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

Research Report 2008

Laboratory of Bacteriophages
Head: Professor Andrzej Górski, M.D.
Research on the biology of bacteriophages and their use in the treatment of bacterial infections

Changes in C-reactive protein (CRP) concentration in serum of patients with chronic symptomatic antibiotic therapy-resistant bacterial infections (inflammation of the bone, peri-prosthesis, or soft tissue) which qualified for phage treatment within the project "Experimental Phage Therapy of Antibiotic Therapy-Resistant Infections, Including MRSA Infections" were retrospectively analyzed. In the majority of cases inflammation was caused by *S. aureus* infection. There was a statistically significant decrease in mean CRP concentration from 42.7±8.5 mg/ml to 24.8±6.7 mg/ml during days 9-32 of treatment in 13 patients who had baseline CRP above 10 mg/l, while in the 13 patients with baseline CRP below 10 mg/l, a insignificant increase from 3.8±0.7 mg/ml to 7.4±2.8 mg/ml was observed. Detailed analysis of the changes in CRP level during treatment depending on the route of administration of the phage preparation showed the greatest decrease in mean CRP serum level during days 9-32 of treatment when topical administration was applied (58.1%, n = 4), while for oral and oral/topical administration the decreases were 31.9% (n = 12) and 9.1%, respectively (n = 4). However, these changes did not reach statistical significance.

Research on the isolation of phages specific to *Enterococcus faecalis* strains was continued with use of 47 environmental samples. Eighteen specific bacteriophages (12 virulent and 6 temperate) were isolated. The range of lytic activity analysis showed sensitivity in 73% of 144 *E. faecalis* strains tested. It was found that temperate bacteriophages demonstrated a broader lytic spectrum (51% positive lytic reaction) than the virulent bacteriophages (38% positive lytic reaction). Electron microscopic study showed that the isolated phages belonged to two families: *Myoviridae* (morphological type A1) and *Siphoviridae* (morphological type B3).

The presence, frequencies, and concentrations of *E. coli* bacteriophages in the stools of healthy volunteers, patients with states predisposing to cancer development (intestinal polyps, Crohn's disease, colitis ulcerosa), and colorectal cancer patients in a Polish population were studied. Stools of 98 individuals of the above groups were studied. Coliphage frequencies for the *E. coli B* strain were lower in the stools of the patients with states predisposing to cancer development and those with colorectal cancer as compared with the healthy individuals. Lower presence of coliphages for *E. coli*

1962 strain was found in the feces samples of the patients with intestinal polyps, Crohn's disease, and colitis ulcerosa, excluding the patients with colorectal cancer, than in healthy individuals. A similar relationship as that found for *E. coli B* was obtained between the frequencies of phages in patients compared with healthy individuals when the data for the two indicator strains *E. coli B* and *E. coli 1962* were combined. The concentrations of coliphages for the *E. coli 1962* and *E. coli B* strains were greater in patients with states predisposing to cancer development and those with colorectal cancer than in healthy volunteers. Our results suggest that there may be associations between phage presence in the human gastrointestinal tract and some gastrointestinal diseases.

Results of grant activities

The alimentary transit of two morphologically different phages was studied. It was observed that neutralization of gastric juice with dihydroxyaluminium sodium carbonate significantly increased the ability of *S. aureus* phage A5 and *E. coli* M13 phage to pass from the stomach to the intestine when the neutralizer was given up to 15 min. before phage administration. It was shown that use of a proton pomp inhibitor to inhibit gastric juice secretion also increased the gastrointestinal transit of phages after oral administration.

In 2008 the first transplantation of olfactory ensheathing cells (OECs) for the treatment of complete spinal cord injury was done in Department of Neurosurgery, Wroclaw Medical University, in a 27-year-old male who had sustained a complete (ASIA-A) thoracic T10-11 injury four years earlier from a knife wound. OECs were isolated at the IIET three times from the patient’s olfactory tissue. The first time they had been cultured in the presence of neurotrophin-3 and frozen in liquid nitrogen to be unfrozen and recultured just before surgery. Three and two weeks before the transplantation a few fragments of the patient’s olfactory mucosa were taken once again using a neuroendoscope and cranial neuronavigation for OEC culture (part of the cultures grew in the presence of supernatants from cadaver-derived OEC cultures). For transplantation, an area of the spinal-cord injury was exposed by a two-level laminectomy, fibrous adhesions were removed, and a suspension of OECs and accompanying olfactory fibroblasts in cell cultures were microinjected into the area surrounding the injury site. Four weeks after the operation there were no adverse effects attributed to the procedures and no adverse complications due to OEC transplantation were noted after transplantation. The patient continues neurorehabilitation. Observation of the safety and functional outcomes will be carried out for two years.

We previously observed binding of T4 phage to the membranes of cancer and normal blood cells. We selected a mutant, HAP1, with enhanced affinity for melanoma cells. Both T4 and HAP1 markedly and significantly inhibited experimental lung metastasis of murine B16 melanoma and

HAP1 was more effective than T4. We also found a mutation in the *hoc* gene that differentiates bacteriophage HAP1 and its parental strain T4: a non-sense type mutation that precludes proper synthesis of gpHoc. In this study we continued investigations of phage-eukaryotic cell interactions. The migration of human (Hs294T) and mouse melanoma (B16) on fibronectin was inhibited by purified T4 and HAP1 bacteriophage preparations; migration of B16 was inhibited by 34% (p=0.0235) and 36% (0.0164), respectively, compared with the control and by 42% (p=0.0008) and 44% (p=0.0006), respectively, compared with 10 U/ml LPS identical to the residual LPS content in the phage preparations. Hs294T migration was decreased by 31% (p=0.0423) by T4 compared with PBS. A significant difference between PBS and HAP1 was not observed (28%, p=0.0859). The migration of human melanoma was also inhibited by the HAP1 phage preparation on matrigel: 48% (p=0.0407). No response of either melanoma cell line to lipopolysaccharide was observed. Therefore the effect of the phage preparations cannot be attributed to lipopolysaccharide. No differences in the effects of T4 and HAP1 on melanoma migration were observed.

DEPARTMENT OF CLINICAL IMMUNOLOGY
Head: Professor Andrzej Lange, M.D.

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

Laboratory of Clinical Immunology continued the collaborative work with the Lower Silesian Center for Cellular Transplantation with the National Polish Bone Marrow Donor Registry in the field of immunology and immunogenetics of hematopoietic stem cell transplantation (HSCT). In 2008 the collaborative studies resulted in the following findings:

1. The function of CD4+CD25++ cells, described as regulatory cells (Tregs) exerting immunosuppression in patients after allogeneic hematopoietic stem cell transplantation (alloHSCT). In this study 74 cases were followed post alloHSCT for the presence of CD4+CD25++, CD134, and FoxP3 lymphocytes in relation to acute graft-versus-host disease (aGvHD), CMV, EBV, and HHV6 reactivation during 100 days post HSCT, and clinical outcome of transplantation. It appeared that patients who developed aGvHD had significantly higher proportions and numbers of CD4+CD25++ cells on the first day of aGvHD manifestation than those without aGvHD when examined at a similar time post-transplant (p=0.05). CD134 co-expression analysis was used to evaluate the activation of CD4+ cells. CD134+ lymphocytes and CD4+CD25+ CD134+ cells followed the pattern of an increase in CD4+CD25++ cells. Patients with aGvHD had higher proportions and numbers of CD4+CD134+ cells than those lacking aGvHD when examined at a similar time post-transplant

(p=0.02). This was also seen in the CD4+CD25++ cell population, which became more frequently CD134+ in individuals with than in those without aGvHD (p=0.03). In addition, significant correlations were found between CD4+CD134+ and CD4+CD25++CD134+ cells regardless of whether proportions (r=0.69, p=0.00002) or counts were considered (r=0.28, p=0.029). Over 76.3% of CD4+CD25++ lymphocytes were FoxP3 positive. This increase in CD4+CD25++ cells in patients after transplantation had a functional impact as it was found that (i) higher proportions of Tregs were associated with reactivation of herpes viruses (EBV or CMV or HHV6) and this relationship was especially visible in EBV positive cases (p=0.046), (ii) the proportion of Tregs inversely correlated with the proportion of EBV pentamers (r=-0.18, p=0.09), (iii) patients with marrow failure or relapse had higher peak values of CD4+CD25++ cells than those without these complications measured during 82 days of observation (p=0.057), and (iv) patients examined two weeks before the fatal outcome of aGvHD had higher proportions of CD4+CD25++ cells than cases responding to the steroids (p=0.02).

2. The hypothesis that the KIR repertoire of the donor might influence the chimerism status of children with immunodeficiencies after HSCT was based on an analyse of 27 children with immunodeficiencies who received allogeneic HSCT. It was observed that a greater number of patients transplanted from donors carrying more than one activating KIR gene (haplotype B) developed complete chimerism, while patients with mixed chimerism had a lower number of donors with this haplotype (p=0.054). Univariate analysis revealed that diagnosis, type of donor, source of transplant material, mean number of CD34+ transplanted cells, and number of procedures did not influence post-transplant chimerism. A multivariate stepwise logistic regression model showed that if more than one activatory KIR gene in donors and/or recipients was lacking C1 or C2 ligands that were present in donors, this had a positive effect on achieving complete chimerism (p=0.005, OR=12.25).

3. The association between microsatellite polymorphism within the first intron of the interferon-gamma (*IFNG*) gene and the proportion of CD4 lymphocytes in peripheral blood with the development of CMV reactivation in patients after HSCT. Individuals having less than 10% CD4+ lymphocytes in blood more frequently presented CMV reactivation (p=0.011) and higher levels of CMV copies (p<0.001) than those with ≥10% CD4+ cells. Similarly, the *IFNG* 3/3 homozygous patients more frequently had CMV reactivation (p=0.022) and higher CMV load (p=0.022). Multivariate analysis confirmed that the *IFNG* 3/3 low-producer genotype (12 CA repeats) (p=0.030) and lower proportion (<10%) of CD4 lymphocytes in blood (p=0.011) constitute two independent factors associated with a higher risk for CMV reactivation.

Laboratory of Immunogenetics
Head: Professor Piotr Kuśnierczyk, Ph.D.
Association of KIR2DS4 and its variant KIR1D with leukemia

We found a difference in the frequencies of partial deletion in *KIR2DS4* gene (p=0.03) and its full-length variant in patients with chronic myeloid leukemia compared with their bone marrow donors. The lack of the transmembrane domain indicates that the product of *KIR1D* is a soluble protein. If it binds to HLA class I and other potential ligands in a way like fully functional KIRs, then it may play a regulatory role by masking ligands for their receptors. Our observation suggests that such interaction may be of particular importance in the absence of intact KIR2DS4 and may decrease the activity of NK cells against CML (*Leukemia*, 2008, 22, 2129-2130).

DEPARTMENT OF MICROBIOLOGY
Head: Professor Jolanta Zakrzewska-Czerwińska, Ph.D.

Laboratory of the Molecular Biology of Microorganisms
Head: Professor Jolanta Zakrzewska-Czerwińska, Ph.D.
The molecular basis of replication and segregation of bacterial chromosomes

Home page: www.iitd.pan.wroc.pl/dept/mic/index.htm

SMC-protein-dependent chromosome condensation during aerial hyphal development in Streptomyces

Members of the SMC (structural maintenance of chromosomes) protein family play a central role in higher-order chromosome dynamics, from bacteria to humans. So far, studies on bacterial SMC proteins have been focused only on unicellular rod-shaped organisms that divide by binary fission. *Streptomyces*, Gram-positive soil bacteria known for their ability to produce many valuable antibiotics and other secondary metabolites, are among the most striking examples of multicellular bacteria. *Streptomyces* colonies consist of a branched vegetative mycelium bearing aerial hyphae that form long chains of exospores. Both vegetative and rapidly growing aerial hyphae are multigenomic, with the latter containing up to 50 uncondensed copies of the linear chromosome in one tip compartment. As an aerial hyphae stops growing, the chromosomes condense and are segregated into unigenomic pre-spore compartments which metamorphose into chains of easily separated spores.

We focused on the contribution of SMC proteins to sporulation-associated chromosome segregation in *S. coelicolor*. Deletion of the *smc* gene causes aberrant DNA condensation and missegregation of chromosomes (7.5% anucleate spores). In vegetative mycelium, immunostained SMC proteins were observed sporadically, while in aerial hyphae about to undergo sporulation they appeared as irregular multiple foci which accompanied, but did not co-localize with, ParB complexes. Our data demonstrate that efficient chromosome segregation requires the joint action of SMC and ParB proteins. SMC proteins, similarly to ParAB and FtsZ, presumably belong to a larger

group of proteins whose expression is highly induced in response to the requirement of aerial hyphal maturation.

Laboratory of Signaling Proteins
Head: Professor Wojciech Gorczyca, Ph.D.
Studies on proteins involved in the activation of proinflammatory transcription factors in immune cells

In 2008 we continued studies on the possible relationship between the expression of proteins belonging to the cAMP/cGMP signaling pathway and the activity of proinflammatory transcription factors. We determined which of the proteins potentially forming the pathway are present in the myeloid and lymphoid cells and analyzed the factors affecting their expression. First, the profiles of activity and the expression of guanylate cyclases (GCs) in rat peripheral blood mononuclear cells (PBMCs) were established. It was found that both the cytosolic (soluble, sGC) and the membrane (GC-A and GC-B) forms of GCs are present in freshly isolated cells. The simultaneous expression of both the cytosolic and membrane forms of GCs distinguishes rat from human PBMCs, which express only active sGC. Therefore rat PBMCs were found to be an useful model for studying the effects of cGMP depending on the site of its synthesis. Using RT-PCR and Western blotting, we also demonstrated the presence of cGMP-dependent protein kinase (PKG-I). It was observed, however, that during prolonged cell culture the rat PBMCs lost the expression of sGC as well as of PKG-I.

In subsequent studies we determined which cyclic nucleotide phosphodiesterases (PDEs) are expressed in the rat PBMCs. It was found that mRNA of isoforms PDE1C, PDE2A, PDE3A, PDE3B, PDE4A, PDE4B, PDE4C, PDE4D, PDE5A, PDE9A, and PDE11A is present in these cells. Using inhibitors selective for PDEs belonging to families 2,3,4, and 9, we found that these PDE are active in rat PBMCs. It has been shown that inhibition of PDEs belonging to the families PDE2, PDE3, and PDE4 results in the suppressed expression of cytokines IL-1β, IL-6, and TNF-α and , in the case of LPS-stimulated cells, in the expression of inducible nitric oxide synthase (iNOS).

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

Laboratory of Immunobiology
Head: Professor Michał Zimecki, Ph.D.
Studies on synthetic and natural immunoregulators of potential application in prevention and therapy

Studies on the mechanism of action of lactoferrin (LF) on the mouse and human immune systems were continued. We demonstrated that recombinant human LF abolishes the suppressive activity of methotrexate in the model of secondary humoral immune response to sheep erythrocytes *in vitro*.

This action was dependent on the presence of sialic acid in the LF molecule. Blocking the sialoadhesin receptor with sialic acid or specific anti-receptor antibodies abrogated the effects of LF. In another experimental model we demonstrated that the suppressive action of LF in mitogen-induced human lymphocyte proliferation may not be caused by the binding of phytohemagglutinin (PHA) by LF since LF also inhibited cell proliferation induced by immobilized anti-CD3 antibodies. It appeared that in this experimental model, several recombinant forms of LF exhibited suppressive activity. That activity did not depend on the presence of sialic acid in the LF molecule.

We also investigated the immunotropic activities of ten compounds from the azophenothiazine family in several *in vitro* and *in vivo* experimental models. We selected one compound which appeared to be a strong suppressor of the humoral and cellular immune response in mice and of the proliferative response of human lymphocytes to PHA and anti-CD3 antibodies. The compound was also low-toxic.

We also continued investigations on the suppressive activity of modified cyclolinopeptide fragments. Some of them are characterized by strong activity and low toxicity.

Studies on the efficacy of phage therapy in immunocompromised mice were performed. Mice were treated with busulfan and cyclophosphamide followed by a syngeneic bone marrow transfer and infected with *Staphylococcus aureus*. The application of specific A5 phages significantly reduced the number of bacterial colony-forming units in the liver and spleen of infected mice. It also provided long-term survival for 80% of the mice infected with a lethal dose of bacteria. In addition, the administration of phages induced characteristic changes in the blood and bone marrow cell type pictures indicating mobilization of myelopoiesis by the phages. The results suggest a benefit of phage therapy in immunocompromised patients.

Other part of research was focused on bone-associated cells, i.e. fibroblast and chondrocytes. These cells possess the ability to acquire a phenotype allowing them to interact with bone matrix and influence bone remodeling or healing. Fibroblasts and chondrocytes are mesenchymal cells. Fibroblasts are cells capable of acquiring a feature typical of other cells of mesenchymal origin, i.e. osteoblasts (bone-forming cells), chondrocytes (cartilage-forming cells), and adipocytes (fat cells). There are several proteins extremely important for osteoblast and osteoclast maturation, differentiation, and activity, such as (i) bone morphogenetic proteins (BMP2 and BMP4), which are chemo-attractive molecules for osteoblasts, (ii) osteopontin (OPN), a protein deposited in bone matrix which determines osteoclast adhesion to bone surface, (iii) ostreoprotegerin (OPG), a soluble ligand which disturbs osteoclast maturation/activation due to interaction with a receptor on their surface, and (v) osteocalcin (OCL), which is highly specific to osteoblast phenotype molecule. Fibroblasts and chondrocytes were examined using RT-PCR or real-time PCR and, in some cases,

Western blot for the expressions of these markers important for bone remodeling or their influence on osteoblasts or osteoclasts. The results showed multipotential capability of all cells to express the abovementioned markers. It seems reasonable to suspect an influence of fibroblasts on bone metabolism *via* regulation of osteoblast and osteoclast activity. This is possible due to BMP and OPG synthesis. The expression of OPN, absent on normal chondrocytes, could be the cause of successful knee joint surface healing after re-implantation of these cells. Moreover, the amount of OPN mRNA increased during chondrocyte propagation. The main aim of this work was to investigate the influence of bone-associated cells on bone metabolism. We tried to demonstrate a regulatory and/or osteoblastic character of fibroblasts as well as the therapeutic usefulness of autologically implanted chondrocytes. The results showed that there is a wide perspective for clinical treatment with these cells. Moreover, these cells appear useful in testing implant materials. The tolerance of implanted material is essential for tissue healing and homeostasis. The implant surface is able to modify the behavior of the recipient's adhering cells and tissue. Therefore the other aim of this study was to examine changes that occur in fibroblasts grown on titanium and steel surfaces and the influence of the roughness of these surface on gene expression of this cells and cell morphology.

Fibroblasts are cells connecting the bone and immunological systems. They possess a specific ligand on the surface called RANKL, which is critical for interaction with dendritic cells from the immune system and osteoclasts as well as their precursors from bone. Fibroblasts are also sensitive to cytokines produced by macrophages and lymphocytes and are able to secrete these factors under inflammatory conditions. The expression of the osteoblast-like phenotype by fibroblasts in response to heterogeneous modulating factors was monitored by RT-PCR and confirmed by real-time RT-PCR. Osteoprotegerin (OPG), osteocalcin (OSC), osteopontin (OPN), and receptor activator for nuclear factor κ B ligand (RANKL), the molecules specific for mature osteoblasts. The cytotoxicity of titanium and steel surfaces were tested in fibroblast culture and compared with cell culture on a plastic surface. The main measured culture parameters were cell number, mortality, and adhesion rate. The results showed good tolerance for the materials used. Fibroblasts were collected and tested for cytokine gene expression and osteoblast-specific gene expression. Our results demonstrate that autological fibroblasts could be a soft-tissue implant interface favorable for implant surface colonization by recipient's tissue, which open new perspectives for optimizing implant surfaces.

Laboratory of Immunopathology
Head: Professor Irena Frydecka, M.D.
Studies on the mechanisms of immune deficiency in neoplastic and autoimmunological diseases

Association studies of CTLA-4, CD28, and ICOS gene polymorphisms with B-cell chronic lymphocytic leukemia (B-CLL) in the Polish population

Abnormal expression of the costimulatory molecules: cytotoxic T-lymphocyte antigen 4 (CTLA-4), CD28, and "inducible co-stimulator" (ICOS), lead to disturbances in immune response and may result in an increased risk of cancer. An extended study was undertaken to evaluate the association between the polymorphisms *CTLA-4c.49A>G*, *CTLA-4g.319C>T*, *CTLA-4 g.*642*AT(8_33), *CD28c.17+3T>C*, and *ICOSc.1554+4*GT(8_15) and B-CLL morbidity in Polish population. Increased frequencies of the *CTLA-4g.319C>T* [T] allele and the *CTLA-4g.319C>T* [T] phenotype were found in B-CLL patients compared with healthy controls (p=0.003, *OR*: 1.73 and p=0.009, *OR*: 1.74, respectively). The presence of the *CD28c.17+3T*>C [C] allele and the *CD28c.17+3T*>C [C] phenotype increased the *OR* of B-CLL to 1.59 (p=0.007) and 1.74 (p=0.007), respectively. Either *CTLA-4g.319C>T* or *CD28c.17+3T*>C was found to associate with time to Rai stage progression. The distributions of the alleles and genotypes of the *ICOS* gene significantly differed in patients and controls (p=0.0009 and p=0.006, respectively). Individuals possessing short alleles were 2.02 times more prone to B-CLL than others (p=0.001), while carriers of long alleles were protected from B-CLL (p=0.02, *OR*: 0.62). Haplotype association study and multivariate analysis confirmed the association of *CTLA-4 g.319C>T* and *ICOS c.1554+4*GT(8_15) gene polymorphisms with B-CLL. The polymorphic sites *CTLA-4c.49A>G* and *CTLA-4 g.*642*AT(8_33) did not correlate with B-CLL. Our results are the first in the literature to report that gene polymorphism of the costimulatory molecules CTLA-4, CD28, and ICOS contributes to the susceptibility to B-CLL.

CTLA-4 gene polymorphisms are associated with CTLA-4 protein expression levels in multiple sclerosis patients and with susceptibility to disease

Cytotoxic T- lymphocyte antigen- 4 (CTLA-4) is an important molecule in the down-regulation of T-cell activation. A study was undertaken to evaluate the association of the *CTLA-4* gene polymorphisms -319C/T, +49A/G, $(AT)_n$, CT60A/G, and Jo31G/T with levels of membrane (mCTLA-4) and cytoplasmic CTLA-4 (cCTLA-4) in CD4+ lymphocyte from multiple sclerosis (MS) patients, with susceptibility to MS, and course of disease. We found that the Jo31GG and the CT60GG genotypes were associated with decreased mean fluorescent intensity (MFI) of total CTLA-4 (sum of mCTLA-4 and cCTLA-4) molecules in CD4+ T cells from both relapsing-remitting (RR) and secondary progressive (SP) patients (total MFI for RR and SP patients: 39.6 vs. 29.6, p=0.03 and 39.6 vs. 33.8, p=0.05, respectively) compared with other patients. Consequently, possessing the Jo31G allele and the CT60G allele was associated with susceptibility to MS (p=0.03 and p=0.04, respectively). Similarly to MFI, the percentage of cells expressing mCTLA-4 and cCTLA-4 in RR patients were higher in individuals possessing the alleles non-predisposing to MS (CT60A and Jo31T) (mCTLA-4: 2.25 vs. 0.6, p=0.05 and 2.20 vs. 0.95, ns, respectively; cCTLA-4:

3.0 vs. 0.9, ns, and 3.0 vs. 1.1, ns, respectively), but, unexpectedly, in SP patients the percentages of the corresponding cells were significantly lower for those patients than in RR patients (medians: CT60A: 2.25 vs. 0.55, p=0.0005 and 3.0 vs. 0.55, p=0.005; Jo31T: 2.2 vs. 0.6, p=0.01 and 3.0 vs. 0.6, p=0.02, respectively). We noted earlier transition to the SP form in these patients. We hypothesized that decreasing frequencies of cells expressing immunosuppressive mCTLA-4 and cCTLA-4 in patients carrying alleles non-predisposing to MS (CT60A and Jo31T) may lead to inadequate down-regulation of ongoing T-cell responses in these patients and consequently earlier progression of disease from the RR to the SP form.

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

Plesiomonas shigelloides, a Gram-negative enterobacterial rod, is responsible for cases of water- and food-borne outbreaks of intestinal infections. *P. shigelloides* is usually found in fish, crabs, prawns, mussels, and oysters. It is one of the most frequent causes of travelers' diarrhea in Japan and China (third position in rankings). Incidents of extra-intestinal infections, most notably meningitidis in neonates, bacteremia, sepsis, and septic shock, were reported for this bacterium. Sepsis and meningitidis caused by *P. shigelloides* are associated with a serious course and high fatality rate. The pathogenicity of *P. shigelloides* is not yet fully understood. Cholera-like toxin, thermostable and thermolabile beta-hemolysin toxins, and cytotoxin complex containing lipopolysaccharide (LPS, endotoxin) were described as possible virulence factors of *P. shigelloides*. Among them, LPS, the main constituent of the outer cell wall, seems to be one of the least characterized virulence factors of *P. shigelloides*. To date only a few structures of *P. shigelloides* LPSs, i.e. the O-specific polysaccharides from strains 22074, 122548, and 302-73 (serotype O1), the core oligosaccharide substituted with one O-repeat for strain O17, and two complete LPSs isolated from *P. shigelloides* O54 and O74, have been elucidated. *P. shigelloides* strain CNCTC 110/92 (O51) was identified as a new example of plesiomonad-synthesizing lipopolysaccharides (LPS) that show preference for a non-aqueous surrounding during phenol/water extraction. Chemical analyses combined with ^{1}H and ^{13}C NMR spectroscopy, MALDI-TOF, and ESI mass spectrometry showed that the repeating units of the O-specific polysaccharides isolated from phenol- and water-phase LPSs of *P. shigelloides* O51 have the same structure: →4)-beta-D-Glc*p*NAc3NRA-(1→4)-alpha-L-Fuc*p*Am3OAc-(1→3)-alpha-D-Qui*p*NAc-(1→, containing rare sugar constituent 2,3-diamino-2,3-dideoxyglucuronic acid

(Glc*p*NAc3NRA) and substituents such as D-3-hydroxybutyric acid (R) and an acetamidino group (Am). The HR-MAS NMR spectra obtained for the isolated LPSs and directly on bacteria indicated that the O-acetylation pattern was consistent throughout the entire preparation. The ^{1}H chemical shift values of the structure reporter groups identified in the isolated O-antigens matched those present on bacteria. We found that the O-antigens recovered from the phenol phase showed a higher degree of polymerization than those isolated from the water phase. As there were no structural differences between the O-repeats obtained from the phenol and water phases, the hydrophobicity of *P. shigelloides* CNCTC 110/92 LPS was correlated with the number of O-repeats in the O-antigens.

Laboratory of Glycoconjugate Immunochemistry
Head: Professor Hubert Krotkiewski, Ph.D.
Immunochemical and genetic studies of human glycophorin and other proteins active in the immune system

Molecular background of NOR polyagglutination. Polyagglutination is an agglutination of erythrocytes by antibodies present in most human sera. NOR polyagglutination is a rare inheritable polyagglutination caused by the presence of the NOR antigen on erythrocytes of NOR-positive individuals. The NOR antigen is a unique carbohydrate sequence (Galα1$\rightarrow$4 GalNAc1-) linked to a ceramide. Such a glycosphingolipid has not been found in mammals except for NOR-positive humans (two families identified so far). We speculate that the NOR antigen appears as a result of a mutation in genes encoding one of the known human glycosyltransferases. To elucidate the possible cause of NOR polyagglutination, we performed the following experiments:

1. we sequenced the exons encoding catalytic fragments of the following glycosyltransferases from NOR-positive individuals:
   a) Forssman transferase (Galα1$\rightarrow$3GalNAc); not active in humans
   b) Pk transferase (Galα1$\rightarrow$4Gal)
   c) ABO transferase (A: GalNAcα1$\rightarrow$3Gal or B: Gal α1$\rightarrow$3Gal)
   and no mutations were found.
2. We sequenced the promoter region of the gene encoding Pk transferase from NOR-positive individuals. No mutations were found.
3. In all NOR-positive individuals of the Polish family we found a copy of O^{1v} gene, which is an allele of *ABO* gene (LOD=2.4). This suggests that the putative "NOR enzyme" may be located on the same (ninth) chromosome as gene *ABO*. We plan to evaluate the following genes located on the same chromosome: α1,3-galactosyltransferase (inactive in humans) and glycosyl-transferase 6 (open reading frame encoding a glycosyltransferase belonging to the same family (6) of glycosyltransfarases).

Glycophorin B, which is a transmembrane glycoprotein of human erythrocytes, carries sS blood group antigens. The peptidic epitopes of eight anti-glycophorin B monoclonal antibodies were identified with the use of peptides synthesized on plastic pins (the *pepscan* method). Five of them with anti-s specificity showed the strongest binding to peptides containing the sequence ^{31}QLVHRF^{36} of the glycophorin B polypeptide chain. One antibody, defined serologically as anti-U (anti-GPB reactive, despite the sS status), recognized an amino-acid sequence common to glycophorins A and B (aa 21-25).

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

A proline-rich polypeptide complex (PRP) with immunoregulatory and procognitive activities, when sublinqually administered, shows beneficial effects in the Alzheimer's disease (AD). The mechanism of action of PRP is not yet fully explained. However, we can assume that the beneficial clinical effects of PRP may involve modification of cytokine release, functional and/or phenotypic differentiation of cells, effect on neurite outgrowth, and reduction of fibril formation and Aβ aggregation. PRP is also involved in the regulation of cellular redox status, NO release, and iNOS activity.

A number of psychiatric and neurodegenerative disorders, including AD, are characterized by abnormalities in the neuronal cytoskeleton. Actin microfilaments are one of its major component. The structural and motile functions of actin are possible through a dynamic conversion between monomeric actin (G actin) and filamentous actin (F actin). Amyloid β peptides forming senile plaques stimulate actin polymerization. Reorganization of the cytoskeleton can affect cell adhesion and modulate cell-cell and/or cell-Aβ interaction.

The cell adhesion process was studied on THP-1 and HL-60 cells undifferentiated and differentiated with vitamin D_3 or PMA in the absence or presence of PRP. The adhesive properties of the cells were studied using culture plates uncoated or coated with fibronectin in the presence or absence of various effectors (PRP, fMLP, and PMA). HL-60 cells, both undifferentiated and differentiated with vitamin D_3, showed a very low adherence and no effect of PRP was observed. However, adherence of vitamin D_3-differentiated cells to uncoated and fibronectin-coated plates was elevated in a statistically significant manner, both in the presence of PMA and PRP. No effect of PRP on adhesion of PMA-differentiated HL-60 cells was observed. In more mature THP-1 cells, adherence to both uncoated and fibronectin coated plates was elevated after incubation with vitamin D_3 or PMA. However, no effect of PRP was observed.

The maturation and differentiation of cells are accompanied by cytoskeletal reorganization, especially in the actin microfilament system. Changes in the levels of G and F actin were monitored during differentiation of HL-60 and THP-1 cells with vitamin D_3 in the absence and presence of PRP. The differentiation of HL-60 cells induced by vitamin D_3 was accompanied by higher F and G actin levels. The elevation was abolished in the presence of PRP. In the case of THP-1 cells the levels of the actin fractions were not changed during the differentiation of the cells.

The results suggest that, similar to the case of functional and phenotypic maturation of cells, PRP can affect the cell skeleton at an early step of differentiation. Cytoskeletal reorganization could be involved in the regulatory effects of PRP on cells' effector functions.

Studies on the transcriptional regulation of the gene encoding the human neonatal Fcγ receptor (hFcRn)

The human neonatal Fcγ receptor (hFcRn), structurally related to the MHC class I proteins, was first discovered in the placenta; however, its expression has recently been identified in a variety of cell types and tissues of humans at all ages. The human neonatal Fcγ receptor is a key receptor involved in the transport of maternal IgG to the fetus, providing protective immunity to the newborn. The second important role of hFcRn is the protection of IgG from catabolism and the maintenance IgG homeostasis. In addition, hFcRn directs the transport of IgG and antigen/IgG complexes across mucosal epithelium and also enhances phagocytosis in neutrophils.

In previous studies, Mikulska et al. isolated the gene from the human genome encoding the heavy chain of the human FcRn and sequenced and determined the organization of the exon/intron. Moreover, we characterized the 5'flanking region of the human gene for hFcRn (mapping the transcription start sites and identifying the hFcRn promoter). These studies provide a starting point for examining the transcriptional regulation of this physiologically important gene. For studies on the regulation of hFcRn transcription, the following human cell lines were chosen: THP1 (human monocyte cell line), Caco-2 (human colon adenocarcinoma cell line), Lu106 (human embryo lungs fibroblasts cell line), and HUVEC (human umbilical vein endothelial cell line). These cell lines were chosen because they represent cell types in which the expression of hFcRn has been detected *in vivo*.

In the first step of our studies we checked the presence of the hFcRn transcript in the cell lines by reverse transcription-polymerase chain reaction (RT-PCR). An RT-PCR product of the expected size (457 bp) was obtained from all lines using a pair of hFcRn-specific primers from exons 3 and 4, respectively. Sequencing of this PCR fragment demonstrated 100% identity with the previously reported sequence of the human FcRn gene. These data showed that these cell lines would be good candidates for studies on the regulation of hFcRn transcription. We prepared nuclear extracts from

the chosen cell lines to begin the examination of the nuclear proteins with the promoter region of the human hFcRn gene.

Laboratory of Glycobiology
Head: Professor Maciej Ugorski, Ph.D., D.V.M.
Organ-specific metastatic growth of human breast cancer cells is dependent on the expression of sialyl Le^a and sialyl Le^x antigens

Several lines of evidence indicate that sialyl Le^a and sialyl Le^x, tumor-associated carbohydrate antigens present on human carcinoma cells, are involved in the formation of metastases. Therefore two different experimental approaches were applied to study whether the metastatic properties of breast cancer are directly affected by changes in the expression of these carbohydrate structures. First, a specific gain-of-function phenotype was developed by transfecting BO2 breast cancer cells with cDNA for α1,3/4-fucosyltransferase (Fut3), which is involved in the synthesis of sialyl Le tetrasaccharides; second, a loss-of-function phenotype was created using the RNAi approach to inhibit the expression of *FUT3* gene in MCF10A (M-IV) cells. When BO2GFP.FUT3 cells, expressing a high level of sialylated Lewis structures, were injected intracardially into athymic nu/nu mice, 62% of the animals developed lung metastases, in contrast to the parental sialyl Lewis-negative BO2.GFP cells which did not form colonies in this organ. In MCF10A (M-IV) breast cancer cells with decreased expression of sialyl Le^a and sialyl Le^x, qualitative differences in the degree of colonization of lung tissues were found when lung metastases were analyzed by fluorescence imaging. The average fluorescence intensity of lungs colonized by control MCF10A.GFP cells was four times higher than that of lungs colonized by modified MCF10A.GFP.shFUT3 cells. In addition, only modified cells developed micrometastases in intestinal lymph nodes, as shown also by fluorescence imaging. Taken together, based on the results of these two experimental models it was found that the expression of sialyl Le^a and sialyl Le^x antigens in breast cancer cells facilitates their entrapment in the lung. It is speculated that the arrest of breast cancer cells in certain organs and therefore their organ-specific localization are at least in part E-selectin mediated and involve their interaction with endothelial cells.

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

Laboratory of Tumor Molecular Immunobiology
Head: Professor Leon Strządała, Ph.D.
The mitochondrial localization of RelB and NFATx in normal and tumor-transformed thymocytes

The strict control of the localization of transcription factors is crucial for their proper function. An activated transcription factor migrates from the cytoplasm to the nucleus, where it regulates gene

expression to initiate the appropriate genetic program. An increasing number of reports reveal that "nuclear" transcription factors can also undergo translocation to the mitochondria under certain conditions. We have shown for the first time the localization of RelB and NFATx in the mitochondrial fractions of normal thymocytes and thymic lymphoma cells. The mitochondrial localization of NFATx was specifically controlled by a calcium signal sensitive to FK506, an immunosuppressant and inhibitor of calcineurin, while RelB localization remained mitochondrial in normal and tumor-transformed thymocytes. Not all the transcription factors were directed to the mitochondria, as we observed no colocalization of Hsp60 with CREB or with p50. These data show that the mitochondrial localization of NFATx is precisely controlled by a calcium signal in immature T cells. It is worth mentioning that the distributions of all the examined proteins were comparable in normal and transformed thymocytes and could therefore be general for immature T cells. These observations are interesting since the subcellular distribution of transcriptions factors is crucial for their function. Do transcription factors deliver any information to the mitochondria as elements of signaling pathways or serve as adaptor proteins at the surface of the mitochondrial membrane? Is it merely an additional way of sequestering them far away from the nucleus (see NFATx after ionomycin with FK506)? Alternatively, mitochondrial NFATx or RelB could be involved in other calcium-regulated and/or energy-dependent biological processes, such as proliferation or differentiation.

Laboratory of Experimental Anticancer Therapy
Acting head: Assistant Professor Joanna Wietrzyk, Ph.D.
Studies of the mechanisms of tumor progression and metastasis and the effects of experimental antitumor therapy

The influence of 1,25- and 1,24-dihydroxyvitamin D_3 on $\alpha_v\beta_3$ integrin expression in cancer cell lines

Integrins are cell-surface receptors engaged in important cancer invasion processes, such as adhesion, migration, proliferation, and differentiation. The aim of this study was to evaluate the effect of 1,25-dihydroxyvitamin D_3 (calcitriol) and its metabolite 1,24-dihydroxyvitamin D_3 (PRI-2191) on $\alpha_v\beta_3$ integrin expression in various cancer cell lines. The expressions of the β_3 and α_v integrins were reduced only in the WEHI-3 and LLC cell lines by both compounds. Calcitriol or PRI-2191 treatment caused differentiation of WEHI-3 mouse leukemia cells, but apoptosis of LLC cells. WEHI-3 and LLC cells exposed to calcitriol or PRI-2191 lost their migratory and adhesive potentials. The inhibition of migratory potential was higher in the LLC cells than in the WEHI-3 cells and appeared to correlate with the increased down-regulation of $\alpha_v\beta_3$ integrin by calcitriol or PRI-2191. It appears that the observed *in vivo* effects (antitumor and antimetastatic) in mice bearing

subcutaneously transplanted LLC cancer could be associated with inhibited migratory potential as a consequence of the lowered integrin expression caused by calcitriol or PRI-2191.

Modulation of antitumor effect of cytostatics by bacteriophage (BP T4) applied in combined treatment of B16 melanoma-bearing mice

The past few years have shown a significant resurgent interest in the old concept of using phages as a therapeutic agent. Some research groups continue to develop whole phages as alternatives to antibiotic antibacterial treatment. However, improvements in the methods of purification of phage preparations opens new opportunities for the successful treatment of antibiotic-resistant bacterial infections. An open question remains whether a bacteriophage T4 preparation (BP T4), prepared and purified at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, could be safely applied in the treatment of patients with bacterial infections as consequences of immunosuppression, for example in the course of anticancer treatment.

The aim of this study was to evaluate the possible modulating effect of BP T4 administered to mice bearing injected *s.c.* or *i.v.* B16 melanoma and treated with known widely used anticancer drugs (cyclophosphamide (CY), cisplatin (CPt), or 5-fluorouracil (5-FU)). Our studies showed that application of BP T4 neither decreased the anticancer effects of CY, CPt, and 5-FU nor the antimetastatic effects of CPt and 5-FU. Moreover, treatment of mice with BP T4 potentiates the antimetastatic effect of CY.

Estimation of the effect of dendritic or tumor cell vaccine on anti-tumor cytolytic response in tumor-bearing mice

In the first study the effect of chemotherapy with CBM-4A ifosfamide derivative and/or IL-12-producing cells on infiltration with CD8+ and CD4+ cells in mice bearing HPV 16-associated tumors was examined. This revealed decreased CD4+ and CD8+ TIL numbers after chemotherapy and their restoration after combined therapy. In the second study the effect of IL-2-producing cell administration on infiltration with CD4+ and CD8+ TIL in mice bearing MC38 colon carcinoma was evaluated. The findings demonstrated a visible increase in CD4+ T-cell influx and slight changes in the number of CD8+ T cells. The increase in TIL influx was accompanied by increased numbers of $CD8^+CD107^+$ and $CD49b^+CD107^+$ cytotoxic cells in restimulated splenocytes from the treated mice.

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

Studies on the methotrexate-fibrinogen conjugates

The Laboratory of Biomedical Chemistry is focused on the development of drug-carrier conjugates for the treatment of experimental cancer and immunological diseases. We investigate the

biochemical properties and biological activities of protein (fibrinogen, albumin, antibodies) and carbohydrate (glucose or mannose polymers) methotrexate and raltitrexed conjugates.

Physiochemical studies of bacteriophages

Besides the chemical modification of macromolecules, we are investigating the physicochemical properties of bacterial viruses, or bacteriophages. In particular, we aim to develop an effective procedure for the purification of bacterial viruses.

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 immune response

The main topics of study in our Laboratory are the mechanisms of pathogenicity of diseases of bacterial etiology, the role of molecular mimicry, bacterial proteins and glycolipids in pathogenicity, and the structure and functions of bacterial capsular antigens and endotoxins. In the search for molecular markers of diseases, a diagnostic potential of oxidative stress markers in children and adolescents with type 1 diabetes was found (*Clin. Biochem*. 2008, 41(1-2), 48-55). The development of protective tools against invading bacteria comprises the determination of structures of molecules involved in infection and immune processes and understanding their biological activities and potential mimicry of human tissue antigens. It was found that antibodies against *Citrobacter braakii* O37 cells recognize the N-glycan of the band 3 glycoprotein of human erythrocyte membrane (*FEMS Immunol. Med. Microbiol*. 2008, 52(3), 352-361). Infection with such mimicking pathogen might induce autoantibodies and autoimmune disease. Regarding the studies on the protective properties of the 38-kDa enterobacterial outer membrane protein, the search of data bases revealed a peptide in this protein containing the protective epitope. In our studies on glycation processes, experiments reveled the inhibition of human muscle-specific enolase by methylglyoxal and irreversible formation of advanced glycation end products (*J. Enzyme Inhib. Med. Chem*. 2008, Oct 1:1. [Epub ahead of print] PMID: 18830874). It was found that the substrate and magnesium ions protect the enzyme activity from glycation with methylglyoxal. This relevant result indicates that the enzyme important for a vital process is protected to a high degree against inhibition by oxidation products.

Laboratory of Virology
Head: Professor Zofia Błach-Olszewska, Ph.D.
Study on nonspecific immunity in viral infection

In our earlier study the resistance of human peripheral blood leukocytes (PBLs) to viral infection was found to be an innate antiviral immune mechanism. To find which subpopulation of PBLs is responsible for the resistance, different cell fractions were separated and the degree of resistance to VSV infection was measured. All cell fractions (adherent, enriched in T or B lymphocytes, NK) expressed higher resistance than whole PBLs population. NK cells were found the most resistant PBLs fraction to VSV infection. The results were published in *Folia Histoch. Cyt*., 2008, 46, 39-43.

In view of innate immunity deficiency observed in elderly people, and also in larygological patients and patients with cancer, the effect of *Scutellaria baicalensis* flavones and donepezil on the resistance of PBL to VSV infection was studied. Inhibition of VSV replication indicates stimulation of PBL resistance. The effect of both agents in PBLs was dose dependent. Inhibition of VSV replication in L_{929} and A_{549} cell lines by both agents was not observed. Both donepezil and the plant flavones reduced or modulated cytokine production (TNF, IFNs, IL-10, IL-12) by non-infected and VSV-infected human PBLs. The results were published in *J. Neurological Scieces*, 2008, 273, 75-80 and in *J. Interferon Cytokine Res.,* 2008, 28, 571-58

Early arthritis is the inflammation of one or more joints which appears for the first time and lasts shortly. The role of STAT proteins, which belong to a family of latent cytoplasmic transcription factors activated in response to cell stimulation through various cytokine receptors, in autoimmune diseases is being widely discussed. It is postulated that STATs play a vital role in rheumatoid arthritis. The aim of the study was to investigate STAT activation levels in the leukocytes of early arthritis patients. The increased activation levels of STAT-3 and STAT-6 in the leukocytes of early arthritis patients confirms the importance of STATs in the pathogenesis of autoimmune arthritis. The lack of differences in STAT-1 activation may result from the early stage of the diseases, the heterogeneity of the patient group, the fact that STAT-1 was predominately expressed in cells which do not migrate to the peripheral blood, or inhibition by *c-fos* gene. The lack of differences in STAT-1, -3, and -6 activation in the subgroups of early arthritis patients supports the hypothesis of the undifferentiated character of the onset of these types of inflammatory arthritis. The results were published in *Adv. Clin. Exp. Med*., 2008, 17(4), 399-404.

The study of Jak/STAT activation in RA (rheumatoid arthritis) describes constitutive STAT-3 DNA binding activity in freshly isolated RA mononuclear cells. Data suggest that rheumatoid arthritis synovial fibroblast survival and their abnormal phenotype are dependent on STAT-3. The objective was to evaluate the activity of STAT-3 in peripheral blood leukocytes of patients with RA

and compare it with parameters of disease activity used in clinical practice. The activity of STAT-3 was higher in RA patients (significantly higher in RA women) than in a group of healthy controls. The results were published in *Adv. Clin. Exp. Med.,* 2008, 17(4), 405-409.

Nitric oxide (NO) is a small, very reactive molecule involved in many physiological processes, including immune response. We previously reported increased levels of NO metabolites in the serum of patients with rheumatoid arthritis (RA) and osteoarthritis. The aim was to evaluate the activity of iNOS in peripheral blood leukocytes (PBLs) in patients with early arthritis (EA) and established RA and to compare it with parameters of disease activity used in clinical practice. As the difference between iNOS expression in PBLs in patients with RA and with EA cannot be attributed to differences in disease activity, further observation is needed to determine whether higher iNOS expression in some patients with early arthritis can predict future RA development. The higher iNOS expression in smokers with EA needs further study aimed at determining whether smoking induces iNOS activity and thereby promotes RA development. The results were published in *Adv. Clin. Exp. Med.,* 2008, 17(4), 415-421.

In another study we evaluated three kinds of restorable materials for bone replacement prepared on the basis of calcium sulfate ($CaSO_4 \cdot 1/2H_2O$ with 0.5% mass $KHSO_4$). One of them was enhanced with poly(alcohol-vinyl) to increase its mechanical resistance. The second was enriched with tri-calcium phosphate, a growth activator of bone tissue. A third material, calcium sulfate without additions, was used as a control. To evaluate their biocompatibility and inflammatory effect we investigated the activity of nuclear factor kappaB (NF-κB) and proinflammatory cytokine concentrations (TNF-α, IL-6, and IL-8) in peripheral human leukocytes after stimulation *in vitro* with the tested materials. In the tests it was observed that the unmodified calcium sulfate materials activated NF-κB after 24-hour incubation and did not significantly decrease its expression after 72 hours. Calcium sulfate materials with an addition of poly(alcohol-vinyl) did not activate NF-κB, while calcium sulfate with tri-calcium phosphate was toxic for leukocytes after both 24 and 72 hours of incubation. The levels of TNF-α, IL-6, and IL-8 after 24 and 72 hours stimulation with gypsum materials were comparable to those of untreated leukocytes. The results were published in (*BIO)Degradable Polymers from Renewable Resources from RENEWABLE RESOURCES*. Editor: A. J. Nadolny. 2008, 83-87.

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

Laboratory of Transgenesis and Lymphocyte Biology
Head: Professor Paweł Kisielow, Ph.D.

Role of NWC transcription in teh regulation of Recombination Activating Gene (RAG) genes in normal and tumor cells: progress report

We continued our efforts to uncover and understand the possible role of *NWC* transcription in the regulation of expression of *RAG* genes by generating genetic constructs which would allow us to monitor the activities of these genes in different cell lineages during the development of transgenic mice made with these constructs. Bacterial artificial chromosome (BAC) containing the entire *RAG* locus, including flanking regulatory sequences, was modified in such a way that the expression of *RAG2* gene was marked by GFP (Green Fluorescent Protein) reporter gene while the expression of *NWC* was marked by YFP (Yellow Fluorescent Protein) reporter gene. We call this construct BAC-NY. Last year we further modified BAC-NY and generated BAC-M1 and BAC-M2 constructs. In BAC-M1, the *RAG-2* intragenic *NWC* promoter, which in non-lymphocytes is active but in lymphocytes is silenced by methylation, was deleted. Therefore the transcription of *NWC* in non-lymphocytes from its own promoter is not possible. In BAC-M2, the *RAG-2* intragenic *NWC* promoter was replaced by the proximal *Lck* promoter, which is constitutively active in lymphocytes and therefore ensures constitutive transcription of *NWC* also in lymphocytes. Transgenic founder mice were obtained by microinjection of the BAC-NY and BAC-M1 constructs into the zygotes of C57/Bl6 mice. Breeding these founder mice resulted in transgenic F1 offspring, born with the expected Mendelian frequency. One of the BAC-M1 transgenic founders developed aggressive thymoma over-expressing RAG genes. The significance of this observation as well as the expression of transgenes during normal development is under study.

As a side track of our major line of investigation we identified a homologue of the *NWC* gene in the sea urchin in which homologues of *RAG* genes were recently discovered. Interestingly, in contrast to vertebrates, *RAG* genes, whose function in sea urchin is unknown, are ubiquitously expressed and *NWC* is not located within the *RAG* locus. This observation would be expected if our hypothesis concerning the role of *NWC* in restricting *RAG* expression to lymphocytes is correct.

DEPARTMENT OF MEDICAL IMMUNOLOGY
Head: Professor Jacek C. Szepietowski, M.D.

Author of report: Janusz Matuszyk, Ph.D.
Study on the mechanisms of neurotrophin-dependent activation of transcription factors

Nur77 (also termed NGFI-B, TR3, NR4A1) and its family members Nurr1 and Nor-1 are orphan nuclear receptors that play important roles in neuronal differentiation, memory consolidation, stress response, and apoptosis. The promoter region of the *nur77* gene contains four near AP-1 (NAP) sites 5'-TGCGTCA. The results of Yoon & Lau (MCB 1994, 14, 7731) supported the hypothesis that JunD, but not CREB, binding to two proximal NAP elements is responsible for the induction of transcription of *nur77* in response to nerve growth factor. However, the results of our study indicate that A-CREB (the dominant negative mutant of CREB) inhibits the activation of the *nur77* promoter in response to the activation of TrkC (receptor of neurotrophin-3). In conclusion it is suggested that activation of the *nur77* promoter in response to neurotrophins requires the cooperation of both CREB and JunD.

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

In 2008 the investigations of the laboratory's members were focused on the presence and possible role of estrogen receptor alpha (ER alpha) in immune cells. The presence of estrogen receptors in immune cells gives the possibility of direct influence of estrogens on immune response development. It seems that ER alpha plays the main role in immune-endocrine interactions mediated by this hormone. We investigated the expression of ER alpha in lymphocyte subpopulations during immunization of male mice with syngenic antigen. Immunization evoked increased expression of ER alpha in all the examined cell subpopulations (CD3+CD4+, CD3+CD8+, CD19+, MHCII+CD86+, and F4/80+MHCII+). On the other hand, orally administered tamoxifen significantly decreased the level of ER alpha in $CD86^{+}$MHCII+ and F480+MHCII+ cells. It was also observed that immunization increased the frequency of $MHCII^{+}CD86^{+}$ cells and $MHCII^{+}CD86^{-}$ cells. In immunized animals treated with tamoxifen the frequency of CD86+MHCII+, but not CD86+MHC^{-}, decreased significantly in comparison with immunized-only animals. The experiment showed that the frequency of MHCII+ cells is dependent on estrogen regulation.

We also investigated the expression of ER alpha in macrophages isolated from lymphoid organs and uterus of cyclic and pregnant mice. We showed that the relative level of ER alpha in splenic and uterine macrophages isolated from female mice is influenced by the ovarian cycle and is lowest

during the estrus phase of the cycle. Moreover, in mated mice the relative level of ER alpha in splenic macrophages was elevated 0.5 days after mating. In contrast, this effect was not observed in uterine macrophages. The elevated level of ERα observed shortly after mating in splenic, but not in uterine, macrophages indicates an early systemic response to male antigens.

It was also shown that the level of ERα was higher in mature macrophages ($F4/80^+MHC\ II^+$ cells) than in immature cells ($F4/80^+MHC\ II^-$) in the examined females. Correlation between the percentage of mature macrophages in the spleen and the level of ERα was observed, which confirmed the interrelation between the presence of ER alpha and MHC II expression in antigen-presenting cells

Laboratory of Cellular Interactions
Head: Associate Professor Danuta Duś, Ph.D.
New markers of tumor progression. Cancer cell-endothelial cell interactions during metastatic spread of cancer cells

The variable outcome of cancer patients with similar clinical status creates a need for the search for new reliable prognostic indicators of tumor progression, recurrence, and survival. The aim of the study was to determine the prognostic value of new molecular traits of tumor cells in breast cancer which could determine tumor progression and metastatic growth. A relationship between the expression of Fas death receptor and/or its cell surface ligand FasL in breast cancer patients and the presence of tumor cells in perilymphatic fat regions were evaluated. Breast carcinoma samples of 147 patients of Dr. M. Bębenek of the Regional Comprehensive Cancer Center in Wroclaw were examined immunohistochemically. The study showed that lack of Fas in primary breast cancer is associated with perilymphatic fat infiltration (p=0.042). Consequently, both the absence of Fas in the primary tumor and the occurrence of neoplatic cells in paranodal fatty tissue should be considered in the prognosis, complementing existing conventional factors. Is should be underlined that presently, no single factor may be considered as an independent predictor of survival in multivariate analysis [Bebenek M. et al., *Adv. Med. Sci*. 2008;53,49-53].

An original model of organ-specific immortalized and stabilized human endothelial cell lines was elaborated for the evaluation of tumor cell-endothelial cell interactions taking place during the metastatic process. EC lines established from human lymph node, appendix, lung, skin, and intestinal microvessels were developed previously on the basis of collaboration with the group of Dr. C. Kieda*. For the purpose of the study a new flow cytometric assay for quantitative determination of adhesive interactions of human endothelial cells with tumor cells was developed. Endothelial cells labeled with a fluorescent dye were grown to confluency in 24-well TC plates. A human colon

adenocarcinoma cell suspension was overlaid onto labeled ECs and allowed to adhere for 20 min under static conditions. Adhering tumor cells together with endothelial cells were then detached from the culture plate. The collected cell fractions were evaluated by flow cytometry. Results were calculated as the ratio (R) of adhering colon carcinoma cells per one endothelial cell. We demonstrated that immortalized human microvascular ECs preserved their organ specificity. Colon carcinoma cells adhered preferentially to ECs of intestinal origin. The organ specificity of the endothelial cell interactions with colon carcinoma cells demonstrated under static conditions was verified and confirmed with a flow adhesion assay. The developed method is suitable for quantifying tumor cells adhering to ECs with simultaneous evaluation of cell surface phenotypic markers of both partner cells participating in adhesive interactions. The immunofluorescent staining of adhering and non-adhering cancer cell subpopulations revealed an augmented level of Lewis(x) antigen on the adhering cancer cells. This assay, validated by comparison with a dynamic shear stress adhesion assay in blood flow reconstituted conditions, greatly facilitates the evaluation of tumor cell-endothelial cell interactions occurring during metastatic processes [Paprocka M. et al., *Microvascular Research,* 2008; 76(2), 134-138].

Endothelial cells are critical in the recruitment and migration of circulating effector cells to sites of inflammation and necrosis as well as in tumor cell extravasation from blood vessels, involving close adhesive interactions of cancer cells with endothelial cells at the site of cancer cell extravasation. The association of chemokines with endothelial cells and extracellular matrices is required for the *in vivo* pro-migratory activity of these molecules. Chemokine receptor expression and chemokine presentation were investigated on organ-specific human endothelial cell lines. Experiments with CCL21 on peripheral lymph node endothelial cells demonstrated that the chemokine did not co-localize with its receptor, but was associated with extracellular matrix components. The specific activity of chemokines was clearly shown to be related to the endothelial cell origin. CX3CL1 and CCL21 promoted lymphocyte recruitment by endothelial cells from the appendix and peripheral lymph nodes, respectively, while CX3CL1 activity was restricted to endothelial cells from the appendix and skin. This unique cellular model demonstrated a fundamental role for chemokines in conferring organ-specificity to the endothelium and its potential for tissue targeting through the selective binding, presentation, and activation properties of chemokines [Crola da Silva C. et al., *Immunology* 2008; Epub].

*Studies on endothelial cells were performed in collaboration with Dr. Claudine Kieda, CBM CNRS UPR 4301, Orleans, France.

Publications - 2008

Published original articles:

1. Baran W., Szepietowski J.C., Mazur G., Baran E.: IL-6 and IL-10 promoter gene polymorphisms in psoriasis vulgaris. *Acta Derm. Venereol*., 2008, 88(2), 113-116 **IF – 1,927**
2. Baran W., Szepietowski J.C., Mazur G., Baran E.: IFN-gamma promoter gene polymorphism in psoriasis vulgaris. *Biomarkers*, 2008, 13(1), 52-58 **IF – 1,978**
3. Bębenek M., Duś D., Koźlak J.: Fas expression in primary breast cancer is related to neoplastic infiltration of perilymphatic fat. *Adv. Med. Sci*., 2008, 53, 49-53
4. Błach-Olszewska Z., Jatczak B., Rak A., Lorenc M., Gulanowski B., Drobna A., Lamer-Zarawska E.: Production of cytokines and stimulation of resistance to viral infection in human leukocytes by *Scutellaria baicalensis* flavones. *J. Interferon Cytok. Res*., 2008, 28, 571-582 **IF - 2,667**
5. Bogacka E., Zwiefka A., Gatsios D.: Problemy diagnostyczne i terapeutyczne POChP w praktyce lekarza rodzinnego. W: „*Wybrane zagadnienia z praktyki lekarza rodzinnego”*. Red. Steciwko A., wyd. Continuo. 2008, vol. 12, 17-31, Eng. Version 105-119
6. Bogunia-Kubik K., Lange A.: Development of the HLA Proficiency Testing for Central and East Europe. *Int. J. Immunogenet*., 2008, 35, 409-416 **IF – 1,279**
7. Bogunia-Kubik K., Mazur G., Wróbel T., Kuliczkowski K., Lange A.: Interleukin-10 gene polymorphisms influence the clinical course of non-Hodgkin's lymphoma. *Tissue Antigens*, 2008, 71(2), 146-150 **IF – 2,245**
8. Bogunia-Kubik K., Middleton P., Norden J., Dickinson A., Lange A.: Association of vitamin D receptor polymorphisms with the outcome of allogeneic haematopoietic stem cell transplantation. *Int. J. Immunogenet*., 2008, 35(3), 207-213 **IF – 1,279**
9. Bonkowska K., Biecek P., Laszkiewicz A., Cebrat S.: Relationship between the selection pressure and the rate of mutation accumulation. *Banach Center Publ*., 2008, 80, 223-228
10. Cebrat M., Cebula A., Łaszkiewicz A., Kasztura M., Miazek A., Kisielow P.: Mechanism of lymphocyte specific inactivation of *RAG-2* intragenic promoter of *NWC*: implications for epigenetic control of *RAG* locus. *Mol. Immunol*., 2008, 45(8), 2297-306 **IF – 3,742**
11. Chaszczewska-Markowska M., Stebelska K., Sikorski A., Madej J., Opolski A., Ugorski M.: Liposomal formulation of 5-fluorocytosine in suicide gene therapy with cytosine deaminase – for colorectal cancer. *Cancer Lett*., 2008, 262, 164-172 **IF – 3,398**
12. Chodaczek G., Zimecki M., Lukasiewicz J., Lugowski C.: Lactoferrin-monophosphoryl lipid a complex enhances immunity of mice to *Plesiomonas shigelloides* CNCTC 138/92. *Acta Biochim. Pol*., 2008, 55, 91-96 **IF –1,261**
13. Choi B-K, Actor J.K., Rion S., d'Anjon M., Stadheim T.A., Warburton S., Giacone E., Cukan M., Li H., Kull A., Sharkey N., Gollnick P., Kocięba M., Artym J., Zimecki M., Kruzel M.L., Wildt S.: Effect of terminal N-acetylneuraminic acid on *in vitro* secondary humoral immune response. *Glycoconjugate J.*, 2008, 25, 581-593 **IF – 1,602**
14. Crola C., Lamerant N., Paprocka M., Mitterrand M., Gosset D., Duś D., Kieda C.: Selective human endothelial cell activation by chemokines as a guide to cell homing. *Immunology*, 2008 sep 16 (Epub ahead of print) **IF – 3,398**
15. Dutko-Gwóźdź J., Gwóźdź T., Orłowski M., Greb-Markiewicz B., Duś D., Dobrucki J., Ożyhar A.: The variety of complexes formed by EcR and Usp nuclear receptors in the nuclei of living cells. *Mol. Cell Endocrinol*., 2008, 294(1-2), 45-51 **IF – 2,971**

16. Dziewicka A., Stawińska N., Kochanowska I.: Osteolytic activity of osteoclast-like cells derived from peripheral blood of different species. EJPAU 2008, 11(1)
17. Ebaid H., Duk M., Gamian A.: Antibodies against *Citrobacter braakii* O37 cells recognize the N-glycan of the band 3 glycoprotein of human erythrocyte membrane. *FEMS Immunol. Med. Microbiol*., 2008, 52(3), 352-361 **IF – 1,928**
18. Ejma M., Misiuk-Hojło M., Gorczyca W.A., Podemski R., Szymaniec S., Kuropatwa M., Rogozińska-Szczepka J., Bartnik W.: Antibodies to 46-kDa retinal antigen in a patient with breast carcinoma and cancer-associated retinopathy. *Breast Cancer Res. Treat*., 2008, 110, 269-271 **IF – 4,453**
19. Giebel S., Nowak I., Wojnar J., Krawczyk-Kulis M., Hołowiecki J., Kyrcz-Krzemień S., Kuśnierczyk P.: Association of KIR2DS4 and its variant KIR1D with leukemia. *Leukemia*, 2008, 22, 2129-2130 **IF - 6,924**
20. Gmyrek G.B., Sieradzka U., Goluda M., Gabrys M., Sozanski R., Jerzak M., Zbyryt I., Chrobak A., Chełmonska-Soyta A.: Flow cytometric evaluation of intracellular cytokine synthesis in peripheral mononuclear cells of women with endometriosis. *Immunol. Invest*. 2008, 37(1), 43-61 **IF – 1,529**
21. Gmyrek G.B., Sieradzka U., Goluda M., Gabryś M., Sozański R., Jerzak M., Zbyryt I., Chrobak A., Chełmońska-Soyta A.: Differential flow cytometric detection of intracellular cytokines in peripheral and peritoneal mononuclear cells of women with endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 2008, 137(1), 67-76 **IF – 1,529**
22. Gogolewski S., Górna K., Zaczyńska E., Czarny A.: Structure-property relations and cytotoxicity of isosorbide-based biodegradable polyurethane scaffolds for tissue repair and regeneration. *J. Biomed. Mater. Res*., (A), 2008, 85, 456-465 **IF – 2,612**
23. Hołowiecki J., Krawczyk-Kuliś M., Giebel S., Jagoda K., Stella-Hołowiecka B., Piątkowska-Jakubas B., Paluszewska M., Seferyńska I., Lewandowski K., Kiełbiński M., Czyż A., Balana-Nowak A., Król M., Skotnicki A.B., Jędrzejczak W.W., Warzocha K., Lange A., Hellmann A.: Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4-2002 MRD Study. *Brit. J. Haematol*., 2008, 142(2), 227-237 **IF – 4,490**
24. Kanska U., Budzynska R., Nevozhay D., Boratynski J.: Preparation of mannan-protein conjucates using high-temperature glycation. *Biotechnol. Appl. Biochem*., 2008, 49, 57-64 **IF – 2,810**
25. Katzenellenbogen E., Kocharova N.A., Korzeniowska-Kowal A., Bogulska M., Rybka J., Gamian A., Kachala V.V., Shashkov A.S., Knirel Y.A.: Structure of the glycerol phosphate-containing O-specific polysaccharide and serological studies on the lipopolysaccharides of *Citrobacter werkmanii* PCM 1548 and PCM 1549 (serogroup O14*). FEMS Immunol. Med. Microbiol*., 2008, 54(2), 255-262 **IF – 1,928**
26. Katzenellenbogen E., Kocharova N.A., Korzeniowska-Kowal A., Gamian A., Bogulska M., Szostko B., Shashkov A.S., Knirel Y.A.: Immunochemical studies of the lipopolysaccharides of *Hafnia alvei* PCM 1219 and other strains with the O-antigens containing D-glucose 1-phosphate and 2-deoxy-2-[(R)-3-hydroxybutyramido]-D-glucose. *Arch. Immunol. Ther. Exp.,* 2008, 56(5), 347-352 **IF – 1,689**
27. Katzenellenbogen E., Toukach P.V., Kocharova N.A., Korzeniowska-Kowal A., Gamian A., Shashkov A.S., Knirel Y.A.: Structure of a phosphoethanolamine-containing O-polysaccharide of *Citrobacter freundii* strain PCM 1443 from serogroup O39 and its relatedness to the *Klebsiella pneumoniae* O1 polysaccharide. *FEMS Immunol. Med. Microbiol*., 2008, 53(1), 60-64 **IF – 1,928**

28. Klopotowska D., Strzadala L., Matuszyk J.: Inducibility of doxycycline-regulated gene in neural and neuroendocrine cells strongly depends on the appropriate choice of a tetracycline-responsive promoter. *Neurochem. Int.*, 2008, 52(1-2), 221-229 **IF - 2,975**
29. Kois A., Świątek M., Jakimowicz D., Zakrzewska-Czerwińska J.: SMC-protein-dependent chromosome condensation during aerial hyphal development in *Streptomyces*. *J. Bacteriol.*, published ahead of print on 17 October 2008, doi:10.1128/JB.00513-08 **IF – 4,013**
30. Kowalik K., Gorczyca W.A.: Badania wpływu inhibitorów fosfodiesteraz cyklicznych nukleotydów (PDE) na ludzkie komórki limfoidalne. *Prace Naukowe Studentów Wydziału Chemicznego Politechniki Wrocławskiej,* 2008, 6, 79-82
31. Krusiński T., Laskowska A., Ożyhar A., Dobryszycki P.: The application of an immoblized molecular beacon for the analysis of DNA binding domains from the ecdysteroid receptor proteins Usp and EcR's interaction with the hasp27 response element. *J. Biomol. Screen*., 2008, 13, 899-905 **IF – 2,148**
32. Krzystek-Korpacka M., Neubauer K., Berdowska I., Boehm D., Zielinski B., Petryszyn P., Terlecki G., Paradowski L., Gamian A.: Enhanced formation of advanced oxidation protein products in IBD. *Inflamm. Bowel Dis*., 2008, 14(6), 794-802 **IF – 4,705**
33. Krzystek-Korpacka M., Boehm D., Matusiewicz M., Diakowska D., Grabowski K., Gamian A.: Paraoxonase 1 (PON1) status in gastroesophageal malignancies and associated paraneoplastic syndromes - connection with inflammation. *Clin. Biochem*., 2008, 41(10-11), 804-811 **IF – 2,072**
34. Krzystek-Korpacka M., Matusiewicz M., Diakowska D., Grabowski K., Blachut K., Kustrzeba-Wojcicka I., Terlecki G., Gamian A.: Acute-phase response proteins are related to cachexia and accelerated angiogenesis in gastroesophageal cancers. *Clin. Chem. Lab. Med*. 2008, 46(3), 359-364 **IF – 1,741**
35. Krzystek-Korpacka M., Matusiewicz M., Diakowska D., Grabowski K., Neubauer K., Kustrzeba-Wojcicka I., Terlecki G., Gamian A.: Respiratory insufficiency related to COPD accelerates systemic inflammation, under-nutrition, and angiogenesis in esophageal malignancies. *Exp. Oncol*., 2008, 30(1), 75-80
36. Krzystek-Korpacka M., Salmonowicz B., Boehm D., Berdowska I., Zielinski B., Patryn E., Noczynska A., Gamian A.: Diagnostic potential of oxidative stress markers in children and adolescents with type 1 diabetes. *Clin. Biochem*., 2008, 41(1-2), 48-55 **IF – 2,072**
37. Kusztal M., Kosmaczewska A., Magott-Procelewska M., Frydecka I., Ciszak L., Bocko D., Patrzalek D., Klinger M.: CD28 downregulation on CD4(+) T cells is associated with age of kidney transplant receipient. *Transpl. Int*., 2008, 7, 661-668 **IF – 2,30**
38. Lange A.: Genetic factors predicting IFN-gamma generation potential in patients with sarcoidosis and after hameatopoietic stem cell transplantation. *Int. J. Immunogenet*., 2008, 35, 385-388 **IF – 1,279**
39. Lange A.: Immunogenetics in hematology and stem cell transplantation. *Int. J. Immunogenet*., 2008, 35, 361 **IF – 1,279**
40. Larsson L., Szponar B., Ridha B., Pehrson C., Dutkiewicz J., Krysińska-Traczyk E., Sitkowska J.: Identification of bacterial and fungal components in tobacco and tobacco smoke. *Tob. Induc. Dis*., 2008, 4(1), 4
41. Leyvraz S., Pampallona S., Martinelli G., Ploner F., Perey L., Aversa S., Peters S., Brunsvig P., Lange A., Yilmaz U., Rosti G.: A threefold dose intensity treatment with Ifosfamide, Carboplatin, and Etoposide for patients with small cell lung cancer: A randomized trial. *J. Natl. Cancer Inst.*, 2008, 100(8), 533-541 **IF – 15,678**

42. Lukasiewicz J., Niedziela T., Jachymek W., Kenne L., Lugowski C.: Two Kdo-heptose regions identified in *Hafnia alvei* 32 lipopolysaccharide: the complete core structure and serological screening of different *Hafnia* O-serotypes. *J. Bacteriol*. (online: 2008, doi:10.1128/JB.00891-08) **IF – 4,013**
43. Łusiak-Szelachowska M., Annabhani A., Weber-Dąbrowska B., Górski A., Bębenek M., Pudełko M., Strutyńska-Karpińska M., Muszyński J., Paradowski L.: *Escherichia coli* bacteriophages in human stool of patients with gastrointestinal tract diseases. *Gastroenterol. Pol.,* 2008, 15, 87-90
44. Łuszczek W., Majorczyk E., Nockowski P., Pluciński P., Jasek M., Nowak I., Wiśniewski A., Kuśnierczyk P.: Distribution of the CTLA-4 single nucleotide polymorphisms CT60G>A and +49A>G in *Psoriasis vulgaris* patients and control individuals from a Polish Caucasian population. *Int. J. Immunogenet.,* 2008, 35, 51-55 **IF - 1,279**
45. Madej J.P., Borkowski J., Boratyński J., Nowacki W.: Oznaczanie testem ELISA poziomu DBP w surowicy klaczy po porodzie. *Medycyna Wet*., 2008, 64(5), 707-712
46. Madej M., Sokalska-Jurkiewicz M., Nowak B., Czarny A., Zaczynska E., Wiland P.: The activity of STAT-3 in patients with rheumatoid arthritis. *Adv. Clin. Exp. Med*., 2008, 17, 4, 405-409
47. Majorczyk E., Łuszczek W., Nowak I., Pawlik A., Wiśniewski A., Jasek M., Kuśnierczyk P.: Distribution of killer cell immunoglobulin-like receptor genes in Poles. *Int. J. Immunogenet*., 2008, 35, 405-407 **IF - 1,279**
48. Malinka W., Gamian A., Redzicka A., Swiatek P.: Pyrido-1,2-thiazines and their *in vitro* antibacterial evaluation. *Acta Pol. Pharm*., 2008, 65(1), 71-74
49. Matusiak L., Bieniek A., Wozniak Z., Szepietowski J.C.: Amelanotic malignant melanoma in an acral location. *Acta Dermatovenerol., Alp. Panonica Adriat*., 2008, 17(2), 72-74 **IF – 1,927**
50. Matusiak Ł., Reich A., Szepietowski J.C.: Dardia Lipo Milk improves skin dryness: a corneometric assessment. *J. Eur. Acad. Dermatol. Venerol*., 2008, 22(11), 1396-1397 **IF – 1,437**
51. Mączyński M., Zimecki M., Ryng S.: A new class of isoxazole derivatives: The M1-9 series of compounds with immunotropic activity. *Acta Pol. Pharm. Drug Res.,* 2008, 65, 241-244
52. Mączyński M., Zimecki M., Taraszkiewicz M., Ryng S.: Synthesis, immunological activity and computational study of 5-amino-3-methyl-4-isoxazolecarboxylic acid semicarbazides and thiosemicarbazides. *Acta Pol. Pharm. – Drug Res*., 2008, 65, 543-549
53. Międzybrodzki R., Świtała-Jeleń K., Fortuna W., Weber-Dąbrowska B., Przerwa A., Łusiak-Szelachowska M., Dąbrowska K., Kurzępa A., Boratyński J., Syper D., Pozniak G., Ługowski C., Górski A.: Bacteriophage preparation inhibition of reactive oxygen species generation by endotoxin-stimulated polymorphonuclear leucocytes. *Virus Res*., 2008, 131, 233-242 **IF - 2,810**
54. Mitkiewicz M., Witwicka H., Siednienko J., Gorczyca W.A.: Guanosine 3', 5'-cyclic monophosphate (cGMP) signaling in inflammation. *Annual Report PAS* 2008, 74-76
55. Mól W., Matyja M., Filip B., Wietrzyk J., Boryczka S.: Synthesis and antiproliferative activity *in vitro* of novel (2-butynyl)thioquinolines. *Bioorg. Med. Chem.,* 2008, 16(17), 8136-41 **IF – 2,662**
56. Nowak B., Sokalska-Jurkiewicz M., Madej M., Zaczynska E., Czarny A., Szechiński J.: iNOS expression in peripheral blood leukocytes of patients with early arthritis and established rheumatoid arthritis. *Adv. Clin. Exp. Med*., 2008, 17(4), 415-421
57. Nowak J., Mika-Witkowska R., Polak M., Zajko M., Rogatko-Koroś M,. Graczyk-Pol E., Lange A.: Allele and extended haplotype polymorphism of HLA-A, -C, -B, -DRB1 and -DQB1 loci in Polish population and genetic affinities to other populations. *Tissue Antigens*, 2008, 71(3), 193-205 **IF – 2,245**

58. Nowak J., Mika-Witkowska R., Polak M., Zajko M., Rogatko-Koroś M., Graczyk-Pol E., Stępkowski T., Lange A.: Road-like pattern of HLA-ABDR-based genetic distances between populations. *Centr. Eur. J. Immunol.,* 2008, 33(3), 114-119
59. Pajtasz-Piasecka E., Rossowka J., Duś D., Weber-Dąbrowska B., Zabłocka A., Górski A.: Bacteriophages suport anti-tumor response initiated by DC-based vaccine against murine transplantable colon carcinoma. *Immunol. Lett*., 2008, 116(1), 24-32 **IF- 2,628**
60. Palus J., Dąbrowska E., Piętka-Ottlik M., Piasecki E., Młochowski J.: Bis(2-amidophenyl) diselenides derived from amino acids and dipeptides: synthesis, antiviral and antimicrobial activity. *Pol. J. Chem*., 2008, 82, 1015-1022 **IF - 0,483**
61. Palusiak A., Dzieciatkowska M., Sidorczyk Z.: Application of two different kinds of sera against the *Proteus penneri* lipopolysaccharide core region in search of epitopes determining cross-reactions with antibodies. *Arch. Immunol. Ther. Exp*., 2008, 56(2), 135-40 **IF – 1,689**
62. Paprocka M., Duś D., Mitterrand M., Lamerant-Fayel N., Kieda C.: Flow cytometric assay for quantitative and qualitative evaluation of adhesive interactions of tumor cells with endothelial cells. *Microvasc. Res*., 2008, 76(2), 134-138 **IF – 2,365**
63. Piętka-Ottlik M., Wójtowicz-Młochowska H., Kołodziejczyk K., Piasecki E., Młochowski J.: New organoselenium compounds active against pathogenic bacteria, fungi and viruses. *Chem. Pharm. Bull.,* 2008, 56, 1423-1427 **IF -1,223**
64. Quintana E.T., Wierzbicka K., Mackiewicz P., Osman A., Fahal A. H., Hamid M. E., Zakrzewska-Czerwińska J., Maldonado L.A., Goodfellow M.: Streptomyces sudanensis sp. nov., a new pathogen isolated from patients with *Actinomycetoma*. *Antonie van Leuwenhoek*, 2008, 93, 305-313 **IF – 1,547**
65. Reich A., Szepietowski J.C.: Vasoactive peptides in the pathogenesis of psoriasis. *G Ital. Dermatol. Venerol*., 2008, 143, 289-198
66. Reich A., Wiśnicka B., Szepietowski J.C.: Haemorrhagic nails in pemphigus vulgaris. *Acta Derm. Venerol*., 2008, 88(5), 58-60 **IF –1,927**
67. Regiec A., Śpiewak M., Jasztold-Howorko R., Międzybrodzki R.: New amides of 5-(4-chloro-benzoyl)aminoorotic acid: Their synthesis and biological activity. *Arch. Pharm*., 2008, 341(11), 677-689 **IF –1,300**
68. Rybka J., Grycko P., da Cruz F.J., Gamian A., Szwajcer Dey E.: Application of supercritical carbon dioxide (scCO2) for the extraction of lipopolysaccharides (LPS) from *Salmonella enterica* subsp. *enterica* PCM 2266. *J. Supercrit. Fluids*, 2008, 45(1) 51-56 **IF – 2,189**
69. Sidoryk K., Kaczmarek Ł., Szczepek W.J., Wietrzyk J., Świtalska M., Peczyńska-Czoch W.: New amino acid derivatives of 6H-indolo[2,3-b]quinolines. *Pol. J. Chem.*, 2008, 82, 2095-2105, 2008 **IF – 0,483**
70. Smietana K., Kasztura M., Paduch M., Derewenda U., Derewenda Z. S., Otlewski J.: Degenerate specificity of PDZ domains from RhoA-specific nucleotide exchange factors PDZRhoGEF and LARG. *Acta Biochim. Pol*., 2008, 55(2), 269-80 **IF –1,261**
71. Sochocka M., Zaczyńska E., Leszek J., Siemieniec I., Błach-Olszewska Z.: Effect of donepezil on innate antiviral immunity of human leukocytes. *J. Neurol. Sci*., 2008, 273, 75-80 **IF - 2,315**
72. Sokalska-Jurkiewicz M.A., Madej M., Nowak B., Czarny A., Zaczynska E., Wiland P.: Activation of STAT-1.-3, and -6 proteins in early arthritis. *Adv. Clin. Exp. Med*., 2008, 17, 4, 399-404
73. Stasik I., Rapak A., Ziolo E., Strzadala L.: The mitochondrial targeting of RelB and NFTAx in immature T cells. *Cell. Mol. Biol. Lett.*, 2008, 13, 493-501 **IF - 1,676**

74. Suwalska K., Pawlak E., Karabon L., Tomkiewicz A., Dobosz T., Urbaniak-Kujda D., Kuliczkowski K., Wolowiec D., Jedynak A., Frydecka I.: Association studies of CTLA-4, CD28 and ICOS gene polymorphisms with B-cell chronic lymphocytic leukemia in the Polish population. *Hum. Immunol.*, 2008, 69(3), 193-201 **IF – 2,901**
75. Szepietowski J.C., Matusiak L.: Trichophyton rubrum autoinoculation from infected nails is not such a rare phenomenon. *Mycoses*, 2008, 51, 345-346 **IF – 1,327**
76. Szepietowski J.C., Salomon J., Pacan P., Hrehorów E., Zalewska A.: Trichophyton rubrum autoinoculation from infected nails is not such a rare phenomenon. *J. Eur. Acad. Dermatol. Venereol*., 2008, 7, 795-799 **IF – 1,437**
77. Szczepanowska J., Reich A., Szepietowski J.C.: Emollients improve treatment results with topical corticosteroids in childhood atopic dermatitis: a randomized comparative study. *Pediatr. Allergy Immunol*., 2008, 19(7), 614-618 **IF – 2,454**
78. Reich A., Trybucka K., Tracinska A., Samotij D., Jasiuk B., Srama M., Szepietowski J.C.: Acne itch: do acne patients suffer from itching? *Acta Derm. Venereol*., 2008, 88(1), 38-42 **IF – 1,929**
79. Tylińska B., Jasztold-Howorko R., Mastalarz H., Szczaurska-Nowak K., Wietrzyk J.: Synthesis and anticancer activity of new 1-substituted-6H-pyrido[4,3-b]carbazole derivatives. *Arch. Pharm.* (Weinheim), 2008, 341(6), 351-356 **IF – 1,300**
80. Wachowiak J., Labopin M., Miano M., Chybicka A., Stary J., Sterba J., Masszi T., Labar B., Maschan A., Kowalczyk J.R., Lange A., Hołowiecki J., Kalman N., Afanassiev B.V., Dini G.; EBMT Paediatric Working Party: Haematopoietic stem cell transplantation in children in eastern European countries 1985-2004: development, recent activity and role of the EBMT/ESH Outreach Programme. *Bone Marrow Transpl*., 2008, 41, suppl. 2, S112-117 **IF – 3,00**
81. Wagner E., Al-Kadasi K., Zimecki M., Sawka-Dobrowolska W.: Synthesis and pharmacological screening of derivatives of isoxazolo[4,5-d]pyrimidine. *Eur. J. Med. Chem*., 2008, 43, 2498-2504 **IF – 3,01**
82. Wietrzyk J., Filip B., Milczarek M., Klopotowska D., Maciejewska M., Dabrowska K., Kurzepa A., Dzimira S., Madej J., Kutner A.: The influence of 1,25-dihydroxyvitamin D3 and 1,24-dihydroxyvitamin D3 on alphavbeta3 integrin expression in cancer cell lines. *Oncol. Rep.*, 2008, 20(4), 941-52 **IF – 1,597**
83. Wietrzyk J., Nevozhay D., Milczarek M., Filip B., Kutner A.: Toxicity and antitumor activity of the vitamin D analogs PRI-1906 and PRI-1907 in combined treatment with cyclophosphamide in a mouse mammary cancer model. *Cancer Chem. Pharmacol*., 2008, 62(5), 787-97 **IF – 2,568**
84. Wodoklis-Kaczyńska E., Zwiefka A.: Projekt RIGHT – wstępna ocena użytkownika. W: „*Wybrane zagadnienia z praktyki lekarza rodzinnego"*. Red. Steciwko A., wyd. Continuo. 2008, vol. 12, 54-61. Eng. Version 142-149
85. Zaczyńska E., Duś D., Paprocka M., Błach-Olszewska Z.: Resistance of human leukocytes to vesicular stomatitis virus infection as one of the innate antiviral immune activities; participation of cell subpopulations. *Folia Histochem. Cytobiol*., 2008, 46(1), 39-43 **IF – 0,897**
86. Zamirska A., Reich A., Berny-Moreno J., Salomon J., Szepietowski J.C.: Vulvar pruritus and burning sensation in women with psoriasis. *Acta Derm. Venereol.,* 2008, 88, 132-135 **IF – 1,927**
87. Zimecki M., Artym J., Kocięba M., Weber-Dabrowska B., Łusiak-Szelachowska M., Górski A.: The concerted action of lactoferrin and bacteriophages in the clearance of bacteria in sublethally infected mice. *Post. Hig. Med. Dośw*, 2008, 62, 42-46
88. Zimecki M., Artym J., Ryng S., Obmińska-Mrukowicz B.: RM-11, an isoxazole derivative, accelerates restoration of the immune function in mice treated with cyclophosphamide. *Pharmacol. Rep.,* 2008, 60, 183-189 **IF – 2,290**

89. Zimecki M., Mączyński M., Artym J., Ryng S.: Very closely related isoxazoles may exhibit opposite immunological activities, *Acta Pol. Pharm – Drug Res*., 2008, 6, 793-794
90. Zwiefka A., Frączkowski K., Klakočar J., Maroszek J., Dudek R.: Biznesowa prognoza dla systemu RIGHT. W: „*Wybrane zagadnienia z praktyki lekarza rodzinnego*". Red. Steciwko A., wyd. Continuo. 2008, vol. 12, 87-96. Eng. Version 174-183
91. Zwiefka A., Klakočar J., Maroszek J., Bogowolska-Wepsięć M., Frączkowski K.: Wpływ wdrażania systemu RIGHT na zmiany modelu świadczeń medycznych. W*: „Wybrane zagadnienia z praktyki lekarza rodzinnego"*. Red. Steciwko A., wyd. Continuo. 2008, vol. 12, 62-68. Eng. Version 150-155
92. Zwiefka A., Klakocar J., Maroszek J., Bogowolska-Wepsięć M., Frączkowski K., Dudek R.: Zastosowanie nowych technologii w ochronie zdrowia w celu redukcji ryzyka diagnozy i leczenia. *Przewodnik Lekarza*, 2008, 109(1), 281-286
93. Zwiefka A., Wodoklis-Kaczyńska E., Frączkowski K.: Redukcja ryzyka w diagnozie i leczeniu w oparciu o system RIGHT. *Family Medicine Primary Care Review*, 2008, 10(3), 1186-1190
94. Żywicka B., Zaczynska E., Czarny A., Pielka S., Jaegermann Z., Karas J., Szymonowicz M.: Activation of transcription nuclear factor NF-κB and induction of inflammatory cytokines in immune response on resorable biomaterials. International Conference (BIO) Degradable Polymers from Renewable Resources. Proceeding of the International Conference Vienna, November 18-21, 2007. Editor: Andrzej J. Nadolny. Scientific Centre of the Polish Academy of Science Vienna, Austria, 2008, 83-87

Published reviews articles:

1. Artym J.: Udział laktoferyny w gospodarce żelazem w organizmie. Część I. Wpływ laktoferyny na wchłanianie, transport i magazynowanie żelaza. *Post. Hig. Med. Dośw*., (online), 2008, 62, 599-612
2. Błach-Olszewska Z., Lamer-Zarawska E.: Come back to root - therapeutic activities of *Scutellaria baicalensis* root in aspect of innate immunity regulation - Part I. *Adv. Clin. Exp. Med*., 2008, 17, 337-345
3. Bujak A., Kałas W.: Chemioterapia metronomiczna jako nowa strategia leczenia chorób nowotworowych. *Post. Hig. Med. Dośw*., (online), 2008, 62, 364-371
4. Fortuna W., Międzybrodzki R., Weber-Dąbrowska B., Górski A.: Bacteriophage therapy in children: Facts and prospects. *Med. Sci. Monit.*, 2008, 14, 126-132 **IF – 1,607**
5. Frydecka I., Kosmaczewska A., Kiełbiński M., Potoczek S.: CD28 expression and function in multiple myeloma. *Acta Haematol. Pol*., 2008, 39, 145-151
6. Kańska U., Boratyński J.: Nowe leki w terapii reumatoidalnego zapalenia stawów. *Post. Hig. Med. Dośw.,* (online)*,* 2008; 62, 94-102
7. Kisielow P., Miazek A., Cebrat M.: *NWC*, a new gene within *RAG* locus: could it keep GOD under control? *Intern. J. Immunogenetics*, 2008, 35, 395-399 **IF – 1,279**
8. Kopij M., Rapak A.: Rola receptorów jądrowych w procesie śmierci komórek. *Post. Hig. Med. Dośw*., (online), 2008, 62, 571-581
9. Kosmaczewska A., Ciszak L., Potoczek S., Frydecka I.: The significance of Treg cells in defective tumor immunity. *Arch. Immunol. Ther. Exp*. 2008, 56, 181-191 **IF –1,689**
10. Kubis A.M., Janusz M.: Choroba Alzheimera – nowe możliwości terapeutyczne oraz stosowane modele eksperymentalne. *Post. Hig. Med. Dośw*., (online), 2008, 62, 372-392

11. Lipka D., Boratyński J.: Metaloproteinazy MMP. Struktura i funkcja. *Post. Hig. Med. Dośw*., (online), 2008; 62, 328-336
12. Lisowska E.: Protein glycosylation, an overview. Encyklopedia of Life Sciences (ELS), J. Wiley & Sons Ltd, 2008, (online) (http://www.els.net/[DOI: 0.1002/9780470015902.a0006211.pub2]
13. Miążek A.: Uzyskiwanie i wykorzystanie myszy zmodyfikowanych genetycznie w badaniach biomedycznych. *Prace i Materiały Zootech.*, 2008, 66, 147-150
14. Pietkiewicz J., Seweryn E., Bartyś A., Gamian A.: Receptors for advanced glycation end products and their physiological and clinical significance. *Post. Hig. Med. Dośw*., (online), 2008, 62, 511-523
15. Rusiecka-Ziółkowska J., Walszewska M., Stekla J., Szponar B.: Rola endotoksyn w patomechanizmie sepsy. *Pol. Merk. Lek*., 2008, 25, 260-265
16. Smolarek D., Krop-Wątorek A., Waśniowska K., Czerwiński M.: Molekularne podstawy układu grupowego ABO. *Post. Hig. Med. Dośw.* (online), 2008, 62, 4-17
17. Zabłocka A., Janusz M.: Dwa oblicza wolnych rodników tlenowych. *Post. Hig. Med. Dośw*., (online), 2008, 62, 118-124
18. Zimecki M.: A proline-rich polypeptide from ovine colostrum: Colostrinin with immunomodulatory activity. *Adv. Exp. Med. Biol.*, Springer, 2008, 606, 241-250 **IF – 0,663**

Published chapters of books:

1. Czarny A., Zaczyńska E., Janicka A., Nawrot U., Włodarska K., Szczepaniak W., Walkowiak W.: Produkcja cytokin przez ludzkie komórki płuc traktowane zanieczyszczeniami powietrza i zarodnikami grzybów, *in vitro*. W: „*Ekotoksykologia w ochronie środowiska*". Red.: B. Kołwzan, K. Grabas. Wydawca: PZITS, 2008, 63-68
2. Czarny A., Zaczyńska E., Janicka A., Szczepaniak W., Walkowiak W.: Wpływ wielopierścieniowych węglowodorów aromatycznych na produkcję TNF-α przez ludzkie komórki płuc, *in vitro*. W: „*Ekotoksykologia w ochronie środowiska*". Red.: B. Kołwzan, K. Grabas. Wydawca: Polskie Zrzeszenie Inżynierów i Techników Sanitarnych Oddział Dolnośląski 2008, 57-62
3. Kołwzan B., Biazik J., Czarny A., Zaczyńska E., Karpenko E.: Ocena toksyczności biosulfaktantów produkowanych przez *Pseudomonas sp*. PS-17. W: „*Ekotoksykologia w ochronie środowiska*". Red.: B. Kołwzan, K. Grabas. Wydawca: Polskie Zrzeszenie Inżynierów i Techników Sanitarnych Oddział Dolnośląski 2008, 191-196
4. Piekarska K., Zaciera M., Czarny A., Zaczyńska E.: Właściwości mutagenne i cytotoksyczne ekstraktów pyłu zawieszonego pobranego na terenie Wrocławia. W: „*Ekotoksykologia w ochronie środowiska*". Red.: B. Kołwzan, K. Grabas. Wydawca: Polskie Zrzeszenie Inżynierów i Techników Sanitarnych Oddział Dolnośląski 2008, 303-312

Articles published by our scientists working abroad without affiliation with our Institute:

1. Barnett B.G., Rüter J., **Kryczek I**., Brumlik M.J., Cheng P.J., Daniel B.J., Coukos G., Zou W., Curiel T.J.: Regulatory T cells: a new frontier in cancer immunotherapy. *Adv. Exp. Med. Biol.* 2008, 622, 255-60 **IF – 0,663**
2. **Grzegorzewicz A.E**., Ma Y., Jones V., Crick D., Liav A., McNeil M. R.: Development of a microtiter plate based assay for lipid linked glycosyl transferase products using the mycobacterial cell wall rhamnosyl transferase, WbbL, *Microbiology*, 2008, 154, 3724-30 **IF – 0,597**

3. **Grzywacz B**., Miller J.S., Verneris M.R.: Use of natural killer cells as immunotherapy for leukaemia. *Best Pract. Res. Clin. Haematol*., 2008, 21(3), 467-83 **IF – 3,589**
4. Khariwala S.S., Dan O., Lorenz R.R., **Klimczak A**., Siemionow M., Strome M.: Donor bone marrow in laryngeal transplantation: results of a rat study. *Am. J. Otolaryngol*. 2008, 29(4), 242-249 **IF – 0,771**
5. Kitov P.I., **Lipinski T**., Paszkiewicz E., Solomon D., Sadowska J.M., Grant G.A., Mulvey G.L., Kitova E.N., Klassen J.S., Armstrong G.D., Bundle D.R.: An entropically efficient supramolecular inhibition strategy for Shiga toxins. *Angew. Chem. Int. Ed. Engl*. 2008, 47(4), 672-676 **IF – 10,031**
6. Kitov P.I., Mulvey G.L., Griener T.P., **Lipinski T**., Solomon D., Paszkiewicz E., Jacobson J.M., Sadowska J.M., Suzuki M., Yamamura K., Armstrong G.D., Bundle D.R.: *In vivo* supramolecular templating enhances the activity of multivalent ligands: a potential therapeutic against the *Escherichia coli* O157 AB5 toxins. *Proc. Natl. Acad. Sci*. USA, 2008, 105(44), 16837-16842 **IF – 9,598**
7. **Kryczek I**., Bruce A.T., Gudjonsson J.E., Johnston A., Aphale A., Vatan L., Szeliga W., Wang Y., Liu Y., Welling T.H., Elder J.T., Zou W.: Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *J. Immunol.*, 2008, 1, 181(7), 4733-41 **IF – 6,068**
8. **Kryczek I**., Wei S., Gong W., Shu X., Szeliga W., Vatan L., Chen L., Wang G., Zou W.: Cutting edge: IFN-gamma enables APC to promote memory Th17 and abate Th1 cell development. *J. Immunol*., 2008, 1,181(9), 5842-5846 **IF – 6,068**
9. **Kubler-Kielb J**., Schneerson R., Mocca C., Vinogradov E.: The elucidation of the structure of the core part of the LPS from *Plesiomonas shigelloides* serotype O17 expressing O-polysaccharide chain identical to the *Shigella sonnei* O-chain. *Carbohydr. Res*., 2008, 343(18), 3123-3127 **IF – 1,723**
10. **Kubler-Kielb J**., Vinogradov E., Ben-Menachem G., Pozsgay V., Robbins J.B., Schneerson R.: Saccharide/protein conjugate vaccines for *Bordetella* species: preparation of saccharide, development of new conjugation procedures, and physico-chemical and immunological characterization of the conjugates. *Vaccine*, 2008, 26(29-30), 3587-3593 **IF –3,377**
11. **Kubler-Kielb J**., Vinogradov E., Hu H., Leppla S.H., Robbins J.B., Schneerson R.: Saccharides cross-reactive with *Bacillus anthracis* spore glycoprotein as an anthrax vaccine component. *Proc. Natl. Acad. Sci*. USA, 2008, 105(25), 8709-8712 **IF – 9,598**
12. Nasir S., Bozkurt M., **Klimczak A**., Siemionow M.: Large antigenic skin load in total abdominal wall transplants permits chimerism induction. *Ann. Plast. Surg*., 2008 61(5), 572-579 **IF – 1,029**
13. Siemionow M., **Klimczak A**., Unal S., Agaoglu G., Carnevale K.: Hematopoietic stem cell engraftment and seeding permits multi-lymphoid chimerism in vascularized bone marrow transplants. *Am. J. Transplant*. 2008, 8(6), 1163-76 **IF – 6,423**
14. Siemionow M., **Klimczak A**.: Basics of immune responses in transplantation in preparation for application of composite tissue allografts in plastic and reconstructive surgery: part I. *Plast. Reconstr. Surg*., 2008, 121(1), 4e-12e. Review **IF – 2,074**
15. Tang Q., **Grzywacz B**., Wang H., Kataria N., Cao Q., Wagner J.E., Blazar B.R., Miller J.S., Verneris M.R.: Umbilical cord blood T cells express multiple natural cytotoxicity receptors after IL-15 stimulation, but only NKp30 is functional. *J. Immunol*., 2008,181(7), 4507-15 **IF – 6,068**
16. Vinogradov E., **Kubler-Kielb J**., Korenevsky A.: The structure of the carbohydrate backbone of the LPS from *Shewanella* spp. MR-4. *Carbohydr. Res*., 2008, 343(15), 2701-2705 **IF – 1,723**

17. Wu X., **Lipinski T**., Paszkiewicz E., Bundle D.R.: Synthesis and immunochemical characterization of S-linked glycoconjugate vaccines against *Candida albicans*. *Chemistry,* 2008, 14(21), 6474-6482
18. **Grzegorzewicz A**., Jones V., Ma Y., Kurosu M., McNeil M.: Development of a high through put assay for mycobacterial rhamnosyl transferase – in press
19. Mailankot M., **Staniszewska M**., Butler H., Caprara M., Howell S., Reneker L., Nagaraj R.H.: Indoleamine 2,3-dioxygenase overexpression causes kynurenine-modification of proteins, fibe cell apoptosis, developmental defects, and cataract formation in the mouse lens. *Lab. Invest*., in press
20. Pozsgay V. **Kubler-Kielb J**.: Conjugation methods towards synthetic vaccines. ACS, 2007, Roy R., Ed., in press