Hirszfeld Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Rudolfa Weigla 12, 53-114 Wrocław

RESEARCH REPORT 2019

Contents Page

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

The role of miR125b in the anti-tumor activity of calcitriol

The research aims to demonstrate the contribution of the miR125b molecule to the anti-tumor activity of calcitriol and its analog PRI-2191 against breast cancer cells. Changes were assessed in the expression of this molecule under the influence of calcitriol and its analog PRI-2191, as well as changes in the expression of target proteins for miR125b at the mRNA and protein level: vitamin D receptor (VDR) and proapoptotic Bak. We found that both calcitriol and PRI-2191 reduced miR-125b expression. In addition, calcitriol and PRI-2191 increased the proapoptotic level of BAK1 protein encoded by the miR-125b target gene. Based on the results obtained, we suggest that calcitriol and PRI-2191 can be used as miR-125b inhibitors in VDR-expressing breast cancer cells.

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

Based on previously obtained results on antiproliferative activity against J774E cells being an *in vitro* model in osteoclast studies, three bisphosphonic acids designated WG9732B, WG8983A and WG9864D were selected. For selected compounds, their ability to inhibit osteoclast differentiation from myeloid precursors ex vivo was pre-determined. Unlike the clinically used bisphosphonates, which belong to hydroxybisphosphonic acids, the tested compounds belong to the less-known group of aminomethylidene bisphosphonic acids. All compounds showed the ability to inhibit the differentiation of mature osteoclasts from BMDM at non-toxic concentrations, with WG9732B and WG8983A, which have an aromatic system in their structure, were significantly more active. In their case, inhibition of differentiation into mature, active osteoclasts was 69% and 56%, respectively.

Determination of the effect of murine dendritic cells of myeloid origin (BM-DC) genetically modified to produce the IL-15/IL-15R complex and stimulated with tumor antigens on activation and differentiation of T cells and ILC (congenital lymphoid cells)

In this reporting period, the focus was on BM-DC modification with lentiviral vectors carrying the interleukin 15 (IL-15) or IL-15 gene sequences in combination with the specific alpha subunit of the receptor for this cytokine (IL-15/IL-15Rα). The level of IL-15 or IL-15/IL-15Rα production was checked by previously introduced signal sequences responsible for transmembrane transport and by the Elisa test. Changes in the surface phenotype of the modified cells have also been characterized. A concentration-dependent toxic effect of IL-15 was observed correlated with high expression of BM-DC/IL-15 co-stimulatory molecules. The IL-15/IL-15Rα complex produced by these cells showed neither such significant toxicity nor high expression of the markers tested on the surface of BM-DC/IL-15/IL-15Rα transductants. The working conclusion highlights that dendritic cells modified to over-produce IL-15 alone become extremely mature as a result of their response to the cytokine and have a shorter lifetime. Meanwhile, BM-DC modified for the production of the complex

show its slower release, lower stimulation, and thus a lower response to the autocrine action of IL-15.

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

Currently, 5-fluorouracil, irinotecan (also known as CPT-11), and oxaliplatin constitute the backbone of chemotherapy for CRC. Because the currently approved therapies fail in a substantial number of CRC patients, new efficient drug combinations are constantly being sought. Emerging data indicate that 5-azanucleosides are able to sensitize cancer cells to the standard chemotherapeutic agents and contribute to overcoming intrinsic or acquired chemoresistance.

Previously, we have demonstrated that pretreatment with DNA demethylating agents, 5-aza-2'-deoxycytidine or 5-azacytidine, sensitizes CRC cells to topoisomerase inhibitors (irinotecan, etoposide, doxorubicin, mitoxantrone), reducing cell viability and clonogenicity and increasing apoptosis more effectively than individual compounds at the same or even higher concentrations.

In the current studies we tested if a similar combination of drugs could be effective against HCT116 cancer cells *in vivo*. The CPT-11 and decitabine were chosen as chemotherapeutics for these studies. The performed experiments led to the conclusions that: a) Decitabine alone was able to slow down tumor growth; b) prior treatment with decitabine improves further effectiveness of CPT-11 even for 30 days; c) early application of decitabine (to tumors smaller than 100 mm^2) allowed to decrease the CPT-11 dose, without detrimental effect on the treatment efficacy; d) the treatment scheme has low toxicity (no extensive weight loss and normal range of ASAT/ALAT ratio).

Simultaneously, we decided to find out if combinational treatment with decitabine and topoisomerase inhibitors would be effective against acute myeloid leukemias (AML). In this regard the efficacy of the previously used scheme was tested on the AML samples obtained from the Department of Hematology, Blood Neoplasm and Bone Marrow Transplantation in Wroclaw.

We did not observe a significant improvement in etoposide treatment as a result of pretreatment with decitabine. On the other hand, we found late and long-term impact of Decitabine on AML, similar to that observed in colon cancer model.

Laboratory of Biomedical Chemistry
Head: Professor Janusz Boratyński, Ph.D., Eng.

The use of icosahedral boron clusters as modifying entities for biologically active molecules

The conjugation of therapeutic peptides with albumin-binding molecules is an effective strategy to improve the half-lives of the peptides. In our studies, we synthesized conjugates of a peptide thymosin β4 (Tβ4) and a metallacarborane $[COSAN]^-$ using a solid-state thermal reaction, allowing us to attach $[COSAN]^-$ at different sites of amino acid sequence of Tβ4. As we have shown in our previous studies, $[COSAN]^-$ has high affinity to serum albumin. By obtaining a library of the conjugates with modifications at different sites, we could study the influence of the modification site on the affinity of the conjugates to HSA and their *in vitro* activity. Using fluorescence quenching of human serum albumin and surface plasmon resonance techniques, we showed that the conjugates have high affinities to human serum albumin. Furthermore, conjugation with $[COSAN]^-$ not only retains but also enhances the

activity of Tβ4. In comparison to unmodified Tβ4, the conjugates showed better prosurvival activity towards cardiomyocytes incubated in stressful conditions and better pro-migratory activity towards fibroblasts. This findings suggest that $[COSAN]^-$ is involved in the formation of interactions with molecular targets of Tβ4, thus leading to a more potent response.

In conclusion, our results show that conjugation with $[COSAN]^-$ has the potential to increase the therapeutic effect of Tβ4 by two mechanisms: first, by conferring on the peptide the ability to bind serum albumin and, potentially, prolonging its half-life; and second, by enhancing the biological activity of the peptide at the site of action. Thus, conjugation with metallacarboranes has a multidimensional influence on the peptide and can increase the activity of the peptide at both molecular and systemic levels. Therefore, metallacarboranes might become a new versatile tool suitable for modifications of therapeutically relevant peptides and proteins, such as interferons, tumor necrosis factors, glucagon-like peptide 1, or insulin, to improve their pharmacological properties.

Bacteriophages

The wide spectrum of bacteriophage applications makes them attractive nanoparticles for the food industry, nanotechnology or medicine. In this context, the stability and effectiveness of bacteriophage preparations is an important issue. Adequate stabilization not only ensures the preservation of antimicrobial activity at a constant level, but also affects the reproducibility of the obtained preparations. Despite the attempts to create new formulations, there is a serious problem with maintaining the lytic activity of bacterial viruses during long storage. Bacteriophages stored in the bacterial lysate maintain their biological activity relatively well; however, due to the presence of bacterial components, these preparations do not meet the standards set for medicines.

Pseudomonas aeruginosa shows a high rate of drug resistance to commonly used antibiotics and causes considerable problems in hospital wards. It is one of the most lethal causative bacteria and it is capable of acquiring resistance to multiple classes of antibiotics. Our research focuses on bacteriophage F8 that infects pathogenic *Pseudomonas* bacteria. We investigated the influence of storage conditions and the presence of selected hydrophobic substances on the bacteriophage's ability to infect bacteria. Using advanced electron microscopy techniques, we examined the effect of hydrophobic substances on the morphology of bacterial viruses.

The proposed research is part of the global trend of research on nanoparticles with particular emphasis on particles with antibacterial potential.

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

The receptor activator for nuclear factor κB (RANK) and its ligand (RANKL) are important signalling proteins involved in osteoclastogenesis and bone loss. It has been shown that patients with multiple myeloma (MM), a haematologic malignancy whose common symptoms are bone lesions and hypercalcaemia, are characterized by higher secretion of RANKL, corresponding with higher bone resorption. Two polymorphisms – rs1805034 located in the gene coding for RANK and rs7325635 located in the gene coding for the

RANK ligand (RANKL) – were analysed in MM patients and healthy individuals. No differences in genotype or allele frequencies were detected between these two groups. The rs1805034 *C* variant was found to be associated with longer overall survival, whereas the rs7325635 *GG* genotype was associated with longer progression-free survival. We also observed that rs1805034 *C*-carrying patients were characterized by slightly higher age at diagnosis. Calcium concentration (calcium being released to blood as a result of bone lesion) was higher in women with allele rs7325635 *G*; however, no such association was observed in in men. These results suggest that analysed genetic polymorphism may function as a marker of bone dysfunction and survival in multiple myeloma.

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 a heterogeneous disease characterized by the presence of uncontrollably proliferating haematopoietic progenitor cells. Telomerase reverse transcriptase (TERT), the telomerase catalytic subunit, is a key regulator of activity and expression of the telomerase holoenzyme. Its ability to maintain telomere length helps in ensuring genome integrity and stability. AML patients and healthy individuals were analysed in the context of *TERT* genetic variability (single nucleotide polymorphisms, SNPs). Two SNPs were analysed – one located in intron 2 of the *TERT* gene (rs2736100) and one in the promoter region (rs2853669). Genotyping results were compared with telomere length and clinical data (i.e. survival, age and sex, FAB subtype and presence of FLT3 and/or NPM1 mutations). Patients carrying the rs2853669 *CC* genotype had shorter survival than those with other genotypes. These *CC* homozygous patients also exhibited shorter telomere length than *TT* and *TC* patients. Patients with the FLT3-ITD mutation had shorter telomeres than patients without this mutation. When stratified according to genetic risk status, high risk FLT3-ITD+/NPM1- patients had shorter telomeres compared to medium risk FLT3-ITD+/ NPM1+ or FLT3-ITD-/NPM1- patients and low risk FLT3-ITD-/ NPM1+ patients. Summarizing, the polymorphism of the *TERT* promoter gene (rs2853669) affects the survival of AML patients and telomere lengths, which vary depending on the presence of mutation status.

Laboratory of Immunogenetics and Tissue Immunology
Head: Professor Piotr Kuśnierczyk, Ph.D.

In 2019, our studies were mostly concentrated on the role of missense single nucleotide polymorphisms of endoplasmic reticulum aminopepidases in several human diseases.

Atopic dermatitis

Atopic dermatitis (AD) is a common inflammatory skin disease of complex aetiology, with interactions between susceptibility genes and environmental factors. We have previously described a protective effect of the *KIR2DS1* gene encoding the natural killer cell receptor, whose ligands are HLA-C molecules. Here, we found an association of *HLA-C*05:01* allele with AD. KIR-HLA-C interactions are affected by peptides presented by HLA-C. The generation of these peptides is strongly influenced by endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and ERAP2). Expression and activity of ERAP molecules depend on the polymorphisms of their genes.

Possible associations of several single nucleotide polymorphisms (SNPs) in the *ERAP1* and *ERAP2* genes with susceptibility to AD were tested. Only one SNP in the *ERAP1* gene, rs26618T>C, causing the amino acid change Ile276Met, had an association with AD. To gain

insight into the functional role of this SNP, we produced recombinant variants differing only at position 276 (Ile or Met) and tested their aminopeptidase activity against a N-terminally extended precursor LIVDRPVTLV of the HLA-C*05:01 epitope IVDRPVTLV. Both ERAP1 variants were able to efficiently generate the epitope, although the 276Ile allotype was able to do this about 50% faster. Furthermore, both variants were quite inefficient in the further degradation of the mature epitope. Finally, we found that the effect of 276Met on susceptibility to AD was seen only in *KIR2DS1*-negative individuals, not protected by this KIR.

In conclusion, associations were found between *HLA-C*05:01* allele and rs26618T>C (Ile276Met) *ERAP1* polymorphism with AD, as well as a significant difference between these two ERAP1 variants in their ability to generate an epitope for the HLA-C*05:01 molecule.

Recurrent spontaneous abortion

Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 trim peptides to generate stable antigenic epitopes for their presentation by HLA class I (HLA-I) molecules to T cell receptor. By influencing the peptide repertoire of HLA-I molecules, they affect also the interactions of HLA-I with killer cell immunoglobulin-like receptors (KIRs) of natural killer (NK) cells. HLA-C is the only polymorphic HLA-I molecule present on the trophoblast.

In this study we investigated the role of *ERAP1* and *ERAP2* polymorphisms in the context of *KIR* and *HLA-C* genes in women suffering from recurrent spontaneous abortion (RSA) in the Polish population. We tested 285 women who experienced recurrent spontaneous abortion (RSA) and 319 fertile women.

We observed a significant association of *ERAP1* rs30187TT genotype with RSA (p = 0.02, OR = 1.89, 95%CI = 1.11–3.21), however the most striking association was found in comparison of patients and controls with *ERAP1* rs30187TT and *KIR* Bx genotypes (p = 0.006, pcorr = 0.036, OR = 2.40, 95%CI = 1.27–4.52). Moreover, this effect was even stronger in *HLA-C2*-positive patients (p = 0.0031, pcorr = 0.019, OR = 3.46, 95%CI = 1.48–8.11). Other weaker associations of the remaining tested ERAP single nucleotide polymorphisms with RSA were also presented.

In conclusion, *ERAP1* rs30187TT genotype itself increased susceptibility to RSA but this effect was much stronger in patients positive for *HLA-C2* and *KIR* Bx genotypes.

Ankylosing spondylitis

The objective of this case-control study was to evaluate the role of four single-nucleotide polymorphisms in the *ERAP1* (rs2287987, rs30187, rs27044) and *ERAP2* (rs2248374) genes and their haplotypes in predicting the risk for ankylosing spondylitis (AS) on a well-defined Polish population. Our study confirmed the strong association between the *HLA-B*27* allele and the disease. For all tested *ERAP1* SNPs, we found significant differences in the minor allele and genotype distribution between patients and controls. The strongest association with AS was observed for rs30187. The minor T allele and homozygous TT genotype of this SNP significantly increased disease risk (OR=1.56, 95%CI = 1.22–1.99, p = 0.0004 and OR = 2.52, 95%CI = 1.50–4.25, p = 0.001, respectively). In the case of rs2287987, minor C allele exerted a protective effect (OR = 0.64, 95%CI = 0.46–0.88, p = 0.008). In contrast to *ERAP1*, we observed no effect of rs2248374 in *ERAP2* on the disease. We also carried out *ERAP1-ERAP2* haplotype analysis to demonstrate a possible association of both genes with AS. Results showed that the haplotype H4, containing *ERAP1* SNPs associated with high enzymatic activity, together with the presence of ERAP2 expression, significantly increased the risk of AS (OR = 1.97, 95% CI = 1.21–3.21, pcorr = 0.048). By contrast, the haplotype H5

coding for low activity of ERAP1 and the lack of ERAP2 expression was strongly protective (OR = 0.41, 95% CI = 0.23–0.72, pcorr = 0.008). To our knowledge, this is the first study describing the effects of *ERAP1-ERAP2* haplotypes implicated in AS susceptibility in case-control study design.

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

In a number of studies we documented the presence of associations between gene polymorphism and the clinical effect, suggesting that a given polymorphic pattern makes a gene more or less prone to activation with the IFNgamma gene as the best example.. For several years we have been focussing on polymorphisms, which involve larger parts of the genome ranging from 5000 bp up to more than 10 Mbp fragments. These fragments may be duplicated or simply deleted. Working with leukemic patients, we and others are professionally involved and obliged to delineate the genetic background to the disease. The gold standard since 1960s is chromosome binding assay. In spite of the substantial progress in lab technology, we are still facing problems, including timing. The optimal timing of the assay must be done with fresh cells and after completion the interpretation of the origin of small fragments. Comparison made between CBA and CNV is as follow:

Among 188 parallel determinations using CNV (only for fragments > 5 Mbp) and CBA in 98 attempts, the results were fully comparable. In addition, in 55 situations the deletions or amplifications seen in the CNV assay was not found in the CBA testing and in 35 instances, because the FISH probes are not suited for detecting these particular CNV. Fourteen abnormalities seen in the CBA testing are not seen in the CNV. Major clinically relevant abnormalities were seen, except 9 abnormalities in both tests.

Analysis of smaller polymorphic fragments (below the level of microscopic resolution) found that:

• CNV deletions/amplifications which may have a polymorphic characteristic (only the patients with normal karyotype were considered) if in an excess favoured leukemic patients survival (50 vs 16%, p = 0.008),

• patients who have t(8:21) (RUNX1/RUNXT1, 67 vs 20%, p = 0.004) enjoy better overall survival. In addition, 67% of patients with t(8:21) had an aberrant expression of CD19 (>22% in the blasts) compared to 5% incidence in patients without translocation (p < 0.001). By contrast, CNV amplifications in the KMT2A gene (9 cases), independently if seen in the FISH assay (3 cases) or not, inversely affected the survival (0 vs 43%, p = 0.032, at the 18 month time point).

Regenerative medicine

We are continuing (with 2003 as a starting time point) our pioneer work in the field of regenerative medicine. In 2019 we performed 56 regenerative medicine attempts in patients suffering from advanced osteoarthritis involving the hip and knee. It is a small surgery which is composed of the following steps: 40 mLs of the marrow is taken from both posterior iliac crests, which is then centrifuged in the gradient of own plasma to deplete the cell suspension with erythrocytes and to decrease the proportions of granulocytes. We increased the proportion of CD45-CD34-, CD45-CD34+ and CD34+, and, also of note, of CD90+ cells (the cells of endothelial progenitor and mesenchymal progenitor cells progeny), (ii) the cells in a volume of 4 – 6 mLs were injected with USG guidance into the affected hip or the knee, (iii) the effect was recorded employing the standard orthopaedic procedures, which showed that 89% of patients reported substantial pain relief and improvement in free movement, including

stairs. The results brought new information about the regenerative potential of identified subpopulation of the marrow cells.

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

1. We continued our studies on the association between different routes of phage administration and anti-phage antibody production in patients receiving phage therapy. In 13 patients receiving intravesical phage therapy low levels of such antibodies were detectable. Those findings suggest that intravesical phage administration does not induce significant antibody responses.In contrast, other topical phage administration (eg wounds, fistulas) frequently results in high antibody levels.

2. One of the current dilammas in the progress of phage therapy is the concomittant use of phages and antibiotics. There are data in the literature suggesting that antibiotics combined with phages both *in vitro* and *in vivo* can have an enhanced anti-bacterial action. However, our *in vitro* studies have revealed a rather complex nature of phage:antibiotics interactions. It appears that the final effect (synergism or antagnonism) is dependent on phage and antibiotic titers. More studies are necessary to characterize those associations and to determine the clinical usefulness of phage:antibiotic combination in therapy.

3. Studies were continued on the immunogenicity of structural proteins of A3R and 676Z phages. The highest antibody responses were elicited by Mcp main head protein and TmpH main tail protein.

4. Further attempts have been made to expand the anti-bacterial spectrum of our phage bank by procuring new phages. Indeed, 15 new phages directed against *Klebsiella pneumoniae* ESBL+ and ESBL- were procured. Moreover, plasmid-prophage-free *Enterococcus* phage preparations were obtained, which should be safer in clinical applications. Our efforts to procure more *Acinobacter* phages were only partly successful. This is an important challenge, as this bacterium is now considered as one of the most dangerous pathogens.

5. Our studies completed so far strongly suggest that, in addition to their well-known antibacterial action, phages also have various immunomodulating functions. What is more, phages may exert phage-specific diverse effects on the immune system, a phenomenon with obvious potential application in clinical immunomodulation. Our relevant results and hypotheses have been published in international journals.

LABORATORY OF BIOLOGY OF STEM AND NEOPLASTIC CELLS
Head: 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)

The human immortalized cell line of adipose tissue-derived mesenchymal stem cells (MSCs): HATMSC1 and HATMSC2, and bone marrow-origin HBMMSC1, were established in our laboratory using the hTERT and pSV402 plasmids. Studies of the immortalized cell lines confirmed the expression of the basic MSCs markers: CD73+, CD90+, CD105+, and the presence of the stemness markers SSEA1, SSEA4 and Oct3/4, which are considered as markers of pluripotent MSCs. To confirm biological stability of immortalized human MSCs lines (HATMSC1, HATMSC2 and HBMMSC1), cells at a dose of $10x10^6$ per mouse, were administered subcutaneously to NOD/SCID strain mice, serving as an *in vivo* model in xenograft transplants. None of the applied cell lines were able to give tumor growth and none of them revealed tumorigenic properties *in vivo*. Histopathological assessment of tissues taken from the site of cell application, i.e., skin and subcutaneous tissue sections, as well as tissues being a potential metastatic target, i.e., liver, lung, kidney, did not show the presence of tumor cells.

This study demonstrated that after MSCs immortalization with the selected vectors, the MSCs lines do not undergo tumorigenic transformation and immortalized cell lines are stable in keeping basic MSCs phenotype.

Biological properties of microvesicles from human immortalized cell lines of endothelial progenitor cells and mesenchymal stem/stromal cells of adipose tissue origin

Endothelial progenitor cells (EPCs) and mesenchymal stem/stromal cells (MSCs) are associated with maintaining tissue homeostasis and tissue repair. Both types of cells contribute to tissue regeneration through the secretion of trophic factors (alone or in the form of microvesicles). This study focused on the isolation and biological properties of microvesicles (MVs) derived from human immortalized MSCs cell line HATMSC1 of adipose tissue origin and EPCs line. The human immortalized cell line HATMSC1 was established in our laboratory using the hTERT and pSV402 plasmids, whereas EPCs line originating from cord blood (HEPC-CB.1) was established in our previous studies. Microvesicles were isolated through a sequence of centrifugations. The content of isolated MVs was analyzed for the presence of cytokines, trophic factors, and microRNA. The EPC-origin MVs showed the expression of 19 out of 43 examined cytokines and trophic factors, whereas the HATMSC1 microvesicles showed the expression of 12 cytokines and trophic factors. Analysis of the protein content of both populations of MVs, using the Membrane-Based Antibody Array, revealed that MVs transported growth factors (e.g., EGF, bFGF) and pro- and anti-angiogenic factors (e.g., IL-8, VEGF, TIMP-1, and TIMP-2). Additionally, EPC-derived microvesicles contained cytokines and molecules that regulate angiogenesis (e.g., GRO, IGF-I, MCP-1, MMP-1, and VEGF-D).

Examination of the miRNA content of isolated MVs revealed the presence of proangiogenic miRNA, i.e., miR-126, miR-296, miR-378, and miR-210, using real-time RT-PCR with the TaqMan technique. The isolated MVs were assessed for their effect on the proliferation and proangiogenic properties of cells involved in tissue repair. It was shown that both EPC- and HATMSC1-derived MVs increased the proliferation of human endothelial cells of dermal origin, and this effect was dose-dependent. In contrast, MVs had a limited impact on the proliferation of fibroblasts and keratinocytes. Both types of MVs improved the proangiogenic properties of human dermal endothelial cells, and this effect was also dose-dependent, as shown in the Matrigel assay.

These results confirm that MVs originated from both cell lines, HEPC-CB.1 and HATMSC1, carry proteins and miRNAs that support and facilitate angiogenic processes, which are important for cutaneous tissue regeneration and may be used as a potential cell-free proangiogenic therapy in ischemic tissues.

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

Mid-upper arm circumference and body mass index as different screening tools of nutritional and weight status in Polish schoolchildren across socio-political changes

Intergenerational changes in many biological traits are indicators of environmental conditions. One of such anthropometric measures is the mid-upper arm circumference (MUAC), which estimates nutritional status. Likewise, Body Mass Index (BMI) is widely used as an anthropometric indicator of relative weight. The aim of this study was to reveal secular trends in MUAC and BMI, as biological indicators of changing living conditions, between 1966 and 2012 among Polish children from different socioeconomic groups. The total sample involved 64,393 schoolchildren aged 7–18 years, investigated in four surveys (1966, 1978, 1988, 2012). The overall socioeconomic status (SES) was divided into two categories: lower and higher (including: urbanization, family size, parental education). The results showed that MUAC and BMI differed significantly with respect to the year of the survey, sex and SES category. Both measures were higher within higher SES group compared to the lower one until 1988, while in 2012 these indicators in both SES categories were observed to have converged. Both the year of survey, sex, SES category and interactions between them had a higher impact on MUAC than BMI (measured by effect size). Our findings revealed that long-term socioeconomic changes affect MUAC more noticeably than BMI. Therefore, MUAC may be a more accurate screening tool.

Sex-dependent effect of post-migration adaptation on height and relative lower leg length in Polish youth

Growth in tibia length is considered to be particularly sensitive to environmental stress. Our aim was to estimate the effect of parental migration status on the relative length of the tibia in their school-age children. Data included a nationwide random sample of 17,155 schoolchildren, 7–18 years of age, examined between 1966 and 1969 in Poland with information on anthropometric measurements and demographic and social characteristics. Parental migration status was based on paternal migration history. After standardisation by LMS method, z-scores of relative tibia length and z-scores of height were used for the analysis. Three-way ANOVA was used to evaluate the influence of migration on tibia length-to-height ratio. Sons of migrants have a significantly higher tibia length-to-height ratio compared to sons of non-migrants. Children of non-migrants were taller than children of migrants among boys in medium SES and among girls in high and low SES. Relative tibia length indicated significant effects of migration among boys in all age categories and in late adolescent girls: sons of migrants had a higher ratio and daughters of migrants had a lower tibia length-to-height ratio. It is possible that migration experiences of the parents may have influenced the growth of their offspring. The results emphasise the potential importance of research addressing the impact of different types of migration on growth of children.

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 replication initiation in bacterial chromosomes 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*). We have recently focused on a detailed analysis of domain I of *H. pylori* DnaA, especially in the context of proper DnaA assembly on bipartite *H. pylori oriC.* We have shown that domain I is responsible for the organisation and stabilisation of the initiation complexes (orisme) and provides cooperativity in DnaA–DNA interactions. Domain I is also important for long-distance DnaA interactions and is essential for orisome assembly on bipartite origins. HobA, which interacts with domain I, increases the DnaA binding to bipartite *oriC*; however, it does not stimulate but rather inhibits DNA unwinding. This suggests that HobA helps DnaA to bind *oriC*, but an unknown factor triggers DNA unwinding. Together, our results indicate that domain I self-interaction is important for the DnaA assembly on bipartite *H. pylori oriC.*

Bacterial response to stress

We are interested in bacterial factors regulating stress response in bacteria belonging to selected species of Campylobacterota. Together with our research partners, we have recently focused on HtrA – a periplasmic serine protease and chaperone that helps bacteria respond to many stress factors; in *H. pylori* and *C. jejuni* HtrA is also an important virulence factor. The unique htrA *H. pylori* deletion mutant created in our laboratory enabled a more detailed characterisation of the role of HtrA in *H. pylori* stress-response and infection. Our results also identified a potential functional relationship between HtrA and the Sec translocon in *H. pylori*, possibly indicating a more general mechanism helping bacteria maintain periplasmic homeostasis.

Secondary metabolism in *Streptomyces*

Streptomyces are gram-positive, filamentous bacteria that are potent producers of secondary metabolites—specialized compounds with adaptive functions, many of which have antibiotic or immunosuppressant activities. We have recently described a zinc-binding regulatory protein HypR from *Streptomyces coelicolor* A3(2). Genes belonging to the regulon of HypR code for enzymes putatively involved in collagen degradation and utilization of L-hydroxyproline (L-Hyp) as concluded from the predicted structure and conserved domains. Their transcription is induced in the wild type strain by the addition of L-Hyp to the culture medium, while knockout of one of the genes from the predicted L-Hyp utilization operon abolished the ability of the strain to grow on L-Hyp as a sole source of carbon. To our knowledge, this work is the first to indicate the existence of the pathway of L-hydroxyproline catabolism in Streptomycetes. We are also continuing an investigation of the regulation of coelimycin (Cpk) biosynthesis by *S. coelicolor* A3(2). We have published a detailed review of the regulatory network governing the expression of *cpk* gene cluster by both cluster situated and "upper level" pleiotropic regulators. Our recently obtained proteomic data indicate the involvement of two SARP proteins encoded within *cpk* gene cluster in the regulation of not only Cpk biosynthesis but also other metabolic processes.

Laboratory of Signaling Proteins/Laboratory of Microbiome Immunobiology
Acting Head: Professor Jakub Siednienko, Ph.D./Professor Sabina Górska, Ph.D

Comparison of immunoregulatory activity of yolkine preparations obtained by SEC chromatography and ethanol extraction on the mouse macrophages BMDM line

The yolkin polypeptide complex isolated from egg yolk by SEC chromatography shows the ability to regulate innate response mechanisms. In 2016, a new, simpler method of isolating the yolkin complex was developed - an ethanol extraction method that can be used on a larger scale (Patent No. 417838). The goal of the research conducted in 2019 as part of statutory activity of the Laboratory was to check whether the yolkin polypeptide complex isolated by ethanol extraction (Yet) is capable of activating the innate response (effect on macrophage proliferation, induction of M1 phenotype, induction of cytokines and NO) compared to the yolkin obtained by complex by SEC (Ysec) chromatography.

The results of the conducted research show that there was no toxic effect of the Ysec and Yet yolkine preparations on the macrophages. The effect of Ysec and Yet on BMDM cell proliferation showed a dose-dependent inhibition of this process. In addition, significant changes were observed in the morphology of macrophages treated by Ysec and Yet after 24h and 48h incubation (spindle shape, increase in the number of cytoplasmic projections, typical changes for the M1 phenotype). In addition, both yolkine preparations have been shown to stimulate BMDM line to secrete significant amounts of TNF-α, while only Ysec stimulates these cells to produce type I interferons, anti-inflammatory IL-10 and nitric oxide, and this effect was dependent on the dose.

The conducted research allowed us to compare the ability of Ysec and Yet preparations to activate innate response in macrophage cells, in terms of their potential use as a nutraceutical supporting the treatment of innate immune deficiencies.

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

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

What difference makes a difference? – in search of the criteria of 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 a nonviable diploid male. The variety of *csd* alleles is associated with the strong polymorphism occurring in the hypervariable region of the gene (due mainly to the presence of insertions/deletions); however, it is not yet a clear big enough difference in the allele sequence is sufficient to trigger the process of female development. It is suggested that the difference in hypervariable region length must be more than 6 nucleotides. It is also known that a smaller difference in the sequence of the *csd* allele pair can lead to female development, but not with 100% probability.

The research conducted so far to determine the criteria of functional heterozygosity consisted solely of the identification of the sequence of *csd* allele pairs found in female genomes, and thus the analysis of functional connections. Given the huge variety of *csd* alleles and the limited number of pairs analyzed, we believe that this approach does not exhaust the problem posed. In our research, we focused on attempting to identify pairs of alleles that are underrepresented in the analyzed bee families, and thus on trying to identify non-functional allele pairs. For this purpose, we determined the genotype of several hundred workers from each of the three families analyzed. Genotyping was carried out in two stages: first by T-RFLP (terminal restriction fragment length polymorphism) we assigned workers to groups depending on the paternal *csd* allele present in their genomes, and then the *csd* alleles present in representatives of each group were cloned and sequenced. As a result of these studies, we were able to identify three cases in which there was a statistically significant disproportion in the frequency of connection between a given paternal allele and maternal alleles. Preliminary sequence analysis of underrepresented allele pairs shows that differences in *csd* allele length are not critical for functional heterozygosity.

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

Development of ELISA assay for assessing levels of soluble dog leukocyte antigen DR (DLA-DR) in blood serum of dogs suffering from B-cell lymphoma

We report on the development of a novel enzyme-linked immunosorbent assay (ELISA) for the detection of circulating soluble DLA-DR (sDLA-DR) complexes in the blood serum of dogs. Physiologically, soluble circulating MHC II molecules (sMHC II) loaded with self-peptides contribute to the maintenance of self-tolerance. They can be released from antigen-presenting cells or tumor B cells as well and suppress T cell immune-surveillance by directly competing with membrane-bound MHC II ligands. We aimed at testing the hypothesis that blood serum levels of sDLA-DR could be indicative of tumor burden in dogs suffering from canine B-cell lymphoma (CBL). To determine immunoassay performance, we analyzed sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using receiver operating characteristic (ROC) analysis; two separate sets of data were analyzed. First, we sought to determine whether elevated serum sDLA-DR levels could be predictive of CBL. The results suggest that this parameter had a strong positive predictive value of 92%, but at the same time it had a relatively low negative predictive value of 56%, and the area under the curve was 0.835. Another set of data was evaluated to see whether the decrease in sDLA-DR level could be used as a biomarker for successful response to chemotherapy. The PPV and NPV parameters and the AUC value equal to 1 indicated that this test could reliably predict the response to chemotherapy. Our observations support sDLA-DR as a potentially useful biomarker for monitoring the outcome of CBL chemotherapy. Overall, our data indicate the potential therapeutic and diagnostic value of anti-DLA-DR-specific antibodies in CBL.

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

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

Effects of lactoferrin on cytotoxicity of natural killer cells

The studies on effects of recombinant lactoferrin from hamster ovary cells (CHOLF) and lactoferrin-Fc immunoglobulin fragment complex (LF-Fc) on natural killer cell activity, were continued. Determination of MAP kinase expression in NK-92 natural killer cells revealed strong increases in expression of ERK1, p38β, p38δ and JNK by CHOLF. These increases were weaker in the case of LF-Fc except that of ERK1.

In addition, we applied a model involving natural killer cells isolated from human blood by means of commercial Miltenuj Biotec kit. CHOLF demonstrated a higher cytotoxic activity towards K-562 cells in comparison to interleukin 2 control. These effects varied among blood donors and LF-Fc was less effective. Nevertheless, LF-Fc strongly stimulated the production of TNFα by human blood mononuclear cells and CHOLF did not. In further studies we will determine identity of cytotoxic factors derived from cultures of monocytes from human blood by LF-Fc and their effects on tumor cell lines.

Studies on activity and mode of action of oxazolone derivatives

The aim of the study was to evaluate immunotropic activities of a series of ten SCM oxazolone derivatives, oxazolo [5,4-*d*] pyridines. Based on their capability to suppress phytohemagglutinin A-induced proliferation of human blood lymphocytes and low cytotoxicity against A-549 cell line, we selected two compounds (SCM5 and SCM9) for further studies. Both compounds inhibited lipopolysaccharide (LPS)-induced proliferation of mouse splenocytes, but only SCM9 moderately suppressed the production of TNF α in LPS-treated human whole blood culture. The compounds inhibited also the growth of several tumor cell lines and replication of HSV-1 virus in A-549 cells.

Molecular studies showed that the compounds elicited differential changes in expression of signaling molecules in Jurkat and WEHI-231 cell lines. SCM5 strongly increased expression of caspases 8 and 9, NF-κB, Bcl 2 and Fas, which suggested a proapoptotic action of the compound towards WEHI 231 cell line. In summary, we described immunosuppressive activity of low toxic oxazolone derivatives, which may find application in therapy of immune disturbances, such as inflammation or autoimmunity, as well as in viral infections.

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

Aberrant expression on mRNA and protein level of co-inhibitory molecules: CTLA-4 and BTLA in chronic lymphocytic leukemia patients

Cell chronic lymphocytic leukemia (CLL) is characterized by the gradual accumulation of mature B cells. Moreover, the imbalance of T-cell subsets, defective immune synapse formation with antigen presenting cells, impaired cytotoxic effector function and high frequency of regulatory T cells (Tregs) is observed in CLL.
The aim of our study was to evaluate the expression of BTLA in CD3 and CD19 subpopulation in CLL patients in relation to another co-inhibitory molecule CTLA-4.
The mRNA of CTLA-4 and BTLA expression level as well as surface BTLA protein expression in T cell and B cells were determined in 20 CLL patients and 17 controls. Moreover, the CTLA-4 surface and intracellular expression on BTLA+ CD19 and BTLA+ CD3 cells was examined by flow cytometry.

Significantly higher BTLA mRNA expression was observed in CLL patients in T and B lymphocytes as compared to controls either in unstimulated and PMA stimulated cells.

However, after PMA stimulation the mRNA BTLA expression decreased significantly in both groups.

In parallel, we observed significantly lower surface BTLA expression in CLL patients in comparison to controls in resting CD19 and CD3 cells. Similarly to mRNA expression after stimulation, the significant decreases of BTLA measured either as proportion of cells or mean fluorescent intensity (MFI) were found.

Moreover, in CLL patients we observed a higher proportion and MFI of surface and intracellular CTLA4+BTLA+ in T cells and a lower proportion and MFI of cells with CTLA4+BTLA+ phenotype within B cells.

Our results indicated impaired expression of BTLA on B cells together with insufficient levels of CTLA-4 on CLL B cells, which might be associated with lowering threshold for B cell activation and proliferation. On the other hand, up-regulation of the expression of CTLA-4 molecule (both surface and cytoplasmic) was found in CLL peripheral BTLA+ T cells, which may reflect a suppression of T cell effector functions, including anti-tumor and anti-inflammatory activities.

$CD4^+CD28^{null}$ T cells are expanded in moderate-active systemic lupus erythematosus and secrete pro-inflammatory IFN-γ depending on the disease activity index

The pathogenic $CD4^+CD28^{null}$ cells are characterized by inflammatory cytokine synthesis and tropism to the inflamed tissues. Recent studies showed the involvement of $CD28^{null}$ T cells in a severe clinical outcome of lupus. However, their role in a moderately active disease is still unresolved.

We examined the levels of circulating $CD4^+CD28^{null}$ cells and $CD8^+CD28^{null}$ suppressor T cells. We also compared the $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T cell functional properties, including the expression of IFN-γ and Ki67 among SLE patients (n=20) and healthy controls (n=20). All the patients were under immunosuppressive treatment and exhibited moderate activity of SLE (median SLE Disease Activity Index (SLEDAI) = 6).

In patients, we found elevated $CD4^+CD28^{null}$ and unchanged levels of suppressor $CD8^+CD28^{null}$ T cells. There was no difference between patients and controls in IFN-γ and Ki67-expressing $CD4^+$, $CD4^+CD28^+$, and $CD4^+CD28^{null}$ T cells, except for higher IFN-γ levels in $CD4^+CD28^+$ T cells in SLE. In each studied group, we observed higher preponderance of IFN-γ- and Ki67-expressing cells among $CD4^+CD28^{null}$ T cells and lower level of IFN-γ in $CD4^+CD28^{null}$ T cells compared to CD28+ subset; similarly, Ki67 intensity was decreased in healthy $CD4^+CD28^{null}$ cells, whereas in patients, comparably high expression was observed in both subsets. IFN-γ intensity in $CD4^+CD28^{null}$ T cells correlated with SLEDAI.

In conclusion, SLE with moderately active clinical course is characterized by PB expansion of $CD4^+CD28^{null}$ T cells and normal abundance of suppressor $CD8^+CD28^{null}$ T cells. The observation that these pathogenic CD4+ T cells, despite the lack of CD28 molecule, maintain the ability to produce pro-inflammatory IFN-γ, positively correlating with disease activity as well as relatively high proliferative capacity, may suggest their potentially predictive role in SLE flares.

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

New research task

Besides the production of antibodies B lymphocytes play other functions as antigen presenting cells and cytokine producers and regulators of immune response.

B-lymphocyte populations that mediate cellular immune suppression are known as B regulatory cells (Bregs). The population of these cells are potent producers of not only IL-10 but also IL-35 which suppresses immune response and alleviate the course autoimmune diseases in animal models.

On the other hand published data indicate that IL-35 is involved in the pathogenesis of endometriosis by suppressing immunoreaction and promoting endometrial cell proliferation. Women with endometriosis showed higher levels of IL-35 in peripheral blood and peritoneal fluid compared to healthy women. The aim of the study was to determine the expression of IL-35 in the following subpopulations of Breg cells: B10 (CD19 + $CD24^{high}$CD27 +), immature B lymphocytes ($CD19^{+}$ $CD24^{high}CD38^{high}$) and plasmoblasts ($CD27^{int}CD38^{high}$) in women with endometriosis and in healthy women (control group).Preliminary analysis showed a higher percentage of IL-35 producing B10 cells ($CD19^{+}$ $CD24^{high}CD27^{+}$ $IL\text{-}35^{+}$) and immature IL-35 expressing B cells ($CD19^{+}$ $CD24^{high}CD38^{high}IL\text{-}35^{+}$) in women with endometriosis. Research continues to obtain the appropriate number of patients in the groups.

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 phage proteins and bacterial glycoconjugates in immune processes. Structural studies of the probiotics surface glycoconjugates, their role in immune processes for therapeutic applications

Studies performed in our Laboratory concern the mechanisms of pathogenicity of diseases with bacterial etiology, the role of bacterial glycoconjugates and proteins in the immune processes, and the structure and functions of bacterial exopolysaccharides, including these from probiotics. Regarding the structural studies of bacterial antigens, a phage protein has been identified and its activity characterized against two strains of multidrug-resistant *Pseudomonas aeruginosa*. The experiments have revealed that bacterial receptors for phages specific for these few clinical *P. aeruginosa* isolates are not polysaccharides but proteins, which also have been characterized. This work has a value for clinical diagnostics. Concerning studies on *Neisseria meningitidis* the protein conjugates have been obtained with oligosaccharide antigens, where protein carrier could be substituted by oligosaccharide antigen with its reducing or non-reducing end. Non-reducing end galactose was oxidized with galactose oxidase to create aldehyde group. Conjugates retained their native epitopes, while devoid of a potential autoantibodies inducing, what makes them safe and potent immunogens for protective vaccine against *Neisseria meningitidis*. In the frame of studies on peptide epitope in OmpC protein of bacteria of *Shigella* genus recognized by umbilical cord blood antibodies, an affinity column has been prepared with immobilized antigens containing epitope present in OmpC protein, which allows us to isolate a specific protective antibody from human sera of blood donors. Such an affinity column serves as a tool to produce from the human blood an intravenous immunoglobulin preparation of protective antibacterial antibodies. Experiments on the determination of the structure and biological activity of

glycolipids from probiotics species allowed us to isolate and purify glycolipids from *Lactobacillus rhamnosus* LOCK 0919. Using cell lines HEK TLR2, TLR4, NOD2, the receptors involved in the recognition of glycolipid were identified and it was found that TLR2 receptor is involved in glycolipid recognition. Furthermore, polysaccharide antigens from *Lactobacillus* were isolated and purified for studies on Tregitopes. These results indicate further directions for studies on properties and biological activities of probiotics glycolipids. Results have a crucial value for understanding the activities of probiotics and an innovative potential for application, especially as therapeutics.

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

Despite the availability of several anti-herpesviral agents, it should be emphasized that the search for new inhibitors is highly encouraged due to the increasing resistant viral strains as well as complications linked with periods of recurring viral replication and reactivation of latent herpes infection. Extract of *Ginkgo biloba* (EGb) is a common phytotherapeutic product around the world with health benefits. Limited studies, however, have addressed the potential antiviral activities of EGb, including herpesviruses such as *Human alphaherpesvirus 1* (HHV-1) and *Human alphaherpesvirus 2* (HHV-2). We evaluated the antiviral activity of EGb and its phytochemical constituents: flavonoids and terpenes against HHV-1 and HHV-2. Pretreatment of the herpesviruses with EGb prior to infection of cells produced remarkable anti-HHV-1 and anti-HHV-2 activity. The extract affected the viruses before adsorption to cell surface at non-cytotoxic concentrations. In this work, through a comprehensive anti-HHV-1 and anti-HHV-2 activity study, it was revealed that flavonoids, especially isorhamnetin, are responsible for the antiviral activity of EGb. Such activity was absent in quercetin and kaempferol. However, EGb showed the most potent antiviral potency compared to isorhamnetin. EGb could augment current therapies for herpes labialis and genital herpes. Moreover, the potential use of EGb in multidrug therapy with synthetic anti-herpes compounds might be considered. The results were published in *Frontiers in Microbiology, 2019; 10: 2367.*

The lack of effective treatment for Alzheimer's disease (AD) stems mainly from the incomplete understanding of AD causes. Neuroinflammation has emerged as an important component of AD pathology, and a vast number of experimental and clinical data have indicated its crucial role in the activation of the innate immune system in disease promotion and symptom progression. Clinical examinations of AD patients in a different stage of disease severity in correlation with the measurement of two innate immune reactions, i.e., peripheral blood leukocyte (PBLs) resistance to viral infection (vesicular stomatitis virus, VSV) ex vivo, and cytokines: TNF-α, IFN-γ, IL-1β, and IL-10, production with enzyme-linked immunosorbent assay (ELISA), have been investigated during this preliminary study before and after 4 weeks of oral treatment with dietary supplement proline-rich polypeptide complex (PRP) (120 μg of PRP/day). The potential effect of PRP on the distribution of PBLs' subpopulations has been specified. We have found a deficiency in innate immune response in AD patients. It was demonstrated for the first time that the degree of PBLs resistance to VSV infection was closely related to the stage of clinical severity of AD. Our study showed significant differences in cytokine production, which indicated that in AD patients innate immune mechanisms are impaired. Administration of PRP to our patients increased innate immune response of PBLs and declined pro- and anti-inflammatory cytokine production, thus subduing the excessively developed inflammatory response, especially among patients with high severity of AD. PRP did not exhibit pro-proliferative activity. It was showed, however, significant influence of PRP on the distribution of PBLs' subpopulations. The findings

mentioned above might be crucial in the context of potential application of immunomodulatory therapy in AD patients and indicated PRP as a potential target for future treatments in neuroinflammatory diseases, such as AD. The results were published in *Journal of Neuroinflammation, 2019; 16: 137*.

One of the most important scientific discoveries of recent years was the disclosure that the intestinal microflora takes part in bidirectional communication between the gut and the brain. Scientists suggest that human gut microflora may even act as the "second brain" and be responsible for neurodegenerative disorders like Alzheimer's disease (AD). Although human-associated microbial communities are generally stable, they can be altered by common human actions and experiences. Enteric bacteria, commensal, and pathogenic microorganisms, may have a major impact on the immune system, brain development, and behavior, as they are able to produce several neurotransmitters and neuromodulators, such as serotonin, kynurenine, catecholamine, as well as amyloids. However, brain destructive mechanisms, which can lead to dementia and AD, start with the intestinal microbiome dysbiosis, development of local and systemic inflammation, and dysregulation of the gut-brain axis. Increased permeability of the gut epithelial barrier results in invasion of different bacteria, viruses, and their neuroactive products that support neuroinflammatory reactions in the brain. It seems that, inflammatory-infectious hypothesis of AD, with the great role of the gut microbiome, starts to gently push into the shadow the amyloid cascade hypothesis that has dominated for decades. It is strongly postulated that AD may begin in the gut, and is closely related to the imbalance of gut microbiota. This is a promising area for therapeutic intervention. Modulation of gut microbiota through personalized diet or beneficial microbiota intervention alters microbial partners, and their products, including amyloid protein, will probably become a new treatment for AD. The results were published in *Molecular Neurobiology, 2019; 56: 1841-1851*.

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

De novo bacteriophage whole genome analysis

DNA libraries of 36 bacterial genomes and two phage genomes were created. Then, sequencing reactions were carried out using second generation sequencers (Illumina MiSeq and NextSeq) and third generation sequencer (MinION). As a result of the reaction, data files in fastq format were obtained. Sequencing quality analysis (FASTQC) was performed for seven genomes followed by low quality reading filtering (Trimming). In the next step, the de novo sequence was assembled using the Spades tool. We are currently assessing the quality of sequence assembly and annotations (PatricRast and proprietary algorithm). Completion of the first seven sequences is planned for March / April 2020.

Differential gene expression analysis between normal and cancer cells

Colorectal cancer (CRC) is one of the most common cancers and the third leading cause of cancer-related deaths, especially in developed countries. The aim of our project was to assess the expression level of the miRNA panel in the serum of patients diagnosed with CRC compared to the serum of healthy patients and to find potential biomarkers.
Serum was collected from 8 patients with CRC and 4 patients from the control group. Expression of 182 free miRNAs was determined using Exiqon diagnostic panels. The ddCt method was used for the analysis and the data were normalized by calculating the LR (Log Ratio). Data were analyzed using the principal components method and hierarchical cluster analysis.

miRNAs': miR-34a, miR-7-1-3p, miR-629-5p, miR-574-5p, miR-543, miR-30c, miR-335-3p, miR-136-3p, miR-20b -5p occurred only in the serum of patients with colorectal cancer. These are potential markers of this disease. Currently, work is underway to extend the validation of selected miRNAs. We plan to expand research with an analysis of the impact of selected miRNAs on transcripts of cancer cells in the next stage.

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

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

Characterization of a monoclonal antibody 5C7 that recognizes catalytic domain of human α-1,4-galactosyltransferase

The mouse monoclonal antibody 5C7 that recognizes human α-1,4-galactosyltransferase was obtained in cooperation with prof. Arkadiusz Miążek (IITD). We showed that the antibody recognizes the catalytic domain of the enzyme, both in the lysate of CHO Lec 2 cells transfected with the gene encoding the full enzyme, and in the supernatant of HEK cells transfected with the gene encoding the catalytic domain. In addition, using the 5C7 antibody we showed α-1,4-galactosyltransferase is located mainly in the endoplasmic reticulum, but also in the Golgi apparatus. We also performed Western blotting analysis of colon cancer tissues from tumors with different metastatic potential and found that most of colon cancer specimens contained increased levels of α-1,4-galactosyltransferase, albeit the level did not correlate with the metastatic potential. We intend to evaluate bigger number of colon cancer samples using a purified 5C7 antibody.

Studies of the specificity and immunogenicity of baculovirus-obtained recombinant binding region of *P. falciparum* EBA-181 ligand - searching for the receptor on human erythrocytes

In order to characterize the *P. falciparum* EBA-181 merozoite ligand specificity, the recombinant binding region (Region II) of EBA-181 ligand was obtained using the baculovirus expression system. Using flow cytometry, we found that the recombinant Region II does not bind to the erythrocytes, possibly because EBA-181 does not recognize the surface receptor but the internal one, which most probably is a cytoskeleton protein. Therefore, we performed Western blotting overlay with SDS-PAGE separated erythrocyte membrane proteins and definitely excluded all glycophorins as receptors for this ligand. However, we observed that EBA-181 binds to an unidentified protein band with molecular weight about 120 kDa. This result may suggest that some interaction exists between EBA-181 and proteins belonging to the spectrin-actin network, which is connected with erythrocyte membrane through band 4.1, GPC, GPA or band 3. We plan to validate this hypothesis using mass spectrometry analysis.

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. This expertise enables us not only to study microbial sugars, but it may also be adopted to eukaryotic glycans.

In 2019, the laboratory collaborated with the Laboratory of Immunobiology of Infections in the Institute of Medical Biology, Polish Academy of Sciences in Łódz. The aim of research was to explain the interactions between malignant cells and tissues and human complement, especially ficolin-3. Ficolin-3 is a pattern-recognition molecule that activates the lectin pathway of complement. It is found in lungs, the liver and blood, but its physiological role is unclear. We have investigated the interaction of recombinant ficolin-3 with human ovarian cancer cell lines and tissues. Recombinant (but not serum-derived) ficolin-3 was found to bind strongly to the ovarian cancer cell lines, SKOV-3, OVCAR-3 and ES-2. Evaluation of interactions pointed out carbohydrates (cell glycans) as ligands of ficolin-3, since interactions were EDTA-dependent and were inhibited by specific carbohydrate ligand of human ficolin-3. O- and N-glycan profiling was performed for SKOV-3 and ES-2 cell lines according to previously described methodology (Jang-Lee J et al. 2006, Methods in Enzymology, 415: 59-86). Permethylated glycans were analysed by MALDI-TOF mass spectrometry and identified using GlycoQuest, the integrated search engine for glycans using the meta-database GlycomeDB and databases included in the GlycomeDB like GlycO. Obtained profiles of O- and N-glycans for both cell lines revealed major differences in N-glycosilation profile. For ES-2 cells incubated in pH 4.5, sialylated glycans and complex glycans were identified, including those substituted by fucose. ES-2 cell line differed by higher content of glycans with terminal galactose and galactosamine residues. Since sialic acids are not ligands for ficolin-3, sialylated glycans might rather prevented interactions with ficolin-3. According to published reports, Gal and GalNAc serve as ficolin-3 ligands, what may explain biological results of complement activation by ovarian cancer cells. Opposite results were obtained for SCOV cell line. Performed preliminary research requires further ESI-MS^n analysis of released glycans to confirm structures predicted by glycan profiling. Basing on data concerning recombinant human ficolin-3, it may be suggested that it is involved in immune response in ovarian cancer. However, unidentified serum factor(s) seem(s) to protect cancer cells from recognition by natural or rficolin-3 (Michalski M, Świerzko AS, Sawicki S, Kałużyński A, Lukasiewicz J, Maciejewska A, Wydra D, Cedzyński M. Interactions of ficolin-3 with ovarian cancer cells. Immunobiology. 2019, 224(2):316-324).