Ludwik Hirszfeld Institute of Immunology and Experimental Therapy
Polish Academy of Sciences

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

Presence of bacteriophages in the alimentary tract of patients with inflammatory bowel diseases

Determination of frequencies and titers of bacteriophage strains – own strain of *E. coli* from examined humans, *E. coli B*, *E. coli 1962* and *E. coli DSM 13127* – in the stools of 28 healthy volunteers and 79 patients with inflammatory bowel diseases (IBD) was performed. We also analyzed the dependence between the number of *E. coli* bacteria, frequencies and titers of coliphages in stools of patients and volunteers. The material from adult patients was obtained from the Clinic of Gastroenterology and Hepatology of the Academic Clinical Hospital of Wrocław.

Frequencies of coliphages detection and isolation of *E. coli* bacteria in stools of patients were lower as compared with volunteers. The titers of coliphages and *E. coli* bacteria in stools were higher in patients than in volunteers. A positive correlation between bacteria concentration, frequencies and titers of coliphages in stools of volunteers and patients with IBD was observed. The differences in results between volunteers and patients were statistically significant for frequencies of coliphages for the following strains: own *E. coli* and *E. coli* DSM 13127.

Phage neutralization by sera of patients receiving phage therapy

The aim of our investigation was to verify whether phage therapy (PT) can induce antiphage antibodies. We determined antiphage activity of sera (AAS) from 176 patients of the Phage Therapy Unit (PTU) in Wrocław before and during PT and from 30 healthy volunteers using a neutralization test. The rate of phage inactivation (K) estimated the level of phages' neutralization by sera. Sera of 12 patients treated with the *S. aureus* MS-1 phage cocktail (consisting of phages 676/Ż, A5/80 and P4/6409) were examined for specific antiphage antibodies IgG, IgA and IgM by ELISA.

Low K rates were found in sera of volunteers as well as in patients before PT. High AAS was observed in 10.8% of patients treated with phages locally (n=17) or locally/orally (n=2) for 15 to 66 days of PT. High AAS was found in patients treated with some *S. aureus*, *P. aeruginosa*, *E. faecalis* and *S. marcescens* phages. Low K rates were observed during oral PT. These results suggest that AAS depends on the route of phage administration and phage type. The induction of AAS during or after PT does not exclude effectiveness of PT. Our

preliminary results from ELISA showed that sera of patients undergoing PT had higher levels of antiphage antibodies during treatment (independently of route of phage administration and clinical outcome of phage therapy). Increasing absorbance during PT was found to be significant ($p<0.05$) except those obtained for *S. aureus* P4/6409 phage for IgG and IgA and corresponded with significantly higher K rates measured by the neutralization test.

In vitro study on the cytotoxic and antibacterial activity of some Pseudomonas bacteriophages using a cell culture model

The aim of the study was to work out *in vitro* conditions for testing cytotoxic and antibacterial bacteriophage activity in a cell culture infection model. The antibacterial effect of three therapeutic phages used for treatment of *Pseudomonas* infections (119x, F-8, and W-31) applied in the form of phage lysates was tested against four *P. aeruginosa* strains isolated from patients admitted to the Phage Therapy Unit in Wroclaw. L929 (mouse fibroblasts) and HACAT (human keratinocytes) cell line cultures on 96-well plates were applied for the tests. After 24 hours of their coincubation in the presence of bacteria and phages the cytopathic effect of bacteria on the cells (on a scale of 0-4) and the intensity of infection (on a scale of 0-3) was assessed under the microscope. The lactate dehydrogenase (LDH) test was also used for quantification of cell viability.

A very differentiated cytopathic effect of the bacteria (at comparable titers) was observed: from intensive cell lysis to intensive infection without any changes in cell morphology. A phage cytoprotective effect was observed when their initial titer was 100 times higher than that of bacteria. Interestingly, phages which did not reveal activity against one of the used *P. aeruginosa* strains in the phage typing test were able to efficiently protect the cell culture against infection with this bacteria. No significant cytotoxicity of the phage lysates was observed. The developed cell culture infection model might be useful for screening phage activity and their selection for application for phage therapy independently of the standard phage typing procedure and for assessment of the virulence/cytopathicity of the bacterial strains *in vitro*.

Combined anticancer and antibacterial activity of bacteriophages engineered with phage display

Dynamic development of novel anticancer strategies has also applied bacteriophages: bacterial viruses are proposed as drug carriers and/or display platforms for various anticancer agents. Phage display has become a powerful technology for selecting and amplifying

peptides; it is also proposed as a relatively cheap and easy technology for production of active peptides in bulk amounts. Peptides (or proteins) can be presented on the surface of phage capsids as a result of molecular engineering. The potential of peptides in cancer treatment is evident due to many studies evolving therapeutic strategies targeting the progression of tumor growth or metastasis formation. Peptides that can target cancer cells or interfere with their functions without affecting normal cells are being developed as an alternative strategy to conventional chemotherapy. At the same time, bacteriophage-based platforms or carriers maintain their natural antibacterial activity that is necessary for phage amplification. We applied an *in vivo* BALB/c mouse model of 4T1 tumor growth accompanied by surgical wound infection to test this combined activity. The wounds were located in the areas of tumors. Bacteriophages (T4) were modified with anticancer YIGSR peptides by phage display and injected intraperitoneally. As a result, tumor growth was decreased in mice treated with YIGSR-displaying phages. The acuteness of wounds, bacterial load and inflammatory markers in phage-treated mice were markedly decreased. Thus, engineered bacteriophages provide a solution for combined antibacterial and anticancer treatment due to combination of their natural and engineered activity. Both aspects should be considered in potential applications of phages as phage display platforms or drug carriers. Combining anticancer (engineered) and antibacterial (natural) phage activity in therapies offers a novel solution for the medicine.

Results of grant activities

A purified stabilized preparation containing staphylococcal phage A5W/80ppf with decreased contamination with other phages and plasmid DNA for use on wounds and skin for experimental treatment was obtained on a production scale under GMP conditions. Comparison of clinical results of the experimental phage therapy in patients with chronic bacterial infections did not reveal that the application of cocktails of staphylococcal phages was superior to the monovalent phage preparations.

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

Laboratory of Microbial Immunochemistry and Vaccines
Head: Professor Czesław Ługowski, Ph.D.
Biochemical characteristics of macromolecules involved in immunological processes. Immunochemical studies of bacterial endotoxins

Infections by Gram-negative multidrug-resistant (MDR) bacteria represent an increasing health care problem worldwide. While there are still some new antibiotics with some degree of efficacy against Gram-positive bacteria, the pipeline of novel drugs being developed to treat Gram-negative pathogens is essentially empty. The potential spread of MDR pathogens and mortality are therefore similar to those associated with infectious diseases in the pre-antibiotic era. A particular concern is the recent emergence of clonal lineages that can balance the normally mutually exclusive phenotypic properties of being MDR with the retention of high virulence potential, a feature that is generally unusual in MDR strains.

The *Escherichia coli* lineage sequence type 131 (ST131)-O25b:H4 is a globally distributed multidrug-resistant clone responsible for a great proportion of extraintestinal infections. This clone alone is responsible for >10% of all extraintestinal *E. coli* infections and accounts for the greatest majority of *E. coli* strains that are resistant to clinically relevant antibiotics. The vast majority of ST131-O25b isolates are resistant to fluoroquinolones. Moreover, approximately 50% of the isolates producing an extended-spectrum β-lactamase (ESBL) that confers resistance to all β-lactam antibiotics, except the carbapenems, originate from this clone. Even more alarming, there are several recent reports that describe representative strains of this lineage expressing various carbapenemases. Consequently, infections by ST131-O25b:H4 strains are a growing concern, with very limited therapeutic options. The obvious pathogenic success of this lineage is conferred by the MDR phenotype and retained virulence potential. However, the range of factors contributing to its virulence still has to be fully elucidated.

Driven by the significant medical needs associated with this successful pathogenic lineage, we were involved in studies aimed at generation of murine monoclonal antibodies (MAbs) against lipopolysaccharide (LPS) of *E. coli* (STI131)-O25b in order to develop quick diagnostic tests. Structural analysis of *E. coli* O25b LPS was important prerequisite for these studies. The structure of O-antigen was elucidated using chemical and instrumental methods. Based on comparative analysis by nuclear magnetic resonance (NMR) and mass spectrometry, the *N*-acetyl-fucose in the O25a O-antigen had been replaced by *O*-acetyl

rhamnose in the O25b repeating unit. The genetic determinants responsible for this structural variation were identified by aligning the corresponding genetic loci and were confirmed by *trans*-complementation of a rough mutant by the sub-serotype-specific fragments of the *rfb* operons.

Murine monoclonal antibodies were generated by immunizing mice with whole killed nonencapsulated ST131-O25b *E. coli* cells and screening hybridoma supernatants for binding to purified LPS molecules obtained from an *E. coli* ST131-O25b clinical isolate. The MAbs selected for further study bound to the surface of live *E. coli* O25b strains irrespective of the capsular type expressed, while they did not bind to bacteria or purified LPS from other serotypes, including the related classical O25 antigen (O25a). Using these specific MAbs, a latex bead-based agglutination assay was developed that has greater specificity and is quicker and simpler than the currently available typing methods. The high specificities of these MAbs can be explained by the novel structure of the O25b repeating unit elucidated in this article.

Laboratory of Glycoconjugate Immunochemistry
Head: Professor Hubert Krotkiewski, Ph.D.

Immunochemical and genetic studies on human glycophorin and other proteins active in the immune system

Purification and characterization of camel glycophorins

Glycophorins are heavily glycosylated proteins incorporated into erythrocyte membranes. Their biological function is unknown, but it seems they take part in creation of the glycocalyx, which is responsible for the elasticity of erythrocyte membranes. Among animal glycophorins characterized so far there are no camel glycophorins. Camels are important animals for the economy of Sub-Saharan countries, and the major threat for camels is surra, a disease caused by *Trypanosoma evansi.* Because in cooperation with INSERM_U665 we plan to obtain naked eye visible agglutination (NEVA) reagents in order to detect *T. evansi* infection, characterization and cloning of camel glycophorins would make a good start for such a reagent. Erythrocytes of the dromedary camel were a gift from Dr. Carlos Guthierrez (University of Las Palmas de Gran Canaria), and glycophorins were obtained by a phenol extraction method. Proteins were fractionated on a Sephadex G-200 column in 1% SDS. SDS-PAGE showed the presence of three bands stained with the PAS method, which were called glycophorins X (29 and 49 kDa), Y (34 and 62 kDa) and Z (108 kDa). In order to evaluate the structures of glycans present on glycophorins, we used lectin blotting with the following biotinylated lectins: *Phaseolus vulgaris* (PHA-E), *Aleuria aurantia* (AAL) and *Arachis hypogaea* (PNA). PHA-E is specific to N-glycans with a structure

Galβ1→4GlcNAcβ1→2Man, AAL recognizes Fucα1→6GlcNAc, while PNA binds O-glycans with the Galβ1→3GalNAc sequence. We found that glycophorins X and Z contain O-linked glycans with the Galβ1→3GalNAc sequence, while glycophorin Y contains only N-linked oligosaccharides. Glycophorin Z contains both types of oligosaccharides. In order to sequence N-terminal fragments of camel glycophorins, they were separated by SDS-PAGE and transferred to PVDF membrane. A preliminary attempt to sequence failed, probably because N-terminal amino acids are blocked. Thus, we plan to analyze peptides obtained by trypsin digestion of purified glycophorins by mass spectrometry.

Interaction of CEA N-terminal domain with endotoxin from Plesiomonas shigelloides

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein attached to the membrane via a GPI anchor. It is composed of seven immunoglobulin-like domains: N-domain (IgV-like) followed by six internal domains A1B1A2B2A3B3 (IgC-like) containing conserved disulphide bridges. CEA is a member of the CEA-related cell adhesion molecule family (CEACAM) and belongs to the immunoglobulin supergene family (IgSF).

CEA is known as a tumor-associated antigen in colon, lung and breast adenocarcinomas. Therefore, the protein is used as a biomarker of tumor progression and metastasis in long-term treatment of cancer patients. However, the biological function of CEA is still unclear. CEA also acts as a receptor for some bacterial pathogens such as *Escherichia coli*, *Haemophilus influenzae* and *Neisseria sp*. Opa proteins expressed by *Neisseria sp.* are bound to the CEA N-terminal domain, which, we suppose, may also be involved in recognition by other bacteria.

In our work we focused on interaction of the CEA N-terminal domain with the main surface antigen of Gram-negative bacteria, lipopolysaccharide (LPS) of *Plesiomonas shigelloides*. It was found using dot blot, ELISA and surface plasmon resonance techniques that the recombinant CEA N-domain interacted with LPS isolated from *Plesiomonas shigelloides* and *Escherichia coli.* The interaction between the protein and LPS involved lipid A, the most conserved part of the LPS. The N-domain was found to contain two potential LPS-binding sites. The first one was in the B strand (^{15}KEVLLLVH^{22}) and the second one in the loop (^{35}KGERVDGNR^{43}) between the C and C' strands. Screening of LPS isolated from various *P. shigelloides* strains by dot blot revealed that two LPS, 85/89 and 144/92, did not interact with the N-domain. The study of the LPS preparations by mass spectrometry and light scattering showed that the lipid A of the first LPS was degraded while the second LPS was highly aggregated, possibly giving false-negative results.

The results presented here may help to clarify the biological function of CEA protein as a possible scavenger of bacterial components. It is possible that CEA could remove LPS from dead and/or dividing bacteria present in human colon.

Laboratory of General Immunochemistry
Head: Professor Maria Janusz, Ph.D.
Studies on the mechanism of action of proline-rich polypeptide complex (PRP)

Studies on the effect of PRP and its nonapeptide constituent (NP) on brain derived nerve growth factor (BDNF) secretion by U87 astrocyte cell line cells

It was shown in our previous studies that among the multidirectional activity of proline-rich polypeptide complex (PRP) and its nonapeptide constituent the most important seems to be a combination of immunoregulatory and antioxidative effects. The role of PRP in the development of the immune and cognitive function allowed it to be provided as orally administered Colostrinin tablets in the case of Alzheimer's disease (AD). In AD pathogenesis the main role is played by astrocytes and microglial cells. Astrocytes secrete trophic factors – nerve growth (NGF) and brain derived nerve growth factor (BDNF) – which are important for the control, development and function of the nerve tissue. In neurodegenerative processes the lowered secretion of trophic factors is observed, especially in the hippocampus region.

In our previous study we found that regulation of NGF secretion can be an additional neuroprotective effect of PRP/NP in the case of AD. In the present study the effect on BDNF secretion by the U87 astrocyte cell line was studied. It was found that U87 cells were able to secrete constitutive BDNF, but at a low level. 26 kDa and 36 kDa pro isoforms were identified in lysates and supernatants collected from cell cultures. No statistically significant influence of PRP and NP on BDNF level was observed.

It can be concluded that regulation of brain derived nerve growth factor secreted by astrocytes is not an element of possible therapeutic effects of PRP/NP in neurodegenerative processes.

Proline-rich polypeptide complex (PRP) and its nonapeptide fragment (NP) influence neuritogenesis and protect against toxic effect of amyloid β 1-42

The results obtained within the grant project demonstrate that both proline-rich polypeptide complex (PRP) and its constituent nonapeptide (NP) are able to regulate neuroprotective processes and mimic nerve growth factor (NGF). It was found that PRP/NP by activation of the nNOS-dependent signaling pathway induces an increase of intracellular NO level. In consequence, cyclic guanyl cyclase is activated and increases the concentration of cyclic GMP and ERK 1/2 kinase activation. These kinases are engaged in activation of transcription factors which control gene expression of proteins playing a role, among others, in survival and neuritogenesis. These effects can shed some light on the mechanism of the therapeutic effect of PRP/Colostrinin in the case of Alzheimer' disease.

The results obtained in PC12 and U87 cell models also indicate that:

- PRP and NP are able to protect cells against toxic doses of Aβ 1-42;
- the intracellular level of NO induced by Aβ 1-42 was lowered when cells were treated with PRP/NP, and a tendency to reduce the level of nitrated proteins was observed;
- the lack of relationship between NO secreted and expression of nNOS suggested that peptides influence enzyme activity rather than its level;
- PRP and NP are able to induce regulation of expression, synthesis and extracellular secretion of NGF in the human astrocyte cell line U87.

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

Laboratory of Tumor Molecular Immunobiology
Head: Professor Leon Strządała, Ph.D.

Induction of apoptosis in canine leukemia/lymphoma cell lines

Hematopoietic neoplasms are the third most common type of tumor diagnosed in the dog, accounting for approximately 8–9% of all canine malignant tumors. Among them lymphoma, which accounts for about 80% of all lymphoid malignancies, is the most commonly diagnosed. Canine neoplasms show strong similarities to their analogs in humans with respect to morphology, tumor genetics, disease progression and response to therapies. The dog

represents a good large animal model for the study of lymphoma in people, including investigation of new therapeutic agents, because of the presence of spontaneous disease in contrast to experimentally induced disease, in xenograft and genetically modified mouse models.

In order to develop specific and targeted anticancer therapies, a panel of different types of neoplastic cell lines is required. Until recently, investigations of spontaneous canine lymphoma and leukemia were limited by a lack of validated, well-characterized and widely available cell lines. So far, a few canine cell lines have been described in the literature. We established and characterized two novel canine cell lines: a B-type leukemia cell line and an NK/T leukemia cell line.

For investigating therapeutic methods and cancer biology, many different cancer cell lines are used. However, chemosensitivity studies carried out on established cell lines do not always reflect the actual patient's situation. Therefore, beside the studies using cell lines, primary culture studies predominate because, in contrast to the cell lines, they better reflect the actual type of malignant cells and their sensitivity to drugs. This is particularly important in those types of cancer which are extremely heterogeneous, such as lymphoma.

The aim of our study was to test the chemosensitivity of canine primary and established leukemia/lymphoma cells (using a modified MTT assay) to the spectrum of cytostatic drugs commonly used in therapy of dogs, as well as to compare the results with those obtained from annexin V and propidium iodide staining. All tested substances exhibited dose-dependent inhibitory effects on the proliferation of the examined cell lines with a different level of apoptosis induction. Vincristine and doxorubicin strongly reduced the viability of canine cell lines, whereas cyclophosphamide induced the highest level of apoptosis.

It was found that T cells show a significantly lower sensitivity to the majority of tested substances than B cells. These results correlate with those of clinical investigation, which showed that in the case of T-cell canine lymphoma, the prognosis value is worse and the pharmacotherapy is poor. The use of in vitro assays, such as the MTT or annexin V/Pi assay, in predicting response to treatment of lymphoma in dogs may be a useful therapeutic tool. Chemosensitivity tests, molecular biology and pharmacogenomic techniques can significantly contribute to improving the treatment outcomes for individual canine patients.

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

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

Anticancer activity of tyrosine kinase inhibitor combined with docetaxel and vitamin D_3 derivative PRI-2191 in A549 non-small cell lung cancer model in vivo

Malignant cells, including lung cancer cells, release many growth factors that are involved in tumor vasculature formation; hence tumor angiogenesis is one of the targets for anticancer therapy. Blocking the signal transduction pathway of VEGF, the main angiogenic mediator, results in endothelial cell apoptosis, which in turn blocks angiogenesis. Endothelial cells appeared to be affected also by chemotherapy, initially designed to destroy cancer cells. In our study we decided to combine tyrosine kinase inhibitor (TKI) with VEGF receptor inhibitory activity, together with docetaxel combined with the vitamin D_3 analog (PRI-2191), in order to strengthen the effectiveness of proposed therapy in a non-small cell lung cancer (NSCLC) model *in vitro* and *in vivo*. Our recent results showed that A549 cells exposed to TKI and/or docetaxel secreted a lower level of VEGF-A than control cells. The strongest effect was observed when a third compound, calcitriol or PRI-2191, was introduced. A similar effect was observed in tumors harvested from mice treated with above-mentioned compounds. Moreover, this repression of VEGF-A secretion by A549 lung cancer cells resulted from up-regulation of p53 and p21 expression in these cells.

1,4- dimethylpyridinium chloride (1,4-DMP), antiplatelet drug (clopidogrel) and a relevant chemotherapeutic as combined treatment in metastasis prevention

Efficiency of metastasis depends mostly on events occurring while cancer cells migrate through the unfavorable environment of flowing blood. Interference with cancer cell and platelet interactions may lead to decreased survival of migrating cells and consequently to a lower number of developed metastases. Mice bearing metastatic prostate, breast or colon tumors were treated with 1,4- dimethylpyridinium chloride (1,4-DMP), which modulates the process of prostacyclin synthesis, an antiplatelet drug (clopidogrel) and a relevant chemotherapeutic. Antitumour and antimetastatic activity together with a systemic toxicity of the proposed treatment regimes were assessed. Results of the present studies indicate that regardless of the tumor type, neither 1,4-DMP nor clopidogrel influenced the growth of primary tumors. However, when applied with 5-fluorouracil, cyclophosphamide or docetaxel, 1,4-DMP increased their activity by 20-30% ($P<0.05$ vs. control). When chemotherapeutic agents were administered with clopidogrel, the observed tumor growth inhibition was slightly

reduced; however, animals among the groups receiving such a combined treatment developed fewer lung metastases. Furthermore, 1,4-DMP diminished elevated blood concentrations of alanine and aspartate aminotransferases, induced by chemotherapy, which may suggest its potential liver protective function.

The present studies indicate that a vascular-oriented treatment, supplemented with drugs having anti-platelet activity, may beneficially influence the overall anticancer effectiveness and alleviate side effects of selected chemotherapeutic drugs, applied in tumors of commonly occurring types.

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

The Laboratory of Biomedical Chemistry is a part of the Integrated Laboratory of Experimental Oncology and Innovative Technologies – "NeoLek". In the laboratory, studies on chemical modifications of biological macromolecules and physicochemical properties of bacteriophages are carried out. Studies are focused on modifications of carriers with biologically active substances. Proteins and oligosaccharides, for example hydroxyethyl sucrose, are used as carriers. Drug conjugates (folic acid derivatives) have higher antitumor activity than unmodified drugs. Studies on binding of boron clusters with carriers are carried out as a result of involvement in development of boron neutron capture therapy (BNCT). Lysozyme modifications with boron clusters showed that 128 arginine and 51 threonine residues are preferentially modified. Studies on chemical modifications of polypeptides with defined biological activity are conducted. Physicochemical properties of bacteriophages are also studied.

In the middle of 2015, NeoLek Laboratory will obtain the GLP certificate. Biological activity of preparations is studied in the Laboratory of Experimental Antitumor Therapy, which is a part of NeoLek. The Lab. of Biomedical Chemistry is equipped with certified research apparatus: HPLC MS/MS with a set of detectors (DAD, fluorescence, corona), UV/Vis spectrophotometer, CD spectrophotometer, viscometer, scales and centrifuges.

Beside basic research, the laboratory collaborates with companies, for example Adamed, Galena and Finepharm.

The laboratory is a place where students can carry out their research for the Bachelor or Master Thesis.

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 pathogenesis of some diseases of bacterial etiology and the role of bacterial surface glycoconjugates and protein antigens in the immune response

The laboratory aims at the clarification of pathogenicity mechanisms of diseases with bacterial etiology, the role of bacterial glycoconjugates and proteins in the immune processes, the structure and functions of bacterial exopolysaccharides and endotoxins. Regarding the studies on the relation of structure of surface antigens of probiotic exopolysaccharides and their role in immune system activation, the structure of polysaccharides (PS) isolated from *Lactobacillus rhamnosus* LOCK 0900 was determined. One of its polymers (I), with a high molecular mass of 830 kDa, is a branched heteropolysaccharide with a unique heptasaccharide repeating unit and pyruvic acid, whereas the second one (II) has a low average molecular mass of 18 kDa and contains a pentasaccharide repeating unit and phosphorus. Both PS neither induce cytokine production and maturation of mouse bone marrow-derived dendritic cells nor induce signaling through TLR2/TLR4 receptors. Exposure to PSI enhanced IL-10 production induced by *L. plantarum* WCFS1, while in contrast, PSII enhanced the production of IL-12p70. Thus PS are able to modulate the immune responses to third-party antigens. The ability to induce regulatory IL-10 by PSI opens up the possibility for its use in therapy of inflammatory conditions, such as IBD, whereas PSII might be useful in reverting the antigen-dependent Th2-skewed immune responses in allergies. Other studies revealed a new type of highly sensitive label-free sensor coated with T4 phage adhesin (gp37) which binds *Escherichia coli* B by recognizing its LPS. The biofunctionalization methodology is based on coating the surface with nickel ions capable of gp37 histidine tag reversible binding. For the first time, a recombinant adhesive phage protein has been used as a receptor molecule in a biosensing scheme. The sensor can measure bacterial contamination in real time and with high accuracy the adhesin binds *E. coli* B LPS in its native or denatured form. The binding is highly specific and irreversible. The applied procedure allows reusable biosensors to be obtained.

Laboratory of Virology
Head: Professor Egbert Piasecki, Ph.D.
Study on nonspecific immunity in viral infections

Scutellariae Radix (root of *Scutellaria baicalensis*) has a long history of application in traditional and modern herbal medications. The major components of Scutellariae Radix are baicalin, baicalein, wogonoside and wogonin. Accumulating evidence demonstrates that *Scutellaria* has immunomodulatory effects and possesses compelling anticancer potential. Treatment of peripheral blood leukocytes (PBLs) with *Scutellaria baicalensis* extract (SBE) enriched in baicalin reduced viability of PBLs obtained from patients with acute lymphoblastic leukemia (ALL). SBE had no impact on the survival of healthy, control leukocytes. The immune system modulation by SBE resulted in increased production of IFN-γ in PBLs, and reduced TNF-α and IL-10 production in bone marrow cells (BMC), in ALL patients. SBE stimulated the nonspecific antiviral immunity, assessed by resistance of PBLs and BMC to vesicular stomatitis virus (VSV) infection. SBE showed pro-apoptotic activity in the NALM-6 cell line (B-type human leukemia). The number of cells expressing annexin V increased from 6% in control cultures to 29% and 52% after treatment with 100 μg/ml and 200 μg/ml respectively. An increased percentage of apoptotic cells was observed when cells were treated with a corresponding concentration of baicalin. SBE enhanced apoptosis of PBLs in BMC of leukemic children. The percentage of PBLs that underwent apoptosis and mean annexin V expression increased from 11% in the control to 17% and 24% for the doses of 100 μg/ml and 200 μg/ml respectively. Importantly, SBE did not induce apoptosis of PBLs in the healthy, control group. The results were published in *International Immunopharmacology, 2014; 23: 558-567.*

The aim of the studies was to examine the potential immunoregulatory activity of Ginkgo biloba extract (EGb 761) on cytokine production, one of the mechanisms of innate antiviral immunity, by human peripheral blood leukocytes (PBLs) ex vivo. PBLs isolated from healthy blood donors were treated with different, nontoxic concentrations of EGb 761. Levels of different cytokines (TNF-α, IFN-α, IFN-γ, IL-10 and IL-12), important in innate immunity development, were determined by ELISA. EGb 761, apart from strengthening of the antiviral response, showed a differential impact on cytokine production by human PBLs ex vivo. It decreased the level of TNF-α and IFN-α but strongly increased the level of IFN-γ in PBLs stimulated by vesicular stomatitis virus (VSV) and non-stimulated PBLs. The extract reduced the production of IL-10 and IL-12 by human PBLs. The results were discussed and compared

with previously published findings on the activity of the synthetic drug donepezil. According to the results from the present study and our previous investigations, we report an immunoregulatory effect of EGb 761 on production of different cytokines by human PBLs ex vivo, which indicates the possibility of using the drug for the treatment of many immune deficiencies or infectious diseases through strengthening of innate immunity reactions. The results were published in *Central European Journal of Biology, 2014; 9: 359-366.*

The main aim of this study was to determine the association between serum antibodies against adenoviruses (AdVs) type 5, 31 and 36 (as a positive control) and obesity in Polish adults and to evaluate the association between anti-AdV antibody status and body mass index (BMI), anthropometric measures, serum lipids and C-reactive protein (CRP). The study included 200 adults, both obese and non-obese. The serum neutralization test was used to assess the presence of anti-AdV antibodies and routine serum chemistry, leptin and CRP were evaluated. The prevalence of anti-AdV5, 31, 36 antibodies ranged from 6.8% to 31.3%. We demonstrated an association between immune response to AdV infection (by establishment of anti-AdV31 and anti-AdV36 antibodies in serum) and obesity in adult Poles. Higher BMI values and WHR (waist to hip ratio) or waist circumference were found in infected versus uninfected ($p<0.05$). All AdV-positive subjects were older than uninfected controls, but sex had no influence on the prevalence of antibodies. We noted that the presence of anti-AdV31 and 36 antibodies was associated with changes in lipid metabolism but kind and severity of lipid metabolism disturbances differed due to the type of infecting virus. Neither elevated CRP nor decreased leptin levels were related to infectobesity. Infections of AdV31 and AdV36 may be associated with obesity in the Polish population and AdV5 is not linked with human obesity. The results were published in *Inter. J. of Virology and Molecular Biology, 2014; 3: 1-8.*

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

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

Studies on the mechanism of action of a cyclic tetrapeptide

Studies on elucidation of the mechanism of action of a cyclic tetrapeptide were continued in several *in vitro* and *in vivo* models including: proliferative response of mouse splenocytes to concanavalin A, proliferation of IL-2-dependent CTLL-2 cells, growth of epidermal A-431

cell line, lipopolysaccharide-induced cytokine production in human whole cell cultures, foot pad carrageenan-induced inflammation and carrageenan-induced inflammation in an air pouch model in mice. The studies were particularly aimed at determination of the role of prostaglandin E2 (PGE2) and its cellular receptors in the anti-inflammatory actions of the tetrapeptide. In these experiments we measured concentrations of inducible PGE2 and evaluated effects of blocking EP3 and EP4 receptors. The results indicate that the anti-inflammatory action of the tetrapeptide is dependent on prostaglandin signaling. In the culture of A-431 cells the production of PGE2 was inhibited. Depending on the model used, we also found that in mediation of the anti-inflammatory actions of the tetrapeptide EP4 and/or EP3 are involved. In addition, parallel experiments conducted in another laboratory proved that the tetrapeptide is a COX-1 and COX-2 inhibitor.

Preliminary studies on susceptibility of mouse splenocytes from young and aged mice to viral infection

We showed that B-cell enriched splenocyte populations from old (more than 12-months) mice were either equally or more resistant to viral infection with EMCV in culture as determined by the survival rate of cells and titer of the virus. In addition, we demonstrated protective, antiviral actions of several types of lactoferrins (LF) such as native bovine and human and recombinant human and mouse in both age categories.

Mechanism of autocrine regulation in HeLa cells, based on the interaction of endogenous bone morphogenetic protein (BMP)

The main purpose of the study was to determine whether elevated levels of ALP and SmadP result from activation of specific receptors by endogenous isoforms of BMP-2 and BMP-4. As previously shown, HeLa cells synthesize and secrete several isoforms of BMPs, and two of them, BMP-2 and BMP-4, possess biological activity *in vitro.* High activity of specific BMP receptor genes and a high level of the signaling molecule – phosphorylated Smad (SmadP) – formed after binding of BMP to a specific receptor were found in HeLa cells. High amounts of phosphorylated Smad and alkaline phosphatase (ALP) synthesized in HeLa cells and resulting from SmadP action on the ALP gene via the cascade effect were significantly diminished after addition of specific monoclonal antibodies against BMP-2 and BMP-4 to the cell culture. The fact of conducting the experiments in serum-free medium led to both disappearance of SmadP and significant lowering of the ALP level after addition of

antibodies. The described results directly showed that HeLa-derived BMPs can affect HeLa cells in an autocrine manner.

The effect of nickel ions, released during the process of corrosion of metal parts of braces, on the patient

The aim of this study was to verify the effect of nickel on a live organism and demonstrate the differences between tissues in the synthesis of metallothionein. Metallothionein plays an important role in immobilization and removal of toxic metals from organisms. The study was conducted on 25 domestic pigs. Thirteen of them possessed the implants from nickel-containing alloys in the oral cavity. Tissue samples from liver, lungs and brain were chosen for the examinations. The liver is an organ of first line defense against toxins and possesses the capacity of regeneration. The lung and the brain are very specialized organs without the ability to regenerate. Statistically significant differences in metallothionein synthesis were noted between the study group and the control group of animals. Significant differences were also noted between the studied tissues. The highest activity for the metallothionein gene was noted in the liver tissue. However, some differences in the activity of that gene in lung and brain tissues, between the studied groups, were also observed.

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

Cytokine gene polymorphisms and serum IL-6 level in schizophrenia patients

An immense body of evidence indicates that dysfunction of the immune system is implicated in the etiology of schizophrenia. Given the strong genetic background of schizophrenia, it might be assumed that aberrant production of cytokines may be the consequence of genetic factors.

This study aimed at investigating the association between schizophrenia susceptibility and selected functional polymorphisms in genes encoding cytokines including interleukin-2 (IL2 –330T>G), interleukin-6 (IL-6 -174G>C), interferon-γ (IFNG +874T>A) as well as for the first time transforming growth factor-β1 (TGFB1 +869T>C and +915G>C). Moreover, the influence of serum IL-6 level together with the IL-6 polymorphism -174G/C and high sensitivity C-reactive protein (hsCRP) levels on clinical manifestation and cognition in schizophrenia patients were investigated.

There was a significant difference in the genotype distribution and allelic frequency of TGFB1 +869T>C between patients with schizophrenia and healthy controls ($p < 0.05$). The risk of schizophrenia was higher in carriers of the T allele (CT+TT genotypes) than in CC homozygotes. Given the gender differences in incidence of schizophrenia, we conducted separate analyses of male and female participants, and we found that the association was significant in females.

Serum IL-6 and hsCRP levels were significantly higher in schizophrenia patients in comparison with healthy controls. Both hsCRP and IL-6 levels were associated with insidious psychosis onset, duration of illness and chronic schizophrenia course with deterioration. After adjustment for age, education level, number of years of completed education, illness duration, total PANSS score, depression severity and chlorpromazine equivalent, there was still a positive association between IL-6 and hsCRP levels and worse cognitive performance. The IL-6 -174G/C polymorphism did not influence IL-6 level, but it was associated with the severity of positive symptoms. Our results suggest that elevated IL-6 levels may play a role in cognitive impairment and serve as a potential inflammatory biomarker of deterioration in schizophrenia.

Proliferative response of peripheral blood malignant lymphocytes from CLL patients with low and high CTLA-4 expression to ex vivo stimulation

Our previous study indicated that the inhibitory CTLA-4 molecule might be an important antiproliferative factor in chronic lymphocytic leukemia (CLL). The present study was undertaken to investigate whether CLL patients with low and high CTLA-4 expression differ in the responsiveness of their cells to ex vivo induced proliferation.

The expression of the CTLA-4 molecule, the cell cycle regulators of G0/G1 phase (cyclins D2 and D3, and $p27^{KIP1}$) and Ki-67 protein in peripheral blood $CD19^+CD5^+$ cells of 38 CLL patients before and after 24 h and 72 h of culture in the presence of immunostimulators (DSP30+rIL-2) were determined by flow cytometry.

In the low CTLA-4-expressing CLL group, ex vivo stimulation did not change the median proportions of $CTLA\text{-}4^+$ cells. In contrast, in the high CTLA-4-expressing CLL group the stimulating culture resulted in a gradual decrease in the median proportions of $CTLA\text{-}4^+$ cells, which became comparable to those observed in the low CTLA-4-expressing CLL group. In both groups of CLL patients, ex vivo stimulation led to a gradual decrease in the median percentages of $p27^{KIP1}$-positive lymphocytes, a significant decline in the median frequencies of cyclin $D2^+$ and cyclin $D3^+$ cells after 72 h of culture, and a marked increase in the median frequencies of $Ki\text{-}67^+$ cells. Furthermore, no significant differences in the expression of studied cell cycle regulators and Ki-67 protein between the two groups of CLL patients at each time point tested were found. Our data indicate that leukemic cells in the low CTLA-4-expressing CLL group as well as in the high CTLA-4-expressing CLL group do not differ in their responsiveness to ex vivo induced proliferation.

Laboratory of Reproductive Immunology
Head: Professor Anna Chełmońska-Soyta, Ph.D., V.D.
Immunological mechanisms associated with reproductive processes in health and disease

Effects of supply of retinoic acid (ATRA) TCD4 lymphocyte functions in female reproductive cycle and early pregnancy

The aim of this study was to determine the changes in the proteome of TCD4 cells of non-pregnant females and changes in the frequency of TCD4 FOXP3 lymphocytes in the female cycle and early pregnancy under the influence of retinoic acid *in vivo*.

The study was conducted in two independent experiments. In the first experiment, non-pregnant C57/BL6 females were treated with retinoic acid (ATRA) orally at a dose of 0.5 mg/kg for a period of two weeks. The study was conducted in collaboration with the Department of Physiology, Cytobiology and Proteomics, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology in Szczecin. CD4 lymphocytes were isolated from the spleen by magnetic sorting. Proteomic studies were performed by 2D technique with subsequent identification of proteins by mass spectrometry. It was found that oral intake of retinoic acid significantly alters the expression of 15 proteins in CD4 lymphocytes, primarily of the group of proteins involved in cellular cytoskeletal organization and cellular metabolism. In a second experiment, ATRA was administered i.p. to transgenic females C57BL6 ^{Foxp3GFP} at a dose of 0.25 mg/kg b.w. during the estrus cycle and in early pregnancy.

The frequency of Treg lymphocytes was determined in the spleen, lymph nodes and the uterus. It was found that intraperitoneal supply of ATRA increases the frequency of Treg in the spleen and lymph nodes in mice in the preimplantation period of pregnancy, which may be a prerequisite for further studies on modulation of the tolerogenic immune response in the peripheral preimplantation period of pregnancy.

DEPARTMENT OF MICROBIOLOGY

Laboratory of Molecular Biology of Microorganisms
Acting Head: Professor Anna Pawlik, Ph.D.
Replication of bacterial chromosomes
Polyketide synthesis and its regulation in Streptomyces

The research activity of the Laboratory of Molecular Biology of Microorganisms (LMBM) is focused on two scientific issues: i/ replication of bacterial chromosomes, and ii/ polyketide synthesis in *Streptomyces*.

DNA replication is an important event of the bacterial cell cycle. The decision to initiate DNA replication is crucial for the cell cycle progression, and depends on both intracellular and environmental signals. In LMBM we are interested in mechanisms of initiation and regulation of bacterial chromosome replication, with special emphasis on characterization of the key factors involved in initiation complex (orisome) formation, namely DnaA (the

initiator protein) and *oriC* (the origin of chromosome replication). We also aim at the identification and functional analysis of regulators which control replication and coordinate the initiation with the cell cycle. We are especially focused on orisome formation in epsilonproteobacteria, amongst which studies on pathogenic *Helicobacter pylori* are the most advanced. Recent analyses allowed us to map all DnaA-binding sites at bipartite *H. pylori oriC*. The most intriguing for *oriC* assembly are the topology-sensitive DnaA boxes (ts boxes) localized at *oriC2* – they are exclusively bound when DNA is supercoiled. The topology-sensitive DnaA-*oriC* interaction is unique for *H. pylori* and suggests that *H. pylori* orisome formation might be regulated by the control of DNA superhelicity. In addition we found that all three DnaA boxes at *oriC2* play important roles in orisome assembly and subsequent DNA unwinding, but different functions can be assigned to individual boxes. This suggests that the *H. pylori oriC* may be functionally divided, similar to what was described recently for *Escherichia coli oriC*. We have also identified a novel factor – the HP1021 protein – which, in addition to HobA, controls initiation complex formation and activity of the orisome. HP1021 is an orphan response regulator which upon binding to *oriC* interferes with interaction between DnaA and its boxes, which, in consequence, inhibits DNA opening at the helically unstable DUE region. It is not known whether some features of orisome formation, thus far unique for *H. pylori*, are also important for orisome assembly in related species. Thus we aim to identify *oriC* regions and analyze orisome formation in a few other selected epsilonproteobacteria. These studies should allow us to propose the initiation mode of the chromosomes of the epsilonbacteria and to indicate the common and unique features for the whole class. We hope that studies on epsilonproteobacteria together with other studies conducted in our laboratory (such as those concerning initiation of *Bdellovibrio bacteriovorus* chromosome replication) will help to extend our knowledge beyond the mechanism of initiation of chromosome replication in *E. coli*.

Bacteria from the genus *Streptomyces* are potent producers of polyketides – a large class of bioactive compounds with extremely diverse structures and functions. They are synthesized as secondary metabolites by giant multienzyme complexes – polyketide synthases. The secondary metabolism of streptomycetes is launched in concert with morphological differentiation, and the regulatory network which governs these processes is best studied in the model organism *Streptomyces coelicolor* A3(2). Our work is focused on a polyketide synthase Cpk from *S. coelicolor* A3(2). Cpk is responsible for the synthesis of a yellow pigment coelimycin. Expression of *cpk* genes is tightly controlled by regulatory proteins encoded by the genes within the *cpk* cluster and probably by several pleiotropic regulators.

We have also found that type II thioesterase ScoT from *Streptomyces coelicolor* A3(2) is required for the production of coelimycin. No production of coelimycin was observed in cultures of the *sco*T disruption mutant. Polyketide production was restored upon complementation with an intact copy of the *sco*T gene. An enzymatic assay showed that ScoT thioesterase can hydrolyze a 12-carbon acyl chain but the activity is too low to play a role in product release from the polyketide synthase. We conclude that ScoT is an editing enzyme necessary to maintain the activity of polyketide synthase Cpk. We provide a HPLC-based method to measure the amount of coelimycin P2 in a culture medium.

Laboratory of Signaling Proteins
Acting Head: Professor Janusz Matuszyk, Ph.D.
Studies on proteins and signaling pathways involved in activation of proinflammatory transcription factors and the response to hypoxia

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in synthesis of catecholamines such as dopamine, norepinephrine and epinephrine (adrenalin). Adenosine (produced from ATP) induces expression of the TH gene as well as synthesis of dopamine in PC12 rat pheochromocytoma cells. Results of our experiments indicate that phosphodiesterase 2 (PDE2) negatively regulates adenosine-induced transcription of the TH gene in PC12 cells. Studies have shown that the cGMP-stimulated PDE2 is involved in the atrial natriuretic peptide (ANP)-triggered reduction in the adenosine-induced accumulation of cAMP. Treatment of PC12 cells with 5,6-DM-cBIMP, a specific synthetic ligand for the GAF domains of PDE2, also reduced the cAMP level produced as a result of stimulation with adenosine. Moreover, the inhibition of PDE2 hydrolytic activity (using Bay 60-7550) abolished ANP (or 5,6-DM-cBIMP)-triggered reduction in the adenosine-induced production of the TH mRNA. The results of research also suggest that cAMP response element (CRE) present in the promoter region of the TH gene is involved in the adenosine-induced activation of the TH gene in PC12 cells, because introduction of a point mutation into the CRE motif in TH gene promoter region not only impairs the response to treatment with adenosine but also abolishes the inhibitory effect of ANP and 5,6-DM-cBIMP on activation of the TH gene promoter. Taken together, the results of our experiments suggest that enhancement of PDE2 hydrolytic activity may possibly lead to inhibition of CREB (the major CRE binding protein)-mediated TH gene transcription in PC12 cells.

DEPARTMENT OF CLINICAL IMMUNOLOGY

Laboratory of Clinical Immunology
Head: Professor Andrzej Lange, M.D.

Study on the pace of cellular immunity recovery in association with CNV sero-status in patients post-allo-HSCT

Laboratory surveillance of Herpes and polyoma viruses: results and clinical consequences
Viral complications constitute one of the main threats after HSCT, especially in patients who received transplants from unrelated donors. Therefore, all patients transplanted in our institution were followed for viral reactivation events including HHV6, CMV, EBV, polyoma BK, and JC.

The study included 156 unrelated donor HSCTs. 90 and 66 patients received myeloablative or reduced intensity conditioning. All patients except 3 received anti-lymphocyte antibodies (ATG [142 patients] and Campath [21]). The latter patients received ganciclovir as a part of microbial prophylaxis (6 mg/kg b.w. for 5 days). Quantitative detection of Herpes virus DNA copies (real-time PCR) was performed routinely at one-week intervals shortly after transplant and then at one-month intervals unless required earlier.

Results. CMV reactivation events were less frequent shortly after transplant than at later times (14% vs. 27%, p=0.003, before and after 30 days post-HSCT). Patients positive for CMV DNA copies (detected at 30-100 days post-HSCT) had higher levels of CD8+CD57+ lymphocytes in the blood than counterparts lacking CMV reactivation at any time after transplant (18.92 +/- 2.14% vs. 8.36 +/- 1.50%, p=0.0075; $186x10^6$ +/- $41.86x10^6$ vs. 84.15 +/- $32.53x10^6$ cells/L, p<0.001). CMV reactivation was associated with the presence of aGvHD grade II-IV (22/52 vs. 22/89, p=0.031).

In contrast to CMV, HHV6 reactivation events were rather more frequent in the early post-HSCT period than at later times (15% vs. 6%, p=0.096). Skin rash was frequent in HHV6-positive patients, but encephalitis was the main concern. Out of 12 encephalitis cases, 6 were HHV6-positive as compared to 22 positive cases out of 133 patients lacking encephalitis (p=0.015). EBV was seen at a similar frequency early and later post-HSCT and resulted in a higher percentage of CD4+CD57+ cells in the blood (3.00 +/- 0.58% vs. 1.42 +/- 0.27%, p=0.033; $56.045x10^6$ +/- $28.95x10^6$ cells/L vs. $8.89x10^6$ +/-$2.39x10^6$ cells/L, p=0.005). EBV reactivation was seen more frequently in patients with aGvHD (15/52 vs. 13/75, p=0.044) and in those with encephalitis (6/12 vs. 21/130, p=0.004) or lymphoproliferative syndrome (7/16 vs. 19/130, p=0.004). Reactivation of polyoma viruses

differed in terms of BK (earlier, median 41 days post-HSCT) and JC (later, median 80 days post-HSCT).

Conclusion: CMV reactivation seen later after transplant and a lack of apparent clinical symptoms may be related to the effect of prophylactic and pre-emptive ganciclovir treatment. The prevalence of CD8+CD57+ lymphocytes in the blood of CMV-positive cases documented the impact of the infection on the immune system. An increase in the proportions and numbers of CD4+CD57+ lymphocytes is a rather novel observation and demonstrates the profound effect of the EBV virus on the lymph nodes.

Reactivation of either HHV6 or EBV constituted the main risk factor of encephalitis. EBV is associated with lymphoproliferative syndrome in spite of the fact that EBV DNA positivity is usually preemptively treated with anti-CD20 antibodies. Overall survival is only marginally affected by reactivation of Herpes viruses, likely due to the pre-emptive treatment followed according to routine laboratory blood work, which includes viral surveillance.

Study on the presence of diversity of immune responsiveness (Th1 vs Th17) and nTrex activity in post-HSCT patients

Hematopoietic stem cell transplantation from anti-CMV-IgG positive donors facilitated post-transplant immunological recovery, which may indicate that chronic CMV infection has an effect on the immune system. This can be seen in the recipients after reconstitution with donor lymphocytes. We evaluated the composition of lymphocytes at hematologic recovery in 99 patients with hematologic malignancies after HSCT.

Anti-CMV IgG seropositivity of the donor was associated with higher proportions of CD4+ ($102.050\pm17.247x10^6$ vs. $227.963\pm304.858x10^6$ cells/l, p=0.009) and CD4+CD25high ($1.589\pm0.218x10^6$ vs. $3.456\pm0.436x10^6$ cells/l, p=0.003) lymphocytes in the blood at hematologic recovery. The latter parameter exerted a diverse influence on the risk of aGvHD if low ($1.483\pm0.360x10^6$ vs. $3.778\pm0.484x10^6$ cells/l, p<0.001) and *de novo* cGvHD if high ($3.778\pm0.780x10^6$ vs. $2.042\pm0.261x10^6$ cells/l, p=0.041). Higher values of CD4+ lymphocytes in patients who received transplants from anti-CMV-IgG-positive donors translated into a reduced demand for IgG support (23/63 vs. 19/33, p=0.048), and these patients also exhibited reduced susceptibility to CMV, EBV and/or HHV6 infection/reactivation (12/50 vs. 21/47, p=0.032). Finally, high levels (≥0.4%) of CD4+CD25high lymphocytes were significantly associated with better post-transplant survival (56% vs. 38%, 4-year survival, p=0.040).

Donors who experience CMV infection/reactivation provide the recipients with lymphocytes which readily reinforce the recovery of the transplanted patients' immune system.

Developing the technology of propagation of mesenchymal stem cells for use as a pre-emptive measure after HSCT

Marrow cells cultured for MSC include a population of cells expressing Oct4, Nanog and Sox2 genes, and this expression declines as the cells reach confluent growth.

Introduction: Bone marrow and mobilized blood constitute a convenient source of the hematopoietic progenitors used in HSCT. In the marrow, in addition to hematopoietic precursors, there reside cells giving rise to endothelial progenitors and stromal/mesenchymal stem cells (MSC) with multipotent characteristics. The latter cells have the ability to mitigate alloreactivity by a direct suppressive effect exerted upon lymphocytes. Therefore, cells reside in the marrow which directly interfere with the immune system and those which have pluripotent differentiation ability. Oct4 and Sox2 genes are essential for the pluripotency and self-renewal of embryonic stem cells. Notably, expression of these genes is critical for reprogramming somatic cells into pluripotent stem cells (induced).

Materials (or patients) and methods: Therefore, in this study we focused on the expression of Oct4 and Sox2 genes in fresh marrow cells, in PBPC mobilized to blood for autologous transplantation and in their offspring cultured in MSC growth-stimulating media. In addition, the expression of LIF, TERT and Rex-1 was investigated. Marrow cells were aspirated from the posterior iliac crest for further culture without any additional processing (5 samples) or from PBPC (2 samples). Cultures were performed in Falcon flasks in the presence of Minimal Essential Medium alpha and human platelet lysate (5%). The cultures were followed for the presence of 100% confluence (reached from 13 to 49 days). The cells after trypsinization were harvested, counted and phenotyped. In the cultures of marrow cells at harvest, CD45- CD34- cells constituted 91% (median value) of the population and CD105+, CD90+ and CD73+ were at 98%, 99% and 96%, respectively. Cells cultured from PBPC at harvest were only 53% CD45- CD34-. Custom-made Real Time Ready PCR included probes and primers for detection of the expression of Nanog, Oct4, Sox2, Rex-1, TERT, and LIF genes in relation to the reference genes (GUSB, RPL 13A). Results: Nanog, Oct4, and Sox2 were expressed in all samples; however, Oct4 expression was closest to the expression of the reference samples (usually in the range of 10E-1). Nanog and Sox2 expression was detected at a lower value range (usually 10E-3). Expression levels of all three of these genes were significantly correlated (R was 0.85, 0.86 and 0.72 for correlation between Oct4 and Nanog, Nanog and Sox2, and Oct4 and Sox2, respectively.)

Cells cultured to confluent growth had a much lower level of expression of the studied genes.

Conclusion: In conclusion, fresh populations of marrow cells and PBPC contain cells which express genes essential for propagation and self-renewal.

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

Immunogenetics of human diseases

Gene–gene interactions between CD40 and CD40L in Polish multiple sclerosis patients

CD40–CD40L interaction is necessary for activation of both the humoral and the cellular immune response and has been suggested to play a role in the pathogenesis of multiple sclerosis (MS). Therefore, we analyzed the combined influence of the CD40 and CD40L variants on MS susceptibility and progression in a well-defined Polish population. Our investigation revealed that CT individuals in the rs1883832 locus of CD40 possessed almost 1.5-fold higher risk for MS than CC individuals (OR = 1.44; 95%CI = 1.03–2.1; p = 0.032), while this risk for TT individuals was almost 2.5-fold higher (OR = 2.36; 95%CI = 1.19–4.78; p = 0.014).

Moreover, for the first time, we observed the association of the CD40 gene with MS development and progression. We observed that for the rs1883832CC individuals the age at diagnosis was on average 2 years lower than for the rs1883832CT and rs1883832TT individuals (95%CI = _3.69 _ (_0.29); p = 0.023). Additionally, we detected that individuals with TT and CT genotypes showed lower risk of developing a secondary progressive course in comparison to those with the CC genotype. For rs1883832TT individuals this risk was 4-fold lower (HR = 0.24; 95%CI = 0.10–0.53; p = 0.00062). Despite the fact that the CD40–CD40L pathway plays a key role in development of autoimmune diseases, we were not able to detect gene–gene interactions between CD40 and CD40L polymorphisms associated with multiple sclerosis.

ALCAM and CD6 — multiple sclerosis risk factors

ALCAM and CD6 may play an important role in the pathogenesis of multiple sclerosis (MS), since they are involved in the transmigration of leukocytes across the blood–brain barrier. In this study, we confirmed our previous findings about the association of the ALCAM gene with risk, development and progression of MS. Additionally, we demonstrated that in the case of the CD6 gene (encoding the receptor of ALCAM) not only polymorphisms but also mRNA expression level are associated with MS. Our analysis revealed that the risk of

the disease for AA individuals in rs12360861 was almost 3.0-fold lower in comparison to GG individuals (OR= 0.34; 95%CI= 0.12; 0.81). Moreover, we observed lower expression of CD6 mRNA in patients than in healthy individuals (T22,74= 6.678; p = 0.002).

Single nucleotide polymorphism -35 kb T>C (rs9264942) is strongly associated with psoriasis vulgaris depending on HLA-Cw⁄06

HLA class I molecules play a role both in viral infection control and in autoimmune disease development. rs9264942T>C polymorphism in the HLA-C gene was found to impact on HLA-C surface expression level and to be associated with HIV-1 control. It was found that those HLA alleles which protect against AIDS are associated with autoimmune disease, e.g. psoriasis vulgaris (PsV). Whether the rs9264942 SNP is associated with PsV was investigated here. rs9264942T>C was genotyped in 292 PsV patients and 254 controls using TaqMan Genotyping Assay.

PsV patients differed from controls in frequencies of rs9264942T>C alleles (p = 3.62 _ 10_16) and genotypes (5.67 _ 10_15). However, the rs9264942C allele predisposed to PsV three times more weakly than HLA-Cw⁄06 (OR = 5.04 vs. OR = 15.61, respectively). In addition, this SNP was described earlier to be in strong linkage disequilibrium (LD) with another SNP, rs67384697 ins/del, which by affecting microRNA binding is responsible for regulating HLA-C expression. However, typing for rs9264942 is cheaper and simpler than that for rs67384697; therefore we think it may substitute for it to some extent.

Presence of the full-length KIR2DS4 gene reduces the chance of rheumatoid arthritis patients to respond to methotrexate treatment

KIR genes coding for natural killer cell immunoglobulin-like receptors (KIR) influence effector and regulatory function of NK cells as well as some subpopulations of T lymphocytes (e.g. CD4+CD28-KIR+) depending on presence of ligands (particularly HLA-C molecules). KIR-KIR ligand interaction may lead to the development of autoimmune disorders, including rheumatoid arthritis (RA). However, their role in the response of RA patients to methotrexate therapy is not known.

KIR genes and KIR-ligand (*HLA-C* C1/C2 allomorphs) genotyping was performed using the PCR-SSP method in 312 RA patients (179 classified as good responders and 133 as poor responders using the ACR20 response parameter). Thus, we evaluated the association of *KIR* genes and *HLA-C* allomorphs with the response to methotrexate (MTX) treatment. We observed that patients possessing the full-length *KIR2DS4* gene (*KIR2DS4f*) had a lower

chance to respond in comparison to *KIR2DS4f*-negative cases. This phenomenon was observed both in erosive disease (ED) and rheumatoid factor (RF) positive and in ED- and RF-negative patients. Interestingly, the observed effect of the *KIR2DS4f* gene was strongest in individuals possessing medium values (20-33 mm/h) of the erythrocyte sedimentation rate (ESR). Patients with high ESR values had a low probability and, in contrast, patients with low ESR had a high probability of MTX response, and the presence of *KIR2DS4f* did not affect their outcome. Additionally, we found that the *KIR2DS4f* effect did not depend on the presence of either C1 or C2 allomorphs. Our results suggest that the response of RA patients with medium ESR values to MTX treatment is dependent on the *KIR2DS4* full-length gene.

Two new cases of *KIR3DP1, KIR2DL4*-negative genotypes, one of which is also lacking *KIR3DL2*

The killer immunoglobulin-like receptor (*KIR*) genes *KIR2DL4*, *KIR3DL2*, and *KIR3DP1* are present in virtually all humans. *KIR2DL4* encodes a receptor present on uterine and decidual natural killer (NK) cells and some peripheral blood NK cells. Its only known ligand is the human leukocyte antigen-G molecule expressed on extravillous trophoblasts, and on tissues in some diseases. KIR3DL2 binds HLA-A*03 and HLA-A*11 as well as HLA-B*27 dimers, and microbial CpG DNA. *KIR3DP1* is a pseudogene. During our immunogenetic studies we found two individuals, one from the Lower Silesia district in Poland, and another from Western Ukraine, who were reproducibly negative for *KIR2DL4* and *KIR3DP1* genes, using three different PCR systems. Both individuals displayed very similar genotypes, possessing only *KIR3DL3*, *KIR2DL3*, *KIR2DP1*, *KIR2DS1*, and probably a rare variant of *KIR2DL1*. The Pole also had *KIR3DL2*, which the Ukrainian was apparently lacking. Lower Silesia was populated after the Second World War by a remarkable percentage of displaced people from Western Ukraine, which might contribute to the genetic similarity of the two individuals described here.

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

VEGF and BFGF gene polymorphisms in patients with multiple myeloma

Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) play an important role in the initiation of angiogenesis. We aimed to assess whether polymorphisms located within the genes coding for these key angiogenic activators (*VEGF* (rs3025039; C>T) and *bFGF* (rs308395; G>C)) contribute to disease susceptibility and/or progression in patients with multiple myeloma (MM).

For this purpose, 133 patients with MM and 122 healthy individuals were typed for the *VEGF* and *bFGF* alleles by the PCR-RFLP technique.

Patients and healthy controls presented with similar distributions of the *VEGF* and *bFGF* alleles and genotypes. No association with disease susceptibility was found.

As in previous studies in patients with NHL (Wróbel et al. *Biomed Res Int*, 2013), a relationship was observed between the rs308395 *bFGF* polymorphism and course of the disease. Patients with the *CC* genotype were more likely to develop III stage of the disease than heterozygous and *GG* homozygous patients (Durie-Salmon criteria, p=0.052). Moreover, patients carrying the *G* allele responded significantly worse to the first line chemotherapy and more frequently developed progressive disease (p=0.022).

The course of disease in patients with multiple myeloma is associated with a polymorphism in the gene coding for bFGF.

Polymorphism and expression of proinflammatory factors in patients with rheumatic diseases treated with TNF-alpha inhibitors

The aim of this study was to analyze the polymorphism of the genes encoding IL-17A, IL-17F and IL-23R, IL-6 and nuclear factor kappa B (nF-κB) in patients with rheumatoid arthritis (RA), in patients with psoriatic arthritis (PsA) and in healthy controls and to determine the relationships between the various polymorphic variants, disease susceptibility, progression, and response to therapy with TNF-alpha inhibitors.

The study included 190 patients with RA, 54 with PsA and 125 healthy individuals. The *IL-17F* (rs763780, 7488 A>G, His161Arg) and *NF-KB1* (rs28362491, -94 ins/del ATTG) polymorphisms were analyzed using PCR-RFLP. For *IL-17A* (rs2275913; -197 G>A), *IL-23R* (rs11209026, 1142 G> A) and *IL-6* (rs1800795; -174 G> C) real-time PCR genotyping was employed, using either LightSNiP (rs2275913; TIB MOLBIOL GmbH, Berlin) or TaqMan SNP Genotyping Assay (rs11209026, rs1800795; Life Technologies, USA).

The presence of the *IL-17F* minor variant (*OR*=3.97; $p<0.001$) and its homozygosity (*OR*=29.62; $p<0.001$) was more frequent among patients than healthy individuals. The *IL-17A* polymorphism was found to affect RA progression and response to anti-TNF treatment. Female patients carrying the *IL-17A* wild-type genotype more frequently presented with stage 4 (8/24 vs. 6/47; $p=0.058$) and were characterized by more active disease (the highest DAS28 score >5.1) after three months of therapy with the TNF inhibitors (12/23 vs. 15/45; p=0.040). The *IL-17F* polymorphism appeared to be associated with susceptibility to the disease. These

results suggest that the polymorphisms within the *IL-17A* and *IL-17F* genes play a significant role in RA (Bogunia-Kubik et al. *Arch Immunol Ther Exp*, in press).

Better response to treatment also characterized patients with the *IL-6* heterozygous genotype (DAS28 <5.1 at 12 months, p=0.037). Moreover, patients with worse response to therapy with TNF-alpha blocking agents more often carried the *ins/ins* homozygous *NF-KB1* genotype, whose presence is associated with better activity of the promoter of this gene (and hence higher expression of pro-inflammatory cytokines) (p=0.05). None of the polymorphisms correlated with the incidence or course of PsA. However, higher IL-17 levels were observed in patients with the *GG* genotype of the IL-17A gene (p=0.069). Thus the *IL-17F* polymorphism was found to affect the RA susceptibility, while the *NF-KB1* and *IL-17A* polymorphic variants (associated with increased expression of these genes) less frequently occurred in patients responding to treatment.

HLA-E polymorphism in patients with rheumatoid and psoriatic arthritis

This study aimed to evaluate the role of the polymorphism within the gene encoding the non-classical HLA-E molecule in rheumatoid (RA) and psoriatic arthritis (PsA).

For this purpose 300 unrelated patients diagnosed with RA, 51 with PsA and 94 healthy controls were investigated and genotyped for the *HLA-E* polymorphism (rs1264457; 01:01/01:03; Arg128Gly) using Light SNiP typing assay. All RA patients included in the study fulfilled the American College of Rheumatology 1987 revised criteria for RA, had a disease activity score (DAS28) >5.1 prior to initiation of anti-TNF therapy and were resistant to treatment with at least at least two disease-modifying anti-rheumatic drugs (DMARDS).

Analysis of distributions of the genotypes and alleles did not reveal any associations between studied polymorphisms and RA risk. However, the clinical outcome of anti-TNF treatment in RA patients was found to be associated with these polymorphisms. After 12 weeks of treatment, the good/moderate response was more frequently displayed by female patients with HLA-E*01:01/01:01 genotype while 01:03 allele carriers were generally unresponsive to treatment (p=0.014). Also the frequency of the HLA-E*01:03/01:03 genotype was overrepresented among the non-responder group of patients in comparison to 01:01/01:01 homozygous patients (p=0.021).

Comparison of the PsA patients (n=51) and healthy control group confirmed the association between HLA-C*06 and PsA (OR=5.16, p<0.0001) and additionally revealed that the HLA-C*02 allele was more frequently observed in PsA patients (OR=5.40, p<0.0005). Interestingly, the HLA-E*01:01 allele was also found to be significantly over-represented

among HLA-C*02-negative patients in comparison to healthy individuals (OR=6.44, p=0.045). Therefore these results suggest that the HLA-E and HLA-C*02 molecules may also play an important role in determination of the immune response contributing to PsA development (Sokolik et al. *Human Immunol*, 2014).

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

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

Ikaros and RAG-2-mediated antisense transcription are responsible for inactivation of the promoter of the NWC gene in lymphocytes

NWC is the third gene within the *RAG-1/RAG-2* locus. It is widely accepted that *RAG-1* and *RAG-2* genes, which are indispensable for V(D)J recombination, have been acquired by jawed vertebrates as a result of a horizontal transfer of a mobile genetic element. The promoter of the *NWC* gene is located in close proximity to the *RAG-2* coding exon, and the structure of the *RAG/NWC* locus is remarkably well evolutionarily conserved in jawed vertebrates; however, homologues of the *NWC* gene can also be found in many invertebrate species. We have found that in contrast to promoters of *RAG* genes, the *NWC* promoter is active in nonlymphoid cells and is inactivated in lymphoid cells. The activity of the *NWC* promoter is controlled by the ZFP-143 transcription factor; its inactivation is controlled by the Ikaros protein competing with ZFP-143 for the binding sites in the promoter and promoter methylation caused by *RAG-2*-driven antisense transcription. We have also demonstrated that the NWC promoter has bidirectional activity which drives the expression of *RAG-2* transcripts in nonlymphoid cells. Taking into consideration all the above facts, we have put forward a hypothesis that the *NWC* locus was the integration site for the *RAG* transposon and the bidirectional activity of the *NWC* promoter (a feature which characterizes many promoters controlling transposon-derived genes) could initially facilitate the integration and survival of the *RAG* transposon, while additional mechanisms controlling the transcription activity of the *RAG* locus (i.e. *RAG* enhancers, inactivation of *NWC* promoter in lymphocytes) were acquired later in evolution to enable proper spatial and temporal expression of *RAG* genes.

To verify this hypothesis, we decided to examine the activity of the *NWC* promoter in several vertebrate (*H. sapiens*, *X. tropicalis*, *D. rerio*) and invertebrate species (*S. purpuratus*, *T. adhaerens*) to check whether the bidirectional activity of the *NWC* promoter is an evolutionarily conserved feature. To do this, we have:

- identified the transcriptional start site of *NWC* transcripts,

- identified the minimal fragments possessing promoter activity,
- characterized fragments indispensable for promoter activity,
- characterized the bidirectional activity of the promoters.

We have found that the *NWC* promoters of *H. sapiens*, *X. tropicalis* and *D. rerio* exhibit bidirectional activity and that the activity of the *H. sapiens* promoter is regulated by the ZNF-143 transcription factor. Although the binding sites for a ZFP-143 homologue (Staf-1) are present in the *X. tropicalis* promoter, their deletion did not significantly affect the promoter activity, suggesting that Staf-1 is not indispensable for the promoter activity. Identification of other factors regulating the *X. tropicalis NWC* promoter and characterization of invertebrate *NWC* promoters are the subjects of current investigations.

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

Dissecting molecular events associated with the development of acute T lymphoblastic leukemia in LAT deficient mice

LAT (Linker for Activation of T Cells) is an integral, transmembrane adaptor protein that coordinates the assembly of multiprotein signaling complexes operated by the T cell receptor (TCR). Preliminary studies showed that LAT knock-out mice but not control CD3ε knock-out mice, both harboring an allele of hyperactivated p56lck kinase (LCK^{Y505F}), develop fully penetrant T-cell acute lymphoblastic leukemia (T-ALL) and die before 17 weeks of age. In order to understand how the loss of LAT translates into oncogenic transformation of early thymocytes, we set out to elucidate the global picture of signaling events triggered by the hyperactivated Lck^{Y505F} kinase in the absence of the LAT signalosome, and in particular to define novel oncogenic substrates of Lck^{Y505F} by phosphoproteome analysis. We also searched for differences in global gene expression in preleukemic and control thymocytes in order to find transcripts specifically induced or suppressed by the Lck^{Y505F} allele. Moreover, we sought to establish novel cell lines derived from the primary thymic lymphomas of LAT KO x LCK^{Y505F} mice as tools for proteomic and transcriptomic analyses. To this end we defined expression levels of 88 genes that were reportedly associated with T-ALL. Among transcripts significantly upregulated in pre-leukemic CD4+CD8+ thymocytes of LAT KO x LCK^{Y505F} mice we found a signature of 8 genes associated with Notch 1 signaling (*Notch1*, *Notch3*, *Hes*, and *Ptcra*) that were strongly overexpressed. Interestingly, the above-mentioned gene signature is consistently found in over 60% of human T-ALL. Therefore the model of LAT KO x LCK^{Y505F} mice closely reflects common signaling events leading to oncogenic transformation of human thymocytes. To dissect alterations in intracellular signaling

associated with loss of the LAT signalosome we measured the activation status of LCK^{Y505F} kinase and found that it was hyperactive. To search for aberrant LCK^{Y505F} kinase substrates we prepared phosphopeptide libraries of control and pre-leukemic thymocytes for protein identification by mass spectrometry. One of the candidate proteins that undergoes LCK^{Y505F} mediated activation is the protein kinase C theta (PKCΘ), which was shown to activate the c-Raf/MEK1/2/Erk pathway in LAT^{Y126F} knock-in mice. We seek to confirm a role played by PKCΘ in the oncogenic transformation of LAT KO x LCK^{Y505F} thymocytes. For this purpose we established 5 novel cell lines from the primary thymic lymphoma of LAT KO x LCK^{Y505F} mice. These cell lines were shown to have productive TCRβ rearrangements and to express markers of CD3-CD4-CD8- (TN) T cell precursors. They should be useful in defining transcriptome profiles and in dissecting signaling pathways associated with T-ALL.

LABORATORY OF GLYCOBIOLOGY AND CELLULAR INTERACTIONS
Head: Professor Danuta Duś, Ph.D.

Mechanisms of tumor progression. Intercellular adhesive interactions during metastatic spread of cancer cells
Biology of endothelial progenitor cells

In 2014 investigations on the biology of endothelial progenitor cells were continued. The studies aimed to determine the participation of these kinds of cells in normal regenerative processes.

Tests were carried out on two human lines of early endothelial progenitor cells: HEPC-CB.1 and HEPC-CB.2 The secretion profile of these cells was determined using a membrane protein RayBio Custom C-Series Human Cytokine Antibody Array. It has been shown that HEPC-CB.1 and HEPC-CB.2 cells produce 13 out of 48 cytokines tested. Cytokines produced in the largest quantities are IL-8 and MCP-1. The production of all cytokines changes over time and depends on oxygen conditions. Similar studies were also performed for two mature endothelial cell (EC) lines: HUVEC (endothelial cells derived from the umbilical vein) and HskMEC.2 (endothelial cells derived from the skin). Comparison of the results leads to the conclusion that only 5 cytokines are shared by both types of cells. EPC produce 8 and EC produce 9 additional cytokines.

Additionally, a functional assay on the reconstituted extracellular matrix (Matrigel) was performed. It was found that the factors secreted by HEPC-CB.1 and HEPC-CB.2 cell lines increase the yield of tubular structure formation on Matrigel by mature endothelial HUVEC cells.

It has been found that the established endothelial progenitor cell lines, as well as the mature endothelial cells, are able to secrete a variety of growth factors and cytokines. Secretion profile analysis distinguishes between EPC and EC. Factors produced by endothelial progenitor cells support mature endothelial cell angiogenesis.

The study will allow for a better understanding of the biology of endothelial progenitor cells. Understanding of the factors supporting angiogenesis, secreted by endothelial progenitors, will allow the future use of these cells for the treatment of diseases involving a damaged endothelial layer (e.g. in myocardial infarction or hard-to-heal wounds).

The role of ceramide galactosyltransferase (UGT8) and galactosylceramide (GalCer) in cellular response and multidrug resistance of breast cancer cells

Our previous studies have shown that the suppression of UDP-galactosylceramide galactosyltransferase (UGT8), which synthesizes galactosylceramide (GalCer), in breast cancer MDA-MD-231 cells has a profound effect on their tumorigenic and metastatic properties. In accordance with this finding, immunohistochemical staining of tumor specimens revealed that expression of UGT8, accompanied by accumulation of galactosylceramide, is associated with a much higher proliferative index and a lower number of apoptotic cells. Based on these results, we proposed that presence of GalCer protects breast cancer cells from cellular stresses induced by the tumor microenvironment, and probably its cytoprotective effect is associated with increased resistance to stress-induced apoptosis. Additional evidence suggesting the involvement of GalCer as an apoptosis "protector" came from our experiments on doxorubicin-induced apoptosis in breast cancer cells. It was found that expression of UGT8 in MDA-MB-231 cells was associated with their resistance to this anti-cancer drug, suggesting that UGT8 may also be involved in multidrug resistance (MDR) of cancer cells. Therefore, the present study was undertaken to identify those cellular stressors or microenvironmental factors which are associated with the anti-apoptotic effect of UGT8 and GalCer, and elucidate the role played by them in multidrug resistance of breast cancer cells. Using three different cellular models, including human breast cancer MDA-*MB*-231 cells with decreased expression of UGT8 and human breast cancer T47D cells and murine mammary carcinoma 4T1 cells with *de novo* expression of UGT8, we have shown that the presence of GalCer makes these cells more resistant to hypoxia-induced apoptosis. Using the same approach, we have also shown that all breast cancer cells with expression of UGT8 and accumulation of GalCer are resistant to apoptosis induced by doxorubicin and paclitaxel.

Publication - 2014

Articles published in the journals from Thomson Reuters Master Journal List:

1. Antoszczak M, Maj E, Napiórkowska A, Stefańska J, Augustynowicz-Kopeć A, Wietrzyk J, Janczak J, Brzezinski B, Huczyński A. Synthesis, anticancer and antibacterial activity of salinomycin *N*-benzyl amides. *Molecules,* 2014, 19, 19435-19459; doi:10.3390/ molecules191219435 **IF – 2,095 (30 pkt)**
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