gene encoding Gb3/CD77 synthase in the common pigeon (*Columba livia*).

Six of the seven genes encoding Gb3/CD77 synthase in pigeons have been amplified by PCR. The genes are remarkably similar, showing 92%–99% homology of the nucleotide sequence in open reading frames (ORFs), as well in the sequences surrounding ORFs. Bioinformatic analysis of the available genomes of birds (*Streptopelia turtur*, *Patagioenas fasciata monilis*) showed that these species contain 4-5 of the genes found by us in *C. livia*, but the sequences of some of these genes are incomplete. Our data show that the number of the *A4GALT* genes in different individuals ranges from 5 to 7, which suggests that these genes duplicated relatively recently. Thus, it may be argued that pigeons became resistant to Shiga toxins by sharing the ecological niche with humans. This basic research informs us about the evolutionary changes and the possible consequences of intentional or unintentional domestication.

Laboratory of Microbial Immunochemistry and Vaccines
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Biochemical characteristics of macromolecules involved in immunological processes: Immunochemical studies of bacterial endotoxins

The expertise of the Laboratory of Microbial Immunochemistry and Vaccines covers variety of preparative, chromatographic, chemical, and instrumental analytical techniques (mass spectrometry, nuclear magnetic resonance spectroscopy, surface plasmon resonance) to elucidate structures of biologically relevant carbohydrates, especially lipopolysaccharides (LPS, endotoxins) of Gram-negative bacteria. Our research concerns Gram-negative species, such as *Klebsiella pneumoniae, Escherichia coli, Shigella sonnei*, *Bordetella* spp. and *Plesiomonas shigelloides,* represent opportunistic human pathogens associated with extraintestinal and nosocomial infections in humans and animals. Moreover, *K. pneumoniae,*

particularly ESBL- and KPC-strains, has been singled out in 2017 as *"priority 1. critical pathogen"* for health care by the WHO, CDC, and the UK Department of Health. *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. holmesii*, are mammalian respiratory pathogens, having substantial economic impact on human health and agriculture. *B. pertussis* is responsible for whooping cough (pertussis) and *B. holm*esii is the second pertussis etiological factor, but the current anti-pertussis vaccines do not provide cross-protection. Major virulence factors and surface antigens of these species are: lipopolysaccharide (LPS, endotoxin, O antigen), capsules (antigen K, i.e. capsular polysaccharide - CPS and exopolysaccharide - EPS), and fimbriae. LPS is built up of lipid A, core oligosaccharide (OS), and O-specific polysaccharide (O-PS). LPS and CPS determine O serotype or K serotype, respectively. Precisely, O serotype is defined by O-PS region built up of carbohydrate repeating units.

In 2021, we have reported series of studies concerning glycoforms and occurrence of enterobacterial common antigen (ECA). ECA is a conserved antigen expressed by enterobacteria. It is built by trisaccharide repeating units: →3)-α-D-Fucp4NAc-(1→4)-β-D-ManpNAcA-(1→4)-α-D-GlcpNAc-(1→ and occurs in three forms: as surface-bound linear polysaccharides linked to a phosphoglyceride (ECA_{PG}) or lipopolysaccharide - endotoxin (ECA_{LPS}), and cyclic form (ECA_{CYC}). ECA maintains outer membrane integrity, immunogenicity, and viability of enterobacteria. A supernatant obtained after LPS ultracentrifugation was reported as a source for ECA isolation, but it has never been assessed for detailed composition besides ECA_{CYC}. We used mild acid hydrolysis and gel filtration, or ZIC®HILIC chromatography combined with mass spectrometry to characterise ECA forms of *Shigella sonnei* and *Escherichia coli* R1 and K12 crude LPS preparations. Presented work is the first report concerning complex characteristic of all ECA forms present in LPS-derived supernatants. We demonstrated the presence of O-acetylated tetrameric, pentameric, and hexameric ECA_{CYC} and linear ECA built from 7 to 11 repeating units. Described results were common for all selected strains. The origin of linear ECA was discussed against the current knowledge about ECA_{PG} and ECA_{LPS} (*Gozdziewicz T.K. et al. Int J Mol Sci, 2021, 22(2): 701*).

We have elucidated polysaccharide structure relevant for virulence and/or viability of pathogenic bacteria. The chemical structure of the lipopolysaccharide O-polysaccharide repeating unit of *Edwardsiella tarda* strain PCM 1155 was elucidated. The rarely occurring monosaccharide, 2,3-diacetamido-2,3,6-trideoxy-l-mannose (L-RhapNAc3NAc) was identified (*Kaszowska M. et al. Carbohydr Res, 2021, 509: 108423*).

Resistance of *K. pneumoniae* 486 strain to a bacteriophage acquired as a result of interaction of bacteria with *Klebsiella* siphovirus KP36 was explained as a loss of capsular antigen. Since the emergence of resistance to antibacterials is generally considered undesirable, in this study, we have investigated the genetic and phenotypic (structural analyses of O and K antigens) characteristics of resistance to the phage-borne CPS-degrading depolymerase along with its effect on *K. pneumoniae* virulence. Genome-driven examination combined with the surface polysaccharide structural analysis of resistant mutant showed the point mutation and frameshift in the *wbaP* gene located within the *cps* gene cluster, resulting in the loss of the capsule. The sharp decline in the yield of CPS was accompanied by the production of a larger amount of smooth LPS. It was showed that the emerging resistance to the antivirulence agent (phage-borne capsule depolymerase) results in beneficial consequences, i.e., the sensitization to the innate immune response (*Kaszowska M. Int J Mol Sci, 2021, 22(21): 11562*).

The comparative structural analysis of *Bordetellae* (*B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. holmesii*) oligosaccharides (OS) revealed that the hexasaccharide, comprising the α-Glc*p*N, α-Glc*p*A, 4,6-disubstituted-β-Glc*p*, 2,7-disubstituted-L-α-D-Hep*p*, 3,4-disubstituted-L-α-D-Hep*p*, and Kdo, constitute the least variable OS segment. This

minimal common element in the structure of lipopolysaccharides of *Bordetellae* could be used to devise a universal cross-protective vaccine component against infections with various bacteria from the genus Bordetella (*Ucieklak K. et al. Int J Mol Sci, 2021, 22(3): 1029*). Additionally

We have devised a model of endosomal-like pre-processing of Bordetella pertussis 186 oligosaccharides (OSs) to verify how it affects the immunogenicity of their conjugates. Glycoproteins are processed endosomally prior to presentation to T cells and subsequent induction of specific antibodies. The sugar part of glycoconjugate may be degraded while the type of the process depends on the features of the particular structure. The generated carbohydrate epitopes may differ from native structures and influence immunogenicity of the antigens. The structural features of the oligosaccharides and their sensitivity to deamination were analyzed by NMR spectroscopy. The distal trisaccharide-comprising pentasaccharide conjugated to a protein was the most effective in inducing immune response against the *B. pertussis* 186 LOS and the immune response to the complete OS conjugates was significantly lower. This could be explained by the loss of the distal trisaccharide during the in-cell deamination process suggesting that the native structure is not optimal for a vaccine antigen. Consequently, our research has shown that designing of new glycoconjugate vaccines requires the antigen structures to be verified in context of possible endosomal reactions beforehand (*Koj S. et al. Vaccines (Basel), 2021, 9: 645)*.

Finally, a report on the structural analysis of LPS was provided for studies on mapping the intestinal plasma cell response to microbial colonization with a single microorganism in mice. Dimeric IgA secreted across mucous membranes in response to nonpathogenic taxa of the microbiota accounts for most antibody production in mammals. In cooperation with University of Bern, Bern (Switzerland) and German Cancer Research Center in Heidelberg, (Germany) recombinant dimeric monoclonal IgAs (mIgAs) were used to identify a range of antigen-specific mIgA molecules targeting defined surface and nonsurface membrane antigens, including LPS. Secretion of individual dimeric mIgAs targeting different antigens *in vivo* showed distinct alterations in the function and metabolism of intestinal bacteria, largely through specific binding. Even in cases in which the same microbial antigen is targeted, microbial metabolic alterations differed depending on IgA epitope specificity. By contrast, bacterial surface coating generally reduced motility and limited bile acid toxicity. The overall intestinal IgA response to a single microbe therefore contains parallel components with distinct effects on microbial carbon-source uptake, bacteriophage susceptibility, motility and membrane integrity (*Rollenske T. et al. Nature, 2021, 598 (7882): 657-661*)