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RESEARCH REPORT 2020

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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.

Studies on the mechanisms of tumor progression, metastasis and on the effects of experimental antitumor therapy

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 study is to determine whether the level of the vitamin D receptor (VDR) influences the effectiveness of 5-FU's anti-cancer effect on colon cancer cells. In this reporting period, the focus was on developing and characterizing human colorectal cancer cell lines with suppressed or overexpressed VDR. The characteristics of the obtained stable cell sublines of colorectal cancer showed silencing of the VDR (HT-29/shVDR, HCT116/shVDR, LS180/shVDR) or its overexpression (HT-29/leVDR, HCT116/leVDR, LS180/leVDR) both at the mRNA and and proteins compared to wild-type cells, and negative controls performed transduction. In all sublines, VDR silencing resulted in a statistically significant acceleration of the cell proliferation rate, while its overexpression significantly slowed down cell proliferation. Further studies were carried out with the wild-type HT-29 cell line showing a mutation in the TP53 gene and its subline (HT-29/shCtrl, HT-29/shVDR, HT-29/leCtrl, HT-29/leVDR). It has been shown that VDR silencing in HT-29 cells significantly increases their clonogenic abilities, while VDR overexpression has the opposite effect and leads to an almost complete abolition of these abilities. HT-29 cells of the wild-type line and its subline after exposure to 5-FU (8 μM, 72 h) show similar caspase-3/7 activity, except for VDR-silenced HT-29 cells in which caspase-3/7 activity is a significantly reduced. The change of experimental conditions (shorter exposure of cells to 5-FU and an increase in its concentration) made it possible to observe that HT-29 cells with VDR overexpression exhibit statistically significantly higher caspase-3/7 activity than control cells. By developing experimental schemes (different exposure times of cells to 5-FU and its concentrations), we showed that VDR-silenced HT-29 cells become resistant to the cytotoxic effect of 5-FU compared to control cells. This effect is exacerbated by prolonged exposure of cells to 5-FU. However, we did not observe any differences in the sensitivity of HT-29 cells overexpressing VDR to the action of 5-FU compared to control cells. Additionally, we showed that in VDR-silenced HT-29 cells, the level of TYMS mRNA increased significantly after exposure to 5-FU. In contrast, HT-29 cells overexpressing VDR showed significantly lower expression of TYMS mRNA after exposure to 5-FU compared to control. Moreover, these cells also showed significantly lower levels of BIRC5 mRNA.

Research on the use of new bisphosphonates in anti-cancer and anti-osteoporotic therapy

The aim of our study was to investigate the antiproliferative activity of newly synthesized compounds designed on the basis of the chemical structure of the compounds tested so far with the highest biological activity. Alendronic acid (alendronate) belongs to the so-called second generation of bisphosphonates containing a nitrogen atom (N-BPS) in their structure. Pharmaceutical preparations containing alendronate in their composition are used in the

treatment and prevention of osteoporosis and Paget's disease. Bisphosphonates also show interesting antiproliferative activity against cancer cells, and one of the most widely and most studied models is the breast cancer model. The most active group of N-BPS are the third generation bisphosphonates, in which the nitrogen atom is incorporated into the appropriate heteroaromatic system (imidazole ring in zoledronate and pyridine in risedronate). Therefore, it was decided to obtain a series of new N-substituted alendronic acid derivatives containing a pyridine and imidazole ring and a simple phenyl substituent, and then to study the influence of such modifications on the anti-osteoporotic and antiproliferative activity against the MCF-7 breast cancer line.

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

Emerging data indicate that 5-azanucleosides are able to sensitize cancer cells to the standard chemotherapeutic agents and contribute to overcoming intrinsic or acquired chemo-resistance. Previously, we have demonstrated that pre-treatment with DNA demethylating agents, 5-aza-2'-deoxycytidine or 5-azacytidine, sensitizes CRC cells to topoisomerase inhibitors. Next, we have shown that a similar combination of drugs could be effective against HCT116 cancer cells *in vivo*. The search for a mechanism of sensitization of cancer cells to anticancer agents revealed that 5-azanucleosides greatly change properties of cancer cells. Once exposed to a low dose of 5-azanucleosides, cancers cells became larger and divided more slowly. Along with increased expression and activity of beta-galactosidse, such morphological features indicate that 5-azanuclesoides induce cellular senescence in the long term.

Also, we examined 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 a limiting factor of accuracy and practicality. Thus, we decided to design 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 in combination with a variety of cancer cells (HTC116, HT29, BxPC3, Caco2, Caki). By using fluorescent labels, we are able to detect the interaction of cancer cells and NK92 in 1:1 and 1:5 ratios (vs. 1:10 up to 1:50 in standard procedures) as fast as 15 minutes incubation. Extending co-incubation of cells up to 2h allows us to detect the cell death of vulnerable cancer cells. Such a new method will allow us to study NK cells activity against cancer cells in various conditions without many practical obstacles found in standard methods.

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

Application of innovative boron compounds in biology and medicine

Boron cluster-based strategies to overcome antibiotic resistance of pathogens

The accelerating emergence of multi-drug resistant (MDR) pathogens poses a great threat to public health and creates a demand for new chemical classes of antibiotics. Boron clusters originate from a different chemical space from conventional antibiotics, providing new opportunities for medicinal chemists in drug design, while simultaneously remaining

a challenge for pathogenic systems of resistance development. Boron clusters have a different chemical composition and structure than organic compounds, which ensures their resistance 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 show antimicrobial activities against Gram-positive and Gram-negative bacteria, as well as fungi. In contrast to antibiotics, the activity of boron cluster-containing compounds remains unchanged when they are used against MDR bacteria. Furthermore, they show anti-biofilm activity. Thus, boron cluster-containing compounds break both the genetic and the phenotypic resistance of pathogens.

A different strategy against MDR bacteria embraces conjugates of short cationic peptides and hydrophobic compounds, whose structure and mechanism of action is based on natural antimicrobial peptides. The cationic side of the conjugate interacts with anionic surfaces of bacteria cells and is responsible for the selectivity of the conjugates, whereas the hydrophobic side interacts with the interior of the cell membrane, which either leads to disruption of the membrane or translocation of the conjugate through the membrane into the cell, where it disrupts bacteria's metabolic pathways. Thus, the selectivity and mechanism of action of the conjugates is based on different molecular principles than conventional antibiotics. Metallacarboranes is a class of boron clusters with a high potential to be used as the hydrophobic side of the conjugates. Metallacarboranes interact with lipid membranes as well as proteins and nucleic acids, which provides the conjugates containing metallacarboranes with multi-target mechanism of action. The bulky structure of metallacarboranes should increase the membrane-disruptive force of the conjugates. Additionally, metallacarborane's ability to interact with serum albumin and to self-assemble should result in a good pharmacokinetic profile of the conjugates.

In our laboratory, we are dedicated to the development of both antimicrobial strategies: low-molecular-weight boron cluster-containing compounds and conjugates of short cationic peptides and metallacarboranes.

Boron carbide and boron nitride nanoparticles as boron donors in boron-neutron capture therapy (BNCT)

In our research on the use of inorganic boron nanoparticles for BNCT, we utilize compounds synthesized in an original way at the AGH University of Science and Technology and at the Military University of Technology. We are conducting research on the biofunctionalization of boron carbide and boron nitride nanoparticle surfaces in order to give them cancer cells targeting properties. The nanoparticles are physicochemically characterized in terms of charge, size, and surface morphology. The interaction of such designed nanoparticles with phagocytic and cancer cells is investigated as well as boron biodistribution in order to verify their usefulness for BNCT.

DEPARTMENT OF CLINICAL IMMUNOLOGY

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

Factors associated with the development and progression of multiple myeloma

Multiple myeloma (MM) is a major haematologic disease characterized by the presence of abnormal plasma cells. It is associated with the activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB). Myeloma cells can activate the NF-κB pathway by interacting with bone marrow stromal cells, leading to the production of proteins such as IL-6 and contributing to MM cell proliferation and survival. Earlier studies on deletion rs28362491 in the promoter of the gene coding for NF-κB (*NFKB1*) showed that it may affect NF-κB expression. In the present study, we aimed to analyse the potential association of this deletion with risk and survival in MM patients. A total of 156 MM patients and 126 healthy individuals were genotyped for the *NFKB1* promoter deletion using the 3500 Genetic Analyzer 8-Capillary Array. We found that patients with the deletion tended to have better overall survival than patients lacking it (p=0.059). However, this association was only present in men (p=0.027), whereas it was completely absent in women (p=0.445). Additionally, we observed that deletion homozygosity tended to be less common in patients diagnosed with stage III (according to Durie-Salmon criteria) than in patients in stages I-II on diagnosis (p=0.095). These results suggest that the *NFKB1* promoter deletion rs28362491 may affect survival in male patients.

Telomere length in the context of genetic variability of the human telomerase reverse transcriptase (hTERT) in patients with blood cancers

Acute myeloid leukaemia (AML) is the most common haematological malignancy associated with short telomeres, whose basic risk stratification and prognostic scoring is based on the presence of the *NPM1* and/or *FLT3* mutations. In addition to *TERT* genetic variants (rs2736100, rs2853669) and telomere length analysis in AML patients with normal karyotype and controls, *TERT* expression (TE), telomerase activity (TA), and telomere length (TL) were also measured in non-stimulated myeloid and normal cell lines cultured *in vitro*. TL was negatively correlated with age in healthy subjects, but not in AML cases. Patients homozygous for the rs2853669 *C* allele were characterized with shorter overall survival. Significant differences were found between patients below and above the median age of 61 years with respect to overall survival and TL. Additionally, the *NPM1* and *FLT3*-ITD mutation status was found to be associated with TL. As for TL, it was found to be increased in AML patients compared to myeloid and normal cell lines (with the shortest TL). Also, TA level was enhanced in myeloid cells compared to normal cell lines while TE was 18 times higher in all myeloid cell lines compared to normal cell lines. The obtained results suggest that TE is an important regulatory element in myeloid cell lines cultured *in vitro*. Additionally, overall survival of AML patients appears to be affected by TL and *TERT* gene variability in addition to other well-established factors such as age or *FLT3*-ITD and *NPM1* mutation status.

Investigating regulatory mechanisms of telomerase activity in a human model of *in vitro* cultured cell lines

An *in vitro* human cell line model was applied to study the role of telomeres and telomerase in various cancers. Cell lines of both solid tumor and haematopoietic origin were investigated. This approach allowed us to study the differences in two experimental groups indicating the relationships between *TERT* gene expression, telomerase activity, and telomere length in the presence of somatic mutations in the *TERT* promoter region.

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

Different impact of polymorphisms in antigen-presenting machinery genes on disease risk and clinical parameters of non-small cell lung cancer in smokers and never-smokers

Lung cancer is strongly associated with cigarette smoking; nevertheless, some never-smokers develop cancer. Immune eradication of cancer cells is dependent on polymorphisms of HLA class I molecules and antigen-processing machinery (APM) components. We compared the distribution of APM polymorphic variants in larger cohorts of Polish patients with NSCLC and controls, stratified according to their smoking status. We found significant but opposite associations in never-smokers and in smokers of all tested SNPs (rs26653, rs2287987, rs30187 and rs27044) but one (rs26618) in ERAP1. No significant associations were seen in other genes. However, haplotype analysis indicated that the ERAP2 SNP rs2248743, determining presence versus absence of the functional enzyme, influenced ERAP1 effect, again in opposite directions in smokers versus never-smokers. We also revealed interesting associations of some APM polymorphisms with: age at diagnosis (ERAP1 rs26653), disease stage (ERAP1 rs27044, LMP2 rs17587), overall survival (ERAP1 rs30187), and response to chemotherapy (ERAP1 rs27044).

Synergy of ERAP1 and ERAP2 polymorphisms in atopic dermatitis

Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 trim peptides to a length of 8–10 amino acids optimal for binding by HLA class I molecules. Although these two enzymes may work separately, they may also form a heterodimer of enhanced trimming efficiency. We have earlier described a role for ERAP1 single nucleotide polymorphism rs26618 and HLA-C*05:01 as risk factors for atopic dermatitis (AD). Here, we examined whether ERAP2 single nucleotide polymorphism rs2248374, determining the presence or absence of the functional form of enzyme, would influence the rs26618 effect. Out of nine rs2248374 – rs26618 genotypic combinations, only one, rs2248374*A/A – rs26618*C/C, was associated with a risk of AD. Interestingly, the odds ratio increased from 1.10 (CI 95% = 0.72; 1.69; $p = 0.657$) for ERAP2 rs2248374*A/A and 1.88 (CI 95% = 1.07; 3.28; $p = 0.025$) for ERAP1 rs26618*C/C to 3.36 (CI 95%: 1.41; 8.01; $p = 0.004$) for their combination, therefore revealing a synergistic effect.

Association of soluble HLA-G plasma level and HLA-G genetic polymorphism with pregnancy outcome of patients undergoing *in vitro* fertilization embryo transfer

Human leukocyte antigen (HLA)-G has been suggested as an immunomodulatory molecule that influences pregnancy outcome. The HLA-G gene encodes either membrane-bound or/and soluble proteins. The aim of this study was to evaluate the role of soluble HLA-G (sHLA-G) and its gene polymorphism in successful implantation after *in vitro* fertilization embryo transfers (IVF-ETs) in different clinical protocols. We tested the HLA-G polymorphism in three positions: rs1632947: c.-964G>A; rs1233334: c.-725G>C/T in

promoter region; and rs371194629:c.∗65_∗66insATTTGTTCATGCCT in 3′ untranslated region of exon 8. We found that certain rs1632947-rs1233334-rs371194629 HLA-G haplotypes and diplotypes were associated with infertility, while others were protective. The lowest secretors of sHLA-G were G-C-ins haplotype carriers (37.21 IU/ml), while the highest - G-C-del carriers (73.80 IU/ml). Regardless of possessed haplotype by the patient, 59.73 IU/ml sHLA-G was the threshold value above which patients had an almost two-fold increased chance to get pregnant than patients who secreted below this value (p = 0.0085; likelihood ratio, 1.74; 95% CI = 0.55–0.78). In addition, IVF patients in frozen/thawed cycles secreted higher sHLA-G than patients in fresh cycles (p = 0.021). Moreover, short ovarian stimulation protocol with GnRH antagonist seemed more beneficial for the outcome of pregnancy than long protocol with GnRH agonist.

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

COVID-19 pandemic surge focused our scientific interest towards studies on the pathomechanism of this life-threatening disease.

Our two first scientific reports were aimed at physicians confronting the new virus we knew so little about. The first paper published on-line was on response of the immune system to viral infections, including SARS-CoV-2, written in an accessible form, suited for public benefit (Monitor Rynkowy, 2020 [1]). The next article, published in Polish Archives of Internal Medicine (2020 Aug 27;130 (7-8):662-667 [2]), presented the cytometry profile with a history of chronic infections. In this paper, the characteristic features of terminally differentiated T cells were depicted. If an excess of these cells are identified in the, the ability to respond to new infectious challenges may be lowered. In addition, with the use of NGS, we showed that, deduced from beta receptor profiling, patterns of different CDR3 representations in the blood help in the estimation of the breadth of the immune TCR beta repertoire, which, if restricted due to the overwhelming use of some receptors, may handicap the ability of an individual to confront successfully new challenges.

The T cell repertoire occupied by previous infectious challenges lacks a sufficient number of T lymphocytes, which have not yet encountered any epitope. If detected, the two features (high proportion of TEMRA cells and restricted TCR beta repertoire) may help in revealing individuals at a higher risk of SARS-CoV-2 infection.

In the next paper published in Transplant Immunology (2021 Apr; 65:101370 [3]), we discovered that monocytes emerging in the blood, which lack HLADR on the cell membrane exert a negative impact on the survival of the patients after alloHSCT, which are regulatory cells, bring down immune response.

The large size of the study cohort (223 patients) and the length of observation (up to 15 years) lend credence to the study results.

We found that the proportions of CD14+HLADR- cells in the blood detected on day 30 after alloHSCT may help in identifying patients at risk of an unfavorable outcome of alloHSCT (the death rate post transplant was significantly higher in the patients with proportions of CD14+HLADR-cells in the blood above than in those with values below (0.81 vs 0.54, $p<0.001$) the threshold (set up at 0.71% by the ROC analysis)

The appearance of CD14+HLADR- cells in the blood soon after receiving conditioning regimen suggests the role of strong inflammatory process followed by emergency myelopoiesis in appearing HLADR negative monocytes. Thus our finding of the negative

impact of CD14+HLADR- cells in blood on the susceptibility to infections may also be valid for their clinical situations in which overwhelming inflammation prevails.

In summary, results of our studies in year 2020 offer the transplantation community the following novel information: (i) the breadth of the T cell repertoire is indicative of the immune system competence, (ii) CD14+HLADR- cells if high in proportions early post transplant is significantly predictive of unfavorable outcome of alloHSCT which is frequently associated with the presence of fatal infections.

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LABORATORY OF BACTERIOPHAGES
Head: Professor Andrzej Górski, Ph.D.

In 2020 our laboratory has been engaged in several projects. The results of some of them have been summarized below:

1. We have analyzed the relationship between anti-phage antibody production and clinical outcome in a group of patients with chronic sinusitis treated with phages against *S. aureus, P. aeruginosa, K. pneumoniae* or *E.coli*. Good results of the therapy were achieved in 8/25 patients. Antibody levels increased in all patients while no correlation has been noted between clinical results and the intensity of the humoral anti-phage response. These results confirm our earlier observations suggesting that the appearance of anti-phage serum antibodies does not interfere with successful phage therapy (Łusiak-Szelachowska et al., *Folia Microbiol*. 2021 Feb;66(1):127-131. doi: 10.1007/s12223-020-00835-z).

2. New methodology (Endoscan) has been applied to study immunogenicity of phage endolysins. Results obtained have allowed for constructing endolysin variants with modified epitopes. It has been established that epitope modification with the aid of Endoscan may modify endolysin structure, allowing for their decreased immunogenicity.

3. *E.coli, K.pneumoniae* and *E.faecalis* strains isolated from patients with urinary tract infections were tested for their sensitivity to phages from our phage bank. Phages were able to lyse 85% of *E.coli* strains whilst other strains were resistant. This data confirms the need to expand our phage collection, especially for phages active against other urinary pathogens.

4. The effects on biofilms of a staphylococcal A5L phage and antibiotics were tested. The phage as well as ampicillin and doxycycline caused a small reduction of biofilms while the combination of phage with antibiotics showed some synergistic action. This suggests that

joint application of phages and antibiotics may be a more efficient means to eradicate bacterial infections than either agent used alone.

5. We have formulated a hypothesis suggesting that phage therapy may be useful in treating COVID-19: immunomodulating and anti-inflammatory activities may interfere with COVID-19 immunopathology, alleviating the symptoms of this syndrome (Górski et al., *Future Microbiol* Aug;15:1095-1100. doi: 10.2217/fmb-2020-0082.; Górski et al., *Pathogens* 2020 Oct 15;9(10):844. doi: 10.3390/pathogens9100844).

6. It is known that so far no clinical trial of phage therapy has confirmed its value according to currently required standards of Evidence-Based Medicine.

The possible causes of this failure have been analyzed in some detail. Furthermore, suggestions how a potentially successful clinical trial should be carried out have been made (Górski et al., *Antibiotics* 2020 Nov 19;9(11):827. doi: 10.3390/antibiotics9110827).

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

Assessment of biological stability of immortalized human mesenchymal stem cells derived from adipose tissue (HATMSC1, HATMSC2) and from bone marrow (HBMMSC1)

Mesenchymal stem cells (MSCs) are characterized with high proliferative activity and production of trophic factors in the early passages; however, when the number of passages increases, the proliferative activity and secretion of bioactive factors decreases significantly. The biological activity of MSCs can be preserved by immortalization of primary cells. However, cell immortalization may result in changes related to the maintenance of genetic stability. Therefore, the proliferative activity and the potential tumorigenic properties of the immortalized MSC lines derived from adipose tissue (HATMSC1, HATMSC2) and bone marrow (HBMMSC1), previously established in our laboratory, was assessed.

The clonogenic potential of the immortalized human MSC lines of HATMSC1 and HATMSC2 derived from adipose tissue and the MSC line obtained from human bone marrow HBMMSC1 was assessed under 2D (standard monolayer culture) and 3D conditions (in MethoCult H4434). The cell lines showed the ability to grow in 2D cultures, forming colonies in a standard monolayer culture, and in 3D cultures in semi-liquid media; however, the ability to form colonies was more effective in 3D culture. Karyotype assessment of examined cell lines was performed by the GTG method. All examined cell lines revealed highly complex hyperploidy, with a chromosome count of 75 to 85. The potential malignant transformation of the cell lines, HATMSC1, HATMSC2 and HBMMSC1, was examined *in vivo* by subcutaneous administration of cells to the NOD / SCID mice strain at a dose of $10x10^6$ per mouse. None of the tested cell lines started to grow and they showed no signs of neoplasia *in vivo* as confirmed by histopathological assessment of tissues taken from the site of cells injection.

The obtained data show that despite the ability to grow in semi-fluid medium and significant genetic (karyotype) defects, immortalized HATMSC1, HATMSC2 and HBMMSC1 cells are unable to form neoplastic tumors in NOD / SCID mice.

Assessment of the effect of microvesicles derived from mesenchymal stem cells in inhibiting the proliferative activity of ovarian cancer cell lines

Transport of bioactive cargo of microvesicles (MVs) into target cells can affect their fate and behavior and change their microenvironment. The effect of MVs derived from mesenchymal stem cells (MSCs) of adipose tissue (HATMS2-MVs) on the biological properties of ovarian cancer cell lines ES-2 (clear cell carcinoma) and OAW-42 (cystadenocarcinoma) was assessed.

The multi-antigenic phenotype of cells, performed by flow cytometry, revealed that ES-2 cell line expressed three markers characteristic of MSCs: CD73, CD90 and CD105, while OAW-42 cells did not express CD105 antigen. Both cell lines were CD44 positive and CD34, CD133, SSEA4 negative. The secretion profile of ovarian cancer cells was evaluated by using protein antibody array and revealed different pattern of secreted cytokines and growth factors between ES-2 and OAW-42 cell lines. Live cells observation, using advanced method of fluorescence microscopy, confirmed that both cell lines internalized HATMSC2-MVs, and this was associated with a decreased metabolic activity of cancer cells. The reduction of the metabolic activity of both cell lines led to an inhibition of the proliferative potential of neoplastic cells. HATMSC2-MVs exerted a pro-apoptotic effect on ES-2 cells, while treatment of OAW-42 cells with HATMSC2-MVs led to an increase in the number of necrotic cells, compared to controls.

In conclusion, we confirmed the inhibition of ovarian cancer cell proliferation via different pathways, apoptosis and/or necrosis, which, with high probability, is associated to the presence of different anti-tumor factors secreted by the ES-2 and OAW-42 cells treated with HATMSC2-MVs.

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

Prenatal and early postnatal exposure to a natural disaster and Attention-Deficit/Hyperactivity Disorder symptoms in prepubertal Indian children

Attention-Deficit/Hyperactivity Disorder (ADHD) is characterized by age-inappropriate levels of inattention and/or hyperactivity/impulsivity and affects the life of patients by increasing significantly the risk of academic failures, conflicts with peers, family members and incarceration. The aim of the research was to assess the relation between early exposure to severe cyclonic storm Aila and the level of ADHD symptoms in children. Indian boys and girls (8-11 y) prenatally (N=336) and early postnatally (N=216) exposed to Aila were compared to the non-exposed control group of their peers (n=285). The main effect of exposure to Aila on ADHD symptoms was significant and independent of controlled factors; nevertheless, the post-hoc analysis revealed sex-specific relations between the timing of the exposure (prenatal vs. early postnatal) and cognitive problems/inattention as well as oppositional symptoms. Exposure to stressful experiences affecting later behavior characteristics is not limited to fetal life but extends at least into infancy.

Blood lead level and nutritional status indicators in pre-adolescent schoolchildren from Copper Basin in southwestern Poland

Environmental pollution with heavy metals may have toxic effects on human health and development. One of the most detrimental is lead exposure, which may disturb

neurodevelopment and linear growth in children. However, data on the effect of lead exposure on nutritional and weight status in children are limited; thus, this study aimed to assess the effect of blood lead (Pb) level on nutritional and weight status in schoolchildren from the industrialized, mining region in southwestern Poland. Our study sample involved N=709 schoolchildren (402 boys and 307 girls) from pre-adolescent developmental period (7-11 years of age for boys and 7-10 years of age for girls). Anthropometric measurements were used to assess nutritional and weight status: body mass index (BMI), mid-upper arm circumference (MUAC) and skinfolds (triceps, subscapular, abdominal). Blood Pb level was evaluated and divided into two groups: above (>3.7) and below median value (≤3.7). Analysis of covariance (with age controlled as a covariate) revealed that children with blood Pb level above median value had significantly lower values of BMI, MUAC and all skinfolds. However, this effect was significant only in boys, whereas in girls no significant differences were found. Nutritional status in children with higher blood Pb level is significantly deteriorated in boys, who appear to be more sensitive to such an environmental factor. Our findings indicate a particular need for nutritional intervention among pre-adolescent children in regions exposed to the higher lead levels.

DEPARTMENT OF MICROBIOLOGY

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

Replication of bacterial chromosomes

We are interested in the 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. Chromosome replication is highly regulated at the initiation step. There are only two known regulators of *H. pylori* and *C. jejuni* chromosome replication initiation, namely *H. pylori* HobA and HP1021 (*C. jejuni* Cj0545 and Cj1509, respectively). We have recently focused on *C. jejuni* Cj0545 protein. We determined that the Cj0545 protein interacted with the initiating protein CjDnaA *in vitro*. The protein formed dimers, resembling the homologous HobA proteins of *H. pylori* and *E. coli* DiaA (both proteins form dimer tetramers). The Cj0545 interacted with initiator proteins from few Campylobacterota species, which indicated the conservative nature of the Cj0545-DnaA interaction surfaces. Unlike the HobA and DiaA proteins, we have not confirmed the involvement of Cj0545 in modulating initiation complex formation in vitro. Since the protein Cj0545 cannot be removed from the chromosome, it is impossible to determine the effect of Cj0545 on the replication rate of the *C. jejuni* chromosome.

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 found that HP1021 helps *H. pylori* respond to oxidative stress. *H. pylori* lacking HP1021 is more sensitive to some oxidative compounds, primarily reactive oxygen species (ROS).

ROS activates a few genes in HP1021-dependent manner wild-type cells. Thus, we conclude that HP1021 is a sensor of oxidative stress.

Secondary metabolism in *Streptomyces*

Bacteria from the genus *Streptomyces* are potent producers of polyketides, a large class of bioactive compounds with highly diverse structures and functions. They are synthesized as secondary metabolites by giant multienzyme complexes – polyketide synthases. Our laboratory focuses on coelimycin synthesis regulation, the earliest colored specialized metabolite synthesized in the life cycle of *Streptomyces coelicolor* A3. *cpkO* and *cpkN* are two SARP activators of the coelimycin synthesis, which raised our particular interest. Deletion of *cpkO* and *cpkN* abolished coelimycin synthesis and resulted in dramatic changes in the later, stationary-phase antibiotics production. Therefore, in our studies, we focused on the underlying mechanisms of these phenotypes. Analysis 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. Moreover, we showed that *quorum* sensing gamma-butyrolactone receptor ScbR regulates *cpkN*.

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. The project was carried out in cooperation with dr hab. Katarzyna Matczyszyn from the Faculty of Chemistry, Wrocław University of Technology, and prof. Ifor Samuel from the University of St Andrews (UK). New photosensitizing compounds are investigated using *Staphylococcus aureus* as a model species and clinical strains - bacteria isolated from patients' diabetic foot. The absorption spectra of these compounds and their efficiency in reducing the survival of selected bacterial strains were characterized.

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

Effect of selected *Bifidobacterium* strains on the production/secretion of brain-derived neurotrophic factor and modulation of nitric oxide production/secretion in experimentally induced inflammatory conditions

Intestinal bacteria have the ability to communicate with the central nervous system (CNS) through numerous mechanisms, including stimulation of the immune system, vagus nerve or production of metabolites such as acetylcholine, catecholamines, gamma-aminobutyric acid or short-chain fatty acids. Some of them are able to cross the intestinal barrier, enter the systemic circulation and may cross the blood-brain barrier. Research in the last decade on the brain-gut axis shows that microbiota plays a key role in shaping the proper development of the brain and its functioning. Brain-derived neurotrophic factor (BDNF) plays a very important role in the proper functioning of the nervous system. It regulates the survival and growth of nerves and is crucial in processes related to memory and learning. In

neurodegenerative processes and during aging, a significant decrease in the level of BDNF is observed, especially in the hippocampus. Recent studies have indicated a relationship between gut dysbiosis and a decrease in the level of BDNF. Thus, it seems that bacterial manipulation, e.g. by specific probiotic strains, could lead to an increased level of BDNF.

In our studies, we tested the ability of selected ten *Bifidobacterium* strains to stimulate the PC12, H19-7 cell lines and human whole blood cells to secrete BDNF. The level of BDNF in cell culture supernatant was determined by ELISA. None of the tested *Bifidobacterium* strains was able to induce BDNF production by PC12 or H19-7 cells. However, we observed a significant influence of *Bifidobacterium* on the secretion of this factor by human whole blood cells. The effect was strain depended, e.g. the highest amounts of BDNF induced strain 370 and 372, whereas the lowest amounts of BDNF were secreted in response to strain 369 and 219.

We also determined the ability of selected bacterial strains to regulate the innate immune response. Macrophages are the first line of defense in infections and play an important role in maintaining tissue homeostasis. In response to the pathogen, they release of a number of pro-inflammatory factors, including NO and pro-inflammatory cytokines. Macrophages were incubated with selected strains in the presence or absence of the SMIU (iNOS inhibitor) and LPS. The level of released NO was determined in supernatant of cell culture by the Griess method. Changes in the expression of iNOS were determined by Western blotting. All tested strain induce the production of NO by macrophages that was regulated by iNOS. The highest levels of NO were released in response to strain 366, whereas the lowest level was in response to 371 and 372. Moreover, all tested strains were able to modulate the production of NO caused by LPS, however the observed activity was district. The strongest inhibitory effect was demonstrated for strains 219 and 371.

DEPARTMENT OF TUMOR IMMUNOLOGY
Head: Professor Paweł Kisielow, Ph.D.

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

Minimal sequence difference is able to establish functional heterozygosity of honey bee complementary sex determiner gene

The complementary sex determiner (*csd*) gene is responsible for sex determination in honey bees and occurs in many polymorphic forms. Bees possessing two different *csd* alleles are females and one allele are males. The occurrence of two identical *csd* alleles in the diploid genome leads to the development of nonviable diploid male. The variety of *csd* alleles is associated with the strong polymorphism occurring in the hypervariable region of the gene (mainly due to the presence of insertions/deletions); however, it is not yet clear what the sufficient difference in the allele sequence is in order to trigger the process of female development. So far, only one case of non-identical *csd* alleles (differing by 3 amino acids) has been shown to not fully determine femaleness and it has been proposed that accumulation of such small (yet not fully functional) differences could be responsible for gradual evolution of *csd* diversity. The aim of our work was to identify statistically significant biases in the frequency of forming pairs between a particular paternal allele and the two maternal alleles present in a given colony. We assumed that, if identified, the absent/underrepresented pairs should be the ones that are not able to fully determine femaleness. This way we wanted to identify more cases of not functional or not fully functional non-identical *csd* pairs.

For this purpose, we determined the genotype of several hundred worker bees from each of the five colonies analysed using T-RFLP method. In total, we have established the maternal and paternal *csd* restrictions patterns of 1,268 worker bees, identifying from 7 to 18 patrilines in a single colony. As a result of cloning and sequencing of the paternal alleles of the representants of each patriline, we have identified 53 distinct paternal alleles and 113 distinct *csd* pairs. Most of the patrilines were characterized by nearly equal frequencies of the presence of maternal alleles; we identified 7 patrilines, which were characterized by significantly different frequency of pairing of the paternal allele with the maternal alleles. We have compared the sequence differences between the *csd* alleles in such pairs to others present in our dataset using two parameters: the difference of the length of the HVRs (given in the number of amino acid residues) and number of amino acid differences (mismatches and indels) outside of the HVRs (the fragments of the RS- and proline-rich domains). The sequence differences of the *csd* alleles forming the pairs underrepresented in our analysis characterized by aforementioned parameters were virtually undistinguishable from the differences characterizing other pairs.

The most startling observation we have made during our screening of *csd* pairs was the identification of a pair encoding proteins differing from each other only by one amino acid. This pair was not underrepresented in the colony, suggesting that it was fully functional. We nonetheless tested whether bee larvae carrying this genotype had the molecular hallmark of female development. This was done by identifying the presence of female and/or male splice variants of the dsx transcript using RT-PCR. In all tested cases the larvae expressed only the female-specific dsx transcript.

Taken together, our research shows that criteria for functional heterozygosity of *csd* alleles cannot be established based on the difference in length and/or number of substitutions in the *csd* sequence alone and suggests the existence of more subtle criteria such as the site of the mutation and its nature. The existence of fully functional pair of *csd* alleles characterized by a minimal difference also shows that functional heterozygosity can be established in a one-step evolutionary event.

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

Enhanced alpha II spectrin destabilization causes ataxia

We report a novel *Sptan1* autosomal dominant mouse mutant strain characterized by a progressive ataxia and tremor. The whole exome sequencing revealed a novel missense mutation in αspectrin; *Sptan1* (NM 001177667): c.3293G>A:p.R1098Q). Homozygous αspectrin *Sptan1* R1098Q $^{-/-}$ neonates die and exhibit craniofacial abnormalities and vascular defects. Heterozygotes are vital but display ataxic movements, tremor and seizures. They perform poorly on the rotarod test and when aged, their brains have significant atrophy of the cerebellum. Signs of cerebellar neurodegeneration include reactive astrogliosis, amyloidosis, aberrant cerebellar cell organization, and Purkinje cell (PC) loss. Electron microscopy and immunohistochemistry highlight irregular morphology of PC endomembranes, dendrites, and a dramatic loss of their postsynaptic densities (PSDs). Sparse immunolabeling of the glutamate transporter, EAAT4, and αspectrin are consistent with loss of PC PSDs and dendritic spines. While molecular modeling of R1098Q does not predict steric clashes, in silico analysis reveals a putative conformational change in the proximal calpain, caspase, calmodulin-binding domain. Data reveal hypersensitive calpain proteolysis and altered calmodulin binding of the mutant peptide. Taken together these results reveal the critical

importance of substrate-level regulation of spectrin cleavage for the maintenance of neuronal integrity.

Since the excessive calpain activation is a common feature of neurodegenerative disease and traumatic encephalopathy, we propose that damage to the membrane-associated periodic spectrin skeleton may contribute to the neuropathology of many disorders.

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

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

Implementation of three-dimensional culture for cell research

Three-dimensional cell culture (3D) with the use of an organic culture framework will avoid cell stress caused by adhesion to the plastic surface, poor exchange of medium, and weak metabolite removal. A durable, biodegradable network with defined mesh was obtained, the size of which corresponds to the size of an average eukaryotic cell. In this case, it is 25 μm. Three short peptides with the RDG sequence are coupled to this mesh, which mediates cell adhesion through transmembrane integrin receptors. Mouse spleen cells briefly exposed to lipopolysaccharide (LPS) acquire the ability to form three-dimensional clusters. This change is permanent, and the tendency to build the spatial structure does not disappear for 12 days even without LPS. Its dimensions after 4 days of cultivation are 68.7 μm and h = 74.5 μm. After 12 days, the culture begins to die, and the first to die are cells not included in the 3D structure.

Biological activity of synthetic fragments of human BMP

In cooperation with the Department of Biomedical Chemistry of the University of Gdańsk, the biological activity of two synthetic peptides with the sequence of BMP-2 fragments was demonstrated.

Effect of lactoferrin on cytotoxicity of human blood monocytes

The studies on the effects of recombinant lactoferrin from hamster ovary cells (CHOLF) and lactoferrin-Fc immunoglobulin fragment complex (LF-Fc) on natural killer cell activity were continued. LF-Fc strongly stimulated production of TNF α by human blood mononuclear cells but CHOLF did not. In addition, we identified another cytotoxic factor derived from cultures of human monocytes by LF-Fc as granzyme B. A cytotoxic effect of the supernatant, derived from culture of monocytes treated with FL-Fc, on viability of Jurkat cells was also found.

Laboratory of Immunopathology
Head: Professor Irena Frydecka, M.D., Ph.D.

Imbalance in PB IL-17-secreting and regulatory cells in pars planitis is associated with dysregulation of IFN-γ-secreting cells, especially in patients with clinical complications

Background/Purpose: Pars planitis (PP) is a chronic intermediate uveitis of undefined cause, although autoimmune etiology is strongly suggested. This study was undertaken to evaluate circulating pro- and anti-inflammatory lymphocyte subpopulations in patients with PP. A better understanding of immune alterations in PP may contribute to the development of an appropriate biologic treatment approach.

Material and methods: In our case control study, samples of peripheral blood were collected from 15 patients with pars planitis and from 17 healthy subjects. The patients underwent a full ophthalmological evaluation including macular optical coherence tomography (OCT) and submacular choroid (with enhanced deep imaging) with the SD-OCT Spectralis system. With flow cytometry, we assessed lymphocyte subpopulations as follows: Th1 subset – CD3+CD8-IFN-γ+ and CCR4-CXCR3+IFN-γ+ cells, Th17subset – CD3+CD8-IL-17+ and CCR4+CCR6+IL-17+ cells, T regulatory (Treg) – CD4+CD25hiCD127- cells, T suppressor (Tsup) – CD8+CD28-FOXP3+ cells, and B regulatory (Breg) – CD19+CD24hiCD38hi cells. The results are presented as the median percentage of immune cells in comparison to those obtained from the healthy control group.

Results: In patients, an increase in the population of Th17-secreting cells negatively correlated with an abundance of both IFN-γ-producing and T regulatory as well as suppressor cells with regard to all the phenotypes studied. Although a strong dependence of the PB Th1 cell compartment on duration of the disease was observed, it was limited to the subgroup of patients with macular edema only. The frequency of B regulatory cells was unchanged compared to controls.

Conclusions: In pars planitis, the alterations in lymphocyte cell distribution affect primarily the T cell repertoire. The imbalance in PB Th1/Th17/Treg cells creates pro-inflammatory conditions, strengthening the suggestion that the immune background may play a role in pars planitis pathogenesis. Also, circulating Th1 level may be of potential clinical relevance in terms predicting a more severe course of the disease.

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

Immunological mechanisms associated with reproductive processes in health and disease

Title: Expression of IL-35 in regulatory B lymphocytes (Breg) in women with endometriosis
Research task continued

The aim of the research was to determine the level of IL-35 expression (Ebi3 and p35 subunit) in regulatory B lymphocytes in the peripheral blood of women with endometriosis compared to healthy women.

Results:

A significant decrease in the percentage of B10 cells (CD19 + CD24highCD27 +) and plasmablasts (CD27intCD38high) was observed along with an increase in the percentage of the same cells expressing IL-35 in women with endometriosis compared to the group of healthy women.

The revised American Society for Reproductive Medicine classification and the score of endometriosis was included in the analysis. A decrease was observed in the percentage of plasmablasts in women with endometriosis both in the early stage (stage I-II) and advanced (stage III-IV) of endometriosis compared to the control group. Moreover, an increase was shown in the percentage of all studied populations (B10, immature B lymphocytes and

plasmablasts) expressing IL-35 in the group of women with endometriosis. The changes in expression of IL-35 in B10 and immature B lymphocytes characterized early stages of endometriosis but in plasmablasts they were independent of the stage of the disease.
A higher proportion of immature B lymphocytes expressing IL-35 has also been observed in women with endometriosis who have over a 50% chance of becoming pregnant within 36 months (EFI = 7-9) compared to women who have up to a 50% chance (EFI = 1-6).
The obtained results indicate the involvement of IL-35 producing cells in the peripheral regulation of the immune response in women with endometriosis as well as the potential use of IL-35 expression level as a peripheral marker of this disease.

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

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

Studies on the pathogenicity mechanisms of some diseases of bacterial etiology, the role of bacterial and phage glycoconjugates and proteins in immune processes as well as the structural studies of the surface glycoconjugates towards their therapeutic applications

Our laboratory is involved in studies on the mechanisms of pathogenicity of diseases with bacterial etiology, the role of bacterial glycoconjugates and proteins in the immune processes, the structure and functions of bacterial exopolysaccharides, including those from actinobacteria. Studies on actinobacteria allowed us to create a comparative set of lipid profiles of MALDI-TOF of *Cutibacterium*, specific finger-prints for strains of diagnostic value. Using an optimized method of vesicles isolation, it will be possible to obtain such nanostructures also from other taxons of *Cutibacterium* and *Propionibacterium*. Studies on phage proteins allowed us to elaborate a detector based on optical fibers to detect bacteria of *Escherichia coli* O157:H7 pathogenic strain. Bacteriophage multifunctional tail proteins express a structural role and also an enzymatic function directed to polysaccharide substrates, which is why investigations of biological, antibacterial functions, and also the mechanisms of activity of these proteins are important. In studies on *Clostridioides difficile* (CD), where an anaerobic pathogen involved in post-antibiotic acute diarrhea leading to severe infection and even to death, it was revealed that protein Cwp22 appeared as a good candidate for vaccine. Studies on advanced glycation end products formed in human tissues revealed in isolation from serum a protein bearing an epitope and allowed the structural characterization of this epitope with NMR and LC-MS/MS methods. Our laboratory was also involved in the development of a vaccine against SARS-CoV-2 virus responsible for Covid-19 disease. In order to find immune reactive viral molecules, a set of convalescent sera have been obtained and tested with viral proteins. Also, recombinant proteins have been produced in several expression systems. The innovative studies performed in our laboratory have applied importance, because these results serve to produce antigens for vaccines against *Clostridioides difficile* bacteria and SARS-CoV-2 virus. Studies on phage proteins and the application of immunoenzymatic assay for the determination of advanced glycation end products AGE are also of practical importance.

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

The COVID-19 pandemic developing rapidly in 2020 is triggered by the emergence of a new human virus SARS-CoV-2. The emergence of a new virus is not an unexpected phenomenon and has been predicted for many years. Since the virus has spread all over the world, it will be very difficult or even impossible to eradicate it. A necessary condition for complete or partial elimination of the virus is to have an effective vaccine. It is possible that SARS-CoV-2 will become milder in the next few years and COVID-19 will then only threaten individuals from risk groups. The review was published in *Archivum Immunologiae et Therapiae Experimentalis, 2020; 68: 35.*

Acute B-lymphoblastic leukemia (B-ALL) is the most common hematologic malignancy in children. Many cases of B-ALL harbor chromosomal translocations, which are often critical determinants of prognosis. Most of them represent altered transcription factors that impact gene transcription or enhance signaling. B-ALLs harboring the mixed-lineage leukemia 1 (*MLL1*) gene rearrangements represent an aggressive, high-risk type of early childhood leukemias that are usually associated with a very poor prognosis. Therefore, there is an urgent need for novel therapeutic agents as well as new treatment strategies.

The objective was to examine the vitro inhibitory effects of *Scutellaria baicalensis* root extract (SBE) in B-ALL cell lines with different chromosomal rearrangements and in leukemic blasts derived from patients' bone marrow (BMCs).

In this study we showed that baicalin, which is the main component of the SBE, possess antitumor activity against all leukemic cell lines, especially those with *MLL* and *PBX1* gene rearrangements. Baicalin inhibited cell proliferation arrested the cell cycle at the G0/G1 phase and induced cell death through caspase 3/7 activation. Moreover, baicalin treatment inhibited the glycogen synthase kinase-3β (GSK- 3β) by suppressing its phosphorylation at Y216, and upregulated the downstream mediator of the cell cycle arrest – cyclin-dependent kinase inhibitor $p27^{Kip1}$. Bone marrow derived blasts from B-ALL patients also exhibited varied sensitivity towards baicalin with 72% of patients sensitive to the SBE and baicalin treatment.

Taken together, our findings provide new insights into the anti-cancer properties of baicalin by showing its diverse mode of action, which might be related to the different genetic background. The results were published in *International Immunopharmacology, 2020; 79: 106114.*

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. Identifying genetic changes, modifying individual response to drugs and identifying potential prognostic and predictive molecular markers (response to treatment) is the key to improving the effectiveness of the treatment. This will allow for the selection of patients that require the introduction of a different therapeutic standard (individualization of treatment time, doses), thus 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 25 samples from patients with breast cancer was performed. The patients were divided into two groups: those responding well to treatment and those responding poorly to treatment (Paclitaxel or Doxorubicin). Using machine learning algorithms, we searched for the relationship between the expression of genes related to the metabolism of both therapeutics and the response to treatment. At the same time, cooperation was established with the Institute of Information Systems and the Computer Center of the University of Zielona Góra to use machine learning algorithms to develop an expression model of breast cancer cells.

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

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

Human blood group antigens: molecular biology, interactions with pathogens
Characteristics of erythroid cells lines JK-1 and BEL-A

The erythroid cell line JK-1 was obtained in 1990 from the cells of a chronic leukemia patient; it was shown that the JK-1 cells, when grown in the presence of erythropoietin, differentiate into immature, nucleated erythrocytes. The BEL-A cell line (Bristol Erythroid Line Adult) is an immortalized, erythroid cell line obtained in 2016 by transduction of human $CD34^+$ bone marrow cells with the gene E6/E7 from human papillomavirus (HPV). The BEL-A cells can be differentiated with erythropoietin to mature reticulocytes. Both cell lines can be genetically modified using standard laboratory procedures. In order to characterize the cells of both lines, we evaluated them using flow cytometry, Western blotting and thin-layer chromatography. We found that undifferentiated JK-1 cells express human glycophorin A antigens M and N, glycophorin C (GPC) and basigin. After treatment with PFI-1, the JK-1 cells differentiation inducer, the expression of the abovementioned blood group antigens did not change significantly.

The undifferentiated BEL-A cells revealed the expression of blood group antigens A, D, N, GPC and P1. After treatment with erythropoietin, the expression of blood group antigens N, GPC and P1 increased considerably. The binding of Shiga toxin subunit Stx1 to the cells also increased. The P1PK genotype of BEL-A cells was determined by the DNA sequencing as P^1P^2. Thin-layer chromatography revealed that the major glycosphingolipids produced by undifferentiated BEL-A cells are GlcCer, LacCer, Gb3 (P^k), Gb4 and P1, and the major glycosphingolipid recognized by Stx1B is Gb3.

We intend to use the JK-1 and BEL-A cell lines to obtain genetically modified cells that will allow us to study interactions between erythrocytes and merozoites of *Plasmodium falciparum*.

Laboratory of Microbial Immunochemistry and Vaccines
Head: Professor Jolanta Łukasiewicz, Ph.D.

Biochemical characteristics of macromolecules involved in immunological processes: Immunochemical studies of bacterial endotoxins

The expertise of the Laboratory of Microbial Immunochemistry covers a 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,* and *Plesiomonas shigelloides,* which 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 a "priority 1 critical pathogen" for health care by the WHO, CDC, and the UK Department of Health. *Bordetella* spp. Represent a group of species causing pertussis. Major virulence factors and surface antigens of these species are the following: lipopolysaccharide (LPS, endotoxin, O antigen), capsules (K antigen, i.e. capsular polysaccharide - CPS and exopolysaccharide - EPS), and fimbriae. LPS consist of lipid A, core oligosaccharide (OS), and O-specific polysaccharide (O-PS). LPS and CPS determine O serotype or K serotype, respectively. Specifically, O serotype is defined by O-PS region consisting of carbohydrate repeating units.

In 2020, we have continued studies of structural diversity of *Klebsiella pneumoniae* O antigens. O- and K-serotypes and encoded by O- or K-loci are promising targets for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics. To make such immunotherapy effective, knowledge about O/K-antigen structures, drift, and distribution among clinical isolates is needed. At present, the structural analysis of O-antigens is efficiently supported by bioinformatics. O- and K-loci-based genotyping by polymerase chain reaction (PCR) or whole genome sequencing WGS has been proposed as a diagnostic tool, including the Kaptive tool available in the public domain. We analysed discrepancies for O2 serotyping between Kaptive-based predictions (O2 variant 2 serotype) and the actual phenotype (O2 variant 1) for two *K. pneumoniae* clinical isolates. The identified length discrepancies from the reference O-locus resulted from insertion sequences (ISs) within *rfb* regions of the O-loci. In silico analysis of 8,130 O1 and O2 genomes available in public databases indicated a broader distribution of ISs in *rfb*s that may influence the actual O-antigen structure. Our results show that current high-throughput genotyping algorithms need to be further refined to consider the effects of ISs on the LPS O-serotype (Artyszuk, D. et al., Int J Mol Sci. 2020; 21(18):6572). Moreover, structures of the new O-specific polysaccharide and core oligosaccharide of *P. shigello*ides CNCTC 90/89 LPS (O22) were elucidated. The pentasaccharide repeating unit of the O-specific polysaccharide is built of one D-QuipNAc and is rich in four D-GalpNAcAN residues. Moreover, the new core oligosaccharide shares common features of other *P. shigelloides* endotoxins, i.e., the lack of phosphate groups and the presence of uronic acids (Maciejewska A et al. 2020, Int J Mol Sci, 21(18):6788). We have broadened the knowledge about LPS structures relevant for developing a vaccine against whooping cough and a pertussis-like illness. Whooping cough is a highly contagious disease caused predominantly by *Bordetella pertussis*, but it also comprises of a pertussis-like illness caused by *B. holmesii*. Since virulence factors of *B. holmesii* and their role in the pathogenesis remained unknown, the structures of the O-specific polysaccharide and the core oligosaccharide of the strain ATCC 51541 have been identified for the first time. The comparative analysis of the NMR spectra of *B. holmesii* core oligosaccharide fraction with this of the *B. pertussis* strain 606 indicated that the investigated core oligosaccharides were identical (Ucieklak K et al. Int. J. Mol. Sci. 2020, 21, 6433). Finally, continued research on enterobacterial common antigen (ECA, ECA-LPS) occurrence, structure and diversity led to the completion of data for *E. coli* R1, R2, R3 and R4 strains. The existence of LPS was hypothesized in the 1960-80s on the basis of serological observations. Only a few *Escherichia*

coli strains (i.e., R1, R2, R3, R4, and K-12) have led to the generation of anti-ECA antibodies upon immunization, excluding ECA_{PG} as an immunogen and conjecturing ECA_{LPS} as the only immunogenic form. Here, we presented a structural survey of ECA_{LPS} in *E. coli* R1, R2, R3, and R4 to correlate previous serological observations with the presence of ECA_{LPS}. The low yields of ECA_{LPS} were identified in the R1, R2, and R4 strains, where ECA occupied outer core residues of LPS that used to be substituted by O-specific polysaccharide in the case of smooth LPS. Previously published observations and hypotheses regarding the immunogenicity and biosynthesis of ECA_{LPS} were discussed and correlated with presented herein structural data (Maciejewska A. et al., Int J Mol Sci. 2020; 21(17):6038). In collaboration with the Department of Chemical Sciences of the University of Naples, we were involved in studies on pairing *Bacteroides vulgatus* LPS structure with its immunomodulatory effects on human cellular models (Di Lorenzo F et al., ACS Cent Sci. 2020;6(9):1602).