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

Research Report 2011

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

The presence of bacteriophages in alimentary tract of patients with inflammatory bowel diseases

The presence, frequency and concentration of *E. coli* bacteriophages in the stools of healthy volunteers and patients with inflammatory bowel diseases (IBD) (Crohn's disease and colitis ulcerosa) as well as the relation between the number of coliphages in intestine and *E. coli* bacteria were studied. Stools of 14 healthy volunteers, 18 patients with Crohn's disease and 21 with colitis ulcerosa were examined for the presence of coliphages for the following strains: own strain *E. coli* from examined patients, *E. coli* B, *E. coli* 1962, *E. coli* DSM 13127. All volunteers and patients were examined for the frequency and concentration of *E. coli* bacteria in stools. The material from adult patients was obtained from the Clinic of Gastroenterology and Hepatology of the Academic Clinical Hospital from Wrocław. Coliphage frequencies were lower in the stools of patients with IBD (7.7%-53.8%) compared with the healthy individuals (14.3%-71.4%). The concentration of coliphages in stools was higher in patients (10^4-10^8 PFU/g) than in healthy volunteers (10^1-10^6 PFU/g). The frequencies of *E. coli* bacteria were 100% in healthy volunteers and 84.6% in patients with IBD. The mean concentration of *E. coli* bacteria was $1.5x10^7$ CFU/g in healthy individuals and $2.4x10^7$ CFU/g in patients with IBD. There was a correlation between *E. coli* bacteria and coliphages for strain *E. coli* DSM 13127 in healthy volunteers and patients with IBD. Under the bacteria concentration 10^6 CFU/g the frequency and concentration of coliphages was low. The differences in the frequency and concentration of phages and bacteria and in the correlation between bacteria *E. coli* and coliphages were statistically insignificant with the exception of results for frequency of coliphages tested on the strain 1962 ($p=0.049$) in patients' stools compared with healthy volunteers.

Neutralization of phages by sera of patients with bacterial infections undergoing phage therapy

The antiphage sera activity in patients with bacterial infections undergoing phage therapy in the Center for Phage Therapy IIET in Wrocław and sera from healthy volunteers was examined.

Antiphage sera activity was determined in 37 patients with *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, *K. pneumoniae* and *M. morganii*. The phages were administered orally, locally or intrarectally. Neutralization studies of *E. coli* T4 bacteriophage by sera of 30 healthy volunteers and *S. aureus* 676/Ż phage by sera of 21 healthy volunteers were performed. 63.3% of examined sera of healthy volunteers caused low neutralization of *E. coli* T4 phage (undiluted sera or diluted 1:10 and 1:100). 57.1% of examined sera of healthy volunteers inactivated *S. aureus* 676/Ż phage (undiluted sera or diluted 1:10). Prior to phage therapy low antiphage activity was observed in 86.5% of examined patients' sera (undiluted or diluted 1:10 and 1:100). During phage therapy (days 7-60) marked antiphage sera activity was observed in 27% of examined patients (dilutions 1:800, 1:1000 and 1:1500). The highest antiphage activity was found in patients with *S. aureus* and *E. faecalis* infections undergoing phage therapy. Furthermore, significant antiphage sera activity was observed in patients using phages locally in contrast to patients receiving phages orally, who usually had low antiphage sera activity.

The influence of phage lysates on the acute inflammatory process

A study on the influence of phage lysates on carrageenan-induced paw edema in rat was performed. The activity of phage 119x lysate of *P. aeruginosa* as well as purified *S. aureus* A5/80, and *E. coli* T4 phages was tested after intraperitoneal and local (paw soaking in the phage preparation) administration. A reduction of edema was observed in the case of intraperitoneally administered 119x phage preparation at 6 hours after edema induction, whereas a sonicate of *P. aeruginosa* increased inflammation after 1 hour. Local application of the phage as well as a sonicate did not increase paw edema. Both purified A5/80 and T4 phages administered intraperitoneally decreased edema at 6 hours after its induction, but this effect was comparable with the one of phosphate buffered saline which was used as a control.

Our observations do not confirm that 119x phage lysate applied both systemically and topically may significantly increase local inflammatory processes, which is an important argument for the safety of phage preparations. The results obtained with the purified A5/80

and T4 phages did not confirm that the anti-inflammatory effect of A5/80 and T4 phage lysates may be attributed to the phage it self.

Results of grant activities

We established protocols for efficient production and purification of high-purity native T4 bacteriophage proteins gp23, gp24, gpHoc and gpSoc that are stable and suitable for further immunological studies. Affinity-tagged (GST) proteins were expressed in *E. coli* from bacterial vectors, combined with chaperones: phage derived gp31 and *E. coli* derived tig and GroEL - GroES. The design of the expression vectors and the conditions of the expression were optimized to obtain high-level expression; approximate rates of total cell proteins were 60% for gp24, 45% for gp23, 50% for Hoc and 10% for Soc. Two-step affinity and size-exclusion chromatography was used for purification. Affinity tags were removed by rTEV proteolytic cleavage. Purity of preparations was determined by SDS-PAGE and analytical gel filtration. Monomeric state of the proteins in solution was verified by dynamic light scattering (DLS) for gp23 and gp24; it was positively verified for both products. LPS content was minimized (less than 1 activity unit per ml).

Native structures of purified proteins were confirmed using circular dichroism (CD) analysis aided by comparison of computed secondary structure content to predictions from the amino acid sequence of studied proteins. Essential gp23 and gp24 are the alpha-structure type, in contrast to non-essential gpHoc in which beta-structure prevails. GpSoc is a mixed type protein. Thermodynamic stability studies were performed by monitoring the CD signal of protein solutions supplemented with an increasing concentration of denaturant (guanidine hydrochloride) and fitting the data to a two-state model to obtain the parameters GdnHCl ½ and deltaGden. Briefly, the least stable were gp23 and Soc, having GdnHCl ½ of 1.08 M and 1.16 M, respectively. Hoc protein had GdnHCl ½ of 2.03 M. Interestingly, gp24 expressed two phases of denaturation, which corresponds well with its structure and existence of two separate domains, a smaller domain A (GdnHCl ½ of 0.133 M) and a larger domain B (GdnHCl ½ of 2.42 M). Interestingly, two essential capsid proteins which are known to be homologous differ substantially in their stability.

29 bacteriophages from the IIET phage bank for *Citrobacter*, *Enterobacter*, *Serratia*, *Escherichia*, *Morganella*, *Pseudomonas* and *Enterococcus* strains have been re-isolated and the lytic activity of 308 bacteriophages has been estimated. 885 different bacterial strains from microbiological laboratories have been collected. For bacteriophage isolation the standard agar plate, broth culture and colorimetric methods were used. 30 specific

bacteriophages for *E. faecalis*, *S. marcescens*, *S. liquefaciens*, *P. aeruginosa*, *E. coli.*, *C. freundii*, *K. oxytoca*, *K. pneumoniae* and *S. pyogenes* have been isolated from different crude city sewage and environmental samples.

The biological characteristics and sensitivity to physico-chemical factors (temperature, chloroform, pH) of newly isolated *Serratia* and *Streptococcus* phages as well as for *Staphylococcus* phages from the phage bank were determined. The highest average adsorption rate of examined phages (99.6%) was observed after 30 minutes incubation of phage with homologous bacterial strain. An apparent decrease of the lytic activity of phages depending on temperature was found: 60°C (for *Serratia* and *Staphylococcus* phages) and 80°C (for *Streptococcus* phages) as well as the chloroform (for *Serratia* phages). The highest (3.5 range) decrease of phage activity and complete phage inactivation was observed at pH 4 for all phages. The studies of morphology and ultrastructure of two *Serratia* phages indicate that they belong to the families of *Myoviridae* and *Caudovirales*. Other phages tested belong to *Myoviridae*, *Siphoviridae* and *Podoviridae* families.

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

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

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

The laboratory is involved in studies of pathogenicity mechanisms of diseases with bacterial etiology, the role of bacterial glycoconjugates and proteins in the immune processes, the structure and functions of bacterial exopolysaccharides and endotoxins. In the framework of these studies the method of determination of four different markers specific for Gram-negative and Gram-positive bacteria has been standardized for serum samples. The work on determination of these chemical markers is important for clinical practice to improve diagnostic analyses in monitoring the clinical pattern of sepsis and septic shock. A difficult problem also concerns identification of actinomycete clinical isolates. Results of experiments on serological characterization and cross reactivity proved that their glycolipids, mycolic acids and also exopolysaccharides are relevant as useful markers which facilitate diagnostic procedures. Studies on probiotics have recently been intensified due to their potential in protection against invading bacteria. In order to understand the mechanisms of this process we

determined the structure of exopolysaccharides produced by several strains, also to determine their role in inflammatory bowel disease, where probiotic microorganisms are involved. The strain *Lactobacillus rhamnosus* KL37B of human origin had two exopolysaccharides; one was composed of nine sugar residues (oligosaccharide repeating units) and the other structure was the same as in *L. rhamnosus* KL37A, and also as in milk strain, found previously. Strains KL37A and KL37B co-aggregated, and were isolated from the same source. These polysaccharides are subjected to studies for potential immunological activities. The other project concerns studies on advanced glycation end-products (AGE). The procedure with the aid of a microwave reactor leads to obtaining antigens and then monoclonal antibodies can be produced, which allows an assay to be developed for determination of AGE and anti-AGE antibodies in serum of diabetic patients.

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

Two mechanisms of innate immunity, i.e. resistance to viral infection and the production of cytokines by leukocytes, were compared in blood isolated from four groups of donors: healthy young (19–35 years old), healthy elderly (over 60), elderly Alzheimer's disease (AD) patients, and elderly patients with alimentary tract cancer (CA).
Peripheral blood leukocytes (PBLs) were isolated by gradient centrifugation in Gradisol G. The degree of resistance was calculated from the kinetics of vesicular stomatitis virus (VSV) replication in the PBLs. Cytokine (TNFα, IFNα, IFNγ, IL-12, and IL-10) levels were determined by ELISA.

The antiviral resistance of the PBLs varied, but a difference was observed only between the young and elderly groups and not between the healthy elderly controls and those with AD or cancer. Differences observed in all the groups concerned the ability and intensity of cytokine production. The most impressive results were obtained for spontaneous TNF and IFNα release. While TNF was released spontaneously by the PBLs of the elderly CA patients and the young healthy group, it was usually undetected in the AD and only sometimes in the healthy elderly group. Leukocytes isolated from the elderly groups responded to VSV infection with more intense IFNα and IFNγ production than the younger group. The results were published in *Experimental Gerontology 2012; 47: 8-13.*

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

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

Bidirectional activity of NWC promoter is responsible for RAG-2 transcription in nonlymphoid cells

Recombination-activating genes *(RAG)-1* and *RAG-2* encode a recombinase responsible for rearrangements of antigen-receptor genes during T and B lymphocyte development. Similarities of *RAG-1* to transposases, ability of the RAG recombinase to catalyze transposition reactions *in vitro* and the highly compact structure of the *RAG* locus have led to the hypothesis that *RAG* genes were acquired by the genome as a result of horizontal transfer of a mobile genetic element. One of the unanswered questions regarding the evolution of the *RAG* gene locus is how the *RAG* transposon was able to avoid host-mediated epigenetic silencing. Recent studies showed that active transposon-derived genes are often associated with genes containing promoters associated with a CpG island to form head-to-head bidirectional pairs. We show that the CpG island-associated promoter of the *NWC* gene (the third evolutionarily conserved gene at the *RAG* locus) which is located in the second intron of the *RAG-2* gene has bidirectional activity and is responsible for unexpected expression of *RAG-2* open reading frame in nonlymphoid cells. We also identified promoter fragments -71/-95 and -20/-45 relative to the *NWC* transcriptional start site as responsible for bidirectional activity, and showed that the ZFP143 transcription factor is the activator of the *NWC*/*RAG-2* promoter.

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

Degradation of the linker for activation of T cells (LAT): molecular switch of T cell function

Degradation of several signaling components of the T cell receptor complex (TCR) was shown to influence the quantity and the quality of lymphocyte signaling. For example, degradation of the adaptor SLP-76 and the Vav-1 molecule were shown to be involved in the active maintenance of T cell unresponsiveness, whereas expression of the degraded fragment of SLP-76 adapter was shown to heavily impair TCR signaling.

In collaboration with Dr Enrique Aguado and his group at the University of Cadiz (Spain) we found that the LAT molecule undergoes rapid degradation when immature or mature T cells are stimulated through the TCR/CD3 or Fas receptors. We developed methods for measuring kinetics of LAT protein degradation upon Fas and TCR/CD3 mediated signaling using flow cytometry and Western blotting. We further mapped sites of cleavage within the LAT molecule and we found that they were conserved among human and murine LAT proteins. Mutated, cleavage-resistant versions of the LAT proteins were produced and their signaling capacities are being evaluated.

Expression and functional characterization of $hLAT_{long}$: an alternatively spliced isoform of the human linker for activation of T cells (LAT)

Although the existence of an alternatively spliced version of the human *Lat* transcript ($hLAT_{long}$) was reported by Erwin Petek's laboratory and entered in the GenBank database, a physiological function of the resulting protein as well as the expression pattern of the $hLAT_{long}$ mRNA has not been established experimentally. To address these issues we performed a semi-quantitative evaluation of $hLAT_{long}$ cytoplasmic mRNA from normal human peripheral blood mononuclear cells (PBMCs) and the Jurkat T cell line. We found that the ratio between the canonical and the $hLAT_{long}$ isoforms was around 100:1. Sorted subpopulations of T cells and NK cells obtained from human PBMCs are being evaluated by real-time PCR in order to confirm the above findings.

We transiently expressed the $hLAT_{long}$ mRNA in mammalian cell lines and found that it produced a distinctive protein that migrated more slowly than the canonical LAT protein when assayed by SDS-PAGE. When compared with canonical LAT protein by fluorescent microscopy, the $hLAT_{long}$ isoform was predominantly targeted to intracytoplasmic and outer membrane compartments. Lentiviral vectors were prepared in order to measure the functional capacities of the $hLAT_{long}$ isoform when transduced to a Lat deficient human T cell line. Peptides containing amino acid residues expressed by $hLAT_{long}$ were obtained and will be used to raise rabbit immune sera.

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

Laboratory of the Molecular Biology of Microorganisms
Head: Professor Jolanta Zakrzewska-Czerwińska, Ph.D.

Chromosome replication initiation in bacteria
Home page: www.iitd.pan.wroc.pl/dept/mic/index.htm

In bacteria, chromosome replication is initiated by cooperative binding of the initiator protein, DnaA, to multiple sequences termed DnaA boxes – 9-mers within the *oriC* (origin of chromosomal replication) region. DnaA activity, like that of other initiator proteins, is regulated by binding and hydrolysis of ATP. DnaA consists of four domains (I-IV): I-dimerization, II–linker, III- and IV- ATP and DNA binding domains, respectively. Only ATP-bound DnaA is able to unwind the duplex DNA within the *oriC* region. Various factors control the ability of DnaA to bind and unwind DNA. Among them, *Escherichia coli* DiaA and *Helicobacter pylori* HobA have been characterized recently. They were found to interact with domain I of DnaA and stimulate DnaA binding to *oriC*. We showed that, despite a high degree of structural similarity, HobA and DiaA are not interchangeable because they are unable to interact with heterologous DnaA proteins. We revealed particular structural differences impeding formation of heterologous complexes and, consistently, we restored DiaA-enhanced *oriC* binding by the hybrid Ec(I)-Hp(II-IV)DnaA protein, i.e. *H. pylori* DnaA in which domain I was exchanged with that of *E. coli*. This proved that DiaA and HobA are functional homologs and upon binding to DnaA they exert a similar effect on orisome formation. Interestingly, we showed for the first time that the dynamics of DiaA- and HobA-stimulated orisome assembly are different. HobA enhances and accelerates HpDnaA binding to oriC, whereas DiaA increases but decelerates EcDnaA binding with *oriC*. We postulate that the different dynamics of orisome formation reflect the distinct strategies adopted by *E. coli* and *H. pylori* to regulate the frequency of the replication of their chromosomes. DiaA/HobA homologs have been identified in many proteobacteria and therefore might constitute a common, though species-specific, factor modulating bacterial orisome assembly.

Zawilak-Pawlik A, Donczew R, Szafrański S, Mackiewicz P, Terradot L, Zakrzewska-Czerwińska J.(2011) DiaA/HobA and DnaA: a pair of proteins co-evolved to cooperate during bacterial orisome assembly. JMol Biol. 408:238-251

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

Our observations and literature data indicate that under certain conditions in human monocytes there may appear activity of membrane-bound guanylyl cyclase type A (GC-A) that could play a role in inhibition of bacterial endotoxin-induced nuclear factor-kappaB activity. It can be assumed that the appearance of GC-A is determined by some unidentified inflammatory factor. The results of our experiments indicate transcription of the gene encoding GC-A in the human monocytic cell line SC and in the human acute monocytic leukemia cell line THP-1 following treatment with phorbol ester as well as a large increase in the activity of GC-A in phorbol ester-treated THP-1 cells.

The orphan nuclear receptor Nur77 plays roles in the processes of differentiation, steroid hormone production and apoptosis, depending on cell type and signaling proteins activated. The results of luciferase reporter assays suggest that cyclic AMP response element binding protein (CREB) plays a significant role in activation of the nur77 gene in response to neurotrophin treatment. Using the method of real-time PCR it has been shown that a dominant negative mutant, A-CREB, inhibited the expression of endogenous nur77 in response to neurotrophins. In conclusion, analysis of both promoter activation and gene expression indicates the role of transcription factor CREB in nur77 gene activation by neurotrophins.

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

Cyclic peptides

The laboratory has been involved for decades in the search for compounds of various origin, both natural and synthetic, with a potential for application in therapy. Among the studied compounds, derivatives of cyclolinopeptide are of particular interest since they may replace calcineurin inhibitors such as cyclosporin A or tacrolimus with well-known side-effects. In 2011 the immunosuppressive and anti-inflammatory properties of several cyclolinopeptide analogs were investigated in *in vitro* and *in vivo* models, such as: proliferation of human peripheral blood mononuclear cells to phytohemagglutinin, the

secondary humoral immune response to sheep erythrocytes, production of proinflammatory cytokines in human whole blood culture, growth of selected tumor cell lines and the carrageenan inflammatory reaction in the air pouch of mice. Two particularly interesting compounds were identified, JK-VDS (strongly antiproliferative) and KJ-6c (strongly anti-inflammatory). The compounds were also low-toxic with regard to human lymphocytes.

Benzoquinothiazines

A second group of 61 compounds from the benzoquinothiazine family was subjected to evaluation in similar models. In the last stage of investigation three compounds (KPC4a, KPS4c, KPC3c) exhibiting the strongest antiproliferative action and low toxicity were used to study their effects in relation to 3 tumor lines (L-1210 lymphoma, SV-948 colon tumor and A-341 epidermal tumor). It appeared that the compounds displayed a comparable or only somewhat lower anti-tumor action than cisplatin. The compounds will be patented.

Bone-associated cells and their suitability for regenerative medicine

Studies were focused on three types of cells supporting the bone system: osteoblasts, chondrocytes, and fibroblasts and factors influencing their condition and biological function. In cooperation with the Department of Maxillofacial Orthopedics and Orthodontics, Wroclaw Medical University, a pilot study on the impact of nickel ions on the condition and functioning of human osteoblasts was conducted. These cell types are under long-term influence of the metal ions in patients with occlusion corrective braces. The oral environment is conducive to corrosion of joining elements. Cell culture techniques demonstrated discrete, adverse effects of nickel on human osteoblasts. In collaboration with the Tissue Bank at the Regional Centre of Blood Donation and Blood Treatment in Katowice, the root regeneration potential of human chondrocytes, used in autologous re-implantations, was proved. This potential is probably based on an autocrine regulation mechanism via autologous biologically active bone morphogenetic proteins (BMP). Two of the most osteogenic isoforms (BMP-2 and BMP-4) were purified from the culture medium of chondrocytes and their biological activities were demonstrated at two molecular levels: signal transduction and an increase in the effector protein concentration due to specific gene activation. In cooperation with "SOL-GEL Laboratory", the Institute of Material Science and Mechanics, Wrocław University of Technology, the influence of silica-coated austenitic steel 316L probes was examined for the reaction of the recipient tissue in fibroblast cultures in vitro. These silica layers were obtained

by a new sol-gel technique making possible immobilization of biologically active particles on the implant surface due to low temperature of coating. The synthesis of two gene constructs for expression of genes of human BMP-2 and BMP-4 in *Yarrowia lipolytica* was started.

Activation of nuclear transcription factor and TNF-α production by human lung cells treated with polycyclic aromatic hydrocarbons and fungal spores

The pulmonary tissue is exposed to inhaled infectious agents and chemical compounds. We previously showed that fungal spores and polycyclic aromatic hydrocarbons (PAHs), byproducts of carbon-based fuel combustion, induced activity of *signal transcription* and activator of transcription (STATs) in human lung epithelial cells. In these studies we investigated PAHs, *Aspergillus fumigates* and *Alternaria sp*. spores for their ability to activate nuclear transcription factor (NF-κB) and stimulate synthesis of inflammatory cytokine TNF-α in the human lung epithelial A549 cell line. The fungal spores and PAHs stimulated activation of the NF-κB system and TNF-α production in these cells. *Aspergillus fumigates* spores induced the highest activity of the transcriptional system and TNF-α synthesis after 24 h. When the lung cells were treated with PAHs and spores, activation of NF-κB and production of TNF-α were lower in comparison to cells incubated separately either with PAHs or spores. In conclusion, the studied environmental pollutants stimulated NF-κB system activation and production of TNF-α by the respiratory tract epithelium. The PAHs suppressed the pulmonary epithelial response to fungal spores, which could promote the development of fungal infections in the lung.

Application of short-term tests in assessment of atmospheric air pollution

The investigations have confirmed high sensitivity of employed *Salmonella typhimurium* strains TA98 and YG1041 tester strains as well as the *Escherichia coli* K12 PQ37 to organic pollutants absorbed on airborne particulates of the PM10 fractions, which provided evidence for occurrence of PAHs and their nitro, amino and hydroxylamino derivatives in atmospheric air. Mutagenic effect of airborne particulates was presented in the form of the mutagenicity ratio (MR) and the genotoxic effect of airborne particulates was evaluated in the form of induction factor (IF). MR and IF values obtained in both bacterial tests indicated that chemical compounds of both promutagen and direct mutagen nature were present in tested samples, and moreover, similar MR values were obtained in the *Salmonella* assay from experiments carried out with metabolic activation by the microsomal fraction S9 as well as in the absence of the S9 fraction. By including the YG series strains in the assay, it was possible to confirm that

moderately and highly polar classes of compounds are responsible for the mutagenic effect of air pollutions, and besides that, to detect the presence of mutagens in very small volumes of sampled air, of the range of 0.0061-0.049 m^3. Considering the results obtained from SOS Chromotest, a higher genotoxicity was found in tests carried out without metabolic activation than in the presence of the microsomal S9 fraction. Among chemical compounds capable of producing such effect are nitro and amino PAH derivatives, polar aromatic compounds, heterocyclic compounds and phenols.

The only way to provide integrated and quick estimation of human exposure to chemicals adsorbed on airborne particulate matter is implementation of biological monitoring. Because of the complexity of the carcinogenesis, it is necessary to adopt suitable organisms, cells and short-term test systems. Use of human cell culture in research, along with bacterial assays, will provide an opportunity to get a correct response of mammal cells to organic compounds that are pollutants of the atmospheric air.

Antifungal activity of new synthetic analogs of allicin – a preliminary examination

Despite tremendous progress achieved in antifungal therapy, there is still a need for the development of efficient and safe antimycotics, for control of fungal grow in infected mucosa and deep-seated tissues. Development of phyto-preparations – highly effective, non-toxic and devoid of side effects – is an urgent task. Thiosulfinates are garlic compounds characterized by the ability to inhibit the growth of different microorganisms. The main active substance of garlic is allicin, one of the esters of thiosulfinic acid. The purpose of the present study was to test *in vitro* the cytotoxicity and antimycotic activity of two new synthesized thiosulfonates and rhamnolipid biosurfactant isolated from *Pseudomonas sp*. PS-17. Thiosulfonates and rhamnolipid were low-toxic. Thiosulfonates showed inhibitory activity against *Candida* and *Aspergillus*, but *A. fumigatus* was more susceptible than *Candida*. Rhamnolipid RL displayed no activity against *C. albicans* or *A. fumigatus*, in the tested range of concentrations. Low concentrations of synthetic thiosulfonates inhibited growth of *C. albicans*, *C. glabrata* and *A. fumigatus*.

New synthetic analogs of garlic biocides with antifungal activity

The incidence of invasive fungal infections caused by *Candida* spp. is increasing worldwide. Emergence of antimicrobial resistance, high toxicity caused by antifungal agents, therapeutic limitations and high cost of treatment justifies the search for new therapeutic agents, especially for herbal products. We investigated the antifungal potential of the *in vitro* activity of *Hydrastis canadensis* (“goldenseal”) tincture, *Casearia sylvestris* (“guacatonga”)

tincture, *Calendula officinalis* ("pot marigold") tincture and *Vaccinium macrocarpon* ("cranberry") in glycerol against ATCC and clinical strains of *Candida* spp. Average diameters of the inhibition zone (37 mm) were observed for *H. canadensis*, clinical and ATCC strains of *Candida* spp. In addition, the antifungal activity of *C. sylvestris* was detected for clinical and ATCC strains of *Candida albicans* and *C. krusei*, with average diameters of the inhibition zone of 20 mm. Interestingly, *C. officinalis* showed inhibition only for ATCC strains of *C. albicans* and *C. glabrata* (10 mm). *Vaccinium macrocarpon* was effective against *C. albicans* from both origins (18 mm). In conclusion, the herbal products exhibited moderate to appreciable antifungal activities. These preliminary data should be supported by further large-scale studies since new molecules can be used for microbiological control, providing a relevant therapeutic alternative for treatment of mycosis, as well as prevention of recurrence.

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

Studies of etiopathogenesis of endometriosis

Cells with the characteristics of stem cells were isolated from endometrial biopsies of the uterine cavity of patients treated for endometriosis, unexplained infertility or simple ovarian cysts. The phenotype of these cells is characterized by flow cytometry as CD34-CD133 + CD271 + CD90 + CD146 + CD105 + CXCR4 + CD13-. No difference was found in the level of expression of studied antigens between the cell lines isolated from women with gynecological diseases listed. Cells in addition to the above phenotype were characterized by formation of colonies, and the possibility of renewal during long culture in vitro. Identified cells resemble stem cells from umbilical cord blood and support evidence of the occurrence in adult body cells in the early stages of differentiation.

The involvement of antigen-presenting cells in establishing peripheral tolerance during preimplantation period of pregnancy in mice. Supported by Gemini Cost Action FA0702 "Embryo-maternal interactions"

The overall aim of the research project is to check whether in the preimplantation period of pregnancy in the mouse in the peripheral compartment the antigen-presenting cell phenotype is changed towards the tolerogenic phenotype. The aims of the study were to: 1 / identify changes in the expression of costimulatory molecules (CD80, CD86, CD40, and MHCII) at the level of protein on the surface of DC (CD11b +) and macrophages (CD11c +

and F4/80 +) (task completed in 2010) 2 / identify changes in the expression of costimulatory molecule transcript levels at the surface of DC and macrophages 3 / determine the ability of dendritic cells and macrophages to present antigen in vitro, 4 / determine in vivo the effect of blocking of costimulatory molecules CD86, CD80 and CD40 on maintenance of pregnancy (number of implantation places), the number of activated Th cells and cytokine concentrations.

It was found that changes in expression of individual molecules are varied depending on the cell population, which may suggest changes in the repertoire of the population of cells involved in antigen presentation at the periphery in the preimplantation period of pregnancy. In all populations of cells expression of CD86 on day 3.5 of pregnancy was increased, while CD80 surface density varies only in CD11b of pregnant females. In *in vitro* experiments the capacity of APC (CD11b + and F4/80 +) for antigen presentation was assessed. For this purpose, at 3.5 days dendritic cells and macrophages from pregnant females and mice in pseudopregnancy were sorted, pulsed with ovalbumin and cultured with haplotype identical, sorted CD4 + T lymphocytes, isolated by MACS from spleens of mice strain C57BL6/J-Tg (TcraTcrb) 1100Mjb/ J. These cells were characterized by a transgenic TCR recognizing OVA fragment. The intensity of TCD4 +, cell proliferation production of cytokines by activated cells and the expression of CD25 activation antigen ware studied. Blocking the interaction of the molecule CD80 with its ligands was manifested by a more than three-fold increase of the rate of lymphocyte proliferation in the presence of F4/80 + as APC. CD80 molecule seems to have no effect on the proliferation of T lymphocytes stimulated by dendritic cells. The opposite effect was observed in the case of blocking the CD86 molecule. Despite sharing the same ligand molecule, CD80 in this case, a decrease in proliferation of lymphocytes activated by all APC populations studied was observed. An analysis of the cytokine secretion profile showed that blocking of the CD80 molecule led to lower secretion of all tested cytokines (IL-10, IL-12, IL-4, IFN-γ, IL-6). Cytometric analysis of activated CD4+ T lymphocytes from co-culture showed a decrease of the number of CD4 + CD25+ after blocking of CD86 on CD11b+ cells. Blocking of CD80 molecules remained without effect on the number of CD4 + CD25 + in all test cultures containing APC, but resulted in a reduction in mean fluorescence intensity for CD25 relative to appropriate controls. In the experiment *in vivo* antibodies selectively blocking costimulatory molecules anti-CD40, anti-CD80, anti-CD86 and the appropriate isotype control were administered to strain C57Bl6 female/ J at 3.5 days after mating. At 10.5 days of gestation, serum cytokine profile of animals, the distribution of T lymphocytes in the spleen and the effect of blocking

costimulation on the number of fetuses were determined. Blocking of costimulatory molecules CD40, CD80 and CD86 had no effect on the number of fetuses compared with appropriate control groups. The *in vivo* experiment revealed little change in the profile of secreted cytokines. However, blocking of CD80 in pregnant mice results in increased concentration of IL-12 and decreased concentration of IL-10. Moreover, observations on changes in the number of activated T cells in the spleen of pregnant females treated with anti-CD80 showed a decrease in the number of these cells in relation to the number of CD4+CD25 + in the control group. In conclusion, the results of the project indicate that in the preimplantation period of pregnancy at the periphery the pattern of costimulatory molecules is changed (advantage of CD86) and favors recognition of exogenous (paternal?) antigens. In turn, it seems that CD80 costimulatory molecule signaling is responsible for cytokine secretion beneficial for pregnancy maintenance.

The development of Lab-on-chip with optical detection – diagnostic device for quick and cheap quality qualification of bovine embryos. Micro- and Nano-Systems in Chemistry and Biomedical Diagnostics. Supported by POIG 01.03.01-00-014/08-00

Apoptosis plays a special role in the differentiation of multicellular organisms and is an important marker of developmental potential of embryos in the preimplantation stage. Most of the research methods of apoptosis require the preservation of biological material or supravital use of dyes that may adversely affect the development of embryos. The aim of this study was to evaluate the lab-on-a-chip microcytometer for supravital assessment of apoptosis in mouse embryos. The study used morula-stage embryos obtained from the mouse strain C57BL/6J. Embryos were obtained post mortem at 2.5 days of gestation and cultured for 10-12 hours in the presence of actinomycin D and a solution of TNF-alpha as an inducer of apoptosis. After the culture the embryos were evaluated morphologically and then stained with annexin V – FITC conjugate. Some of the embryos were cryopreserved. The research intensity of fluorescence staining of mouse embryos was determined on silicon-glass lab-on-chip with integrated optical fibers. Mean fluorescent intensity of embryos incubated with TNF-α or actinomycin D was significantly higher than in the non-stained control. The level of PS expression in TNF-α treated embryos was comparable to the actinomycin D treated group. However, developmental progress of TNF-α treated embryos was similar to the negative control (medium alone), in contrast to the actinomycin D treated group where all cultured embryos degenerated after 24 hours of culture. In conclusion, we developed a chip which enables assessment of mean fluorescence intensity of stained single embryos. This device

may serve as a useful tool for monitoring of spontaneous and induced apoptosis in live embryos.

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

Comparative analysis of cytokine profile in sera from rheumatoid arthritis (RA) patients before and after treatment with methotrexate and/or TNF-alpha inhibitors

Cytokine profile may provide a suitable environment for differentiation of Th1, Th17, and Treg cells in peripheral blood (PB) of RA patients. We performed analysis of serum concentrations and modifications of TNF-alpha, IL-17, VEGF, IL-6, IFN-gamma, and IL-2 after therapy with methotrexate (MTX) and/or TNF-alpha inhibitors in RA patients.

Thirty-six RA patients and 13 healthy controls were included. Nineteen patients were treated with MTX (MTX group); TNF-alpha inhibitors were administered to the other 17 patients (iTNF group). Serum levels of studied factors were detected before and after 4-6 months of therapy by using cytometric bead array (CBA).

Before therapy, all patients showed higher concentrations of IL-6 and VEGF. Serum levels of TNF-alpha and IL-17 were similar in patients and controls. In progressive RA (iTNF group), we observed lower levels of IL-2 and IFN-gamma compared to the MTX group and controls. After treatment, there was a decrease of IL-6 in the MTX group, but its levels did not normalize in all patients. We noted a trend towards VEGF decline in this group. Both Th1 cytokines, IL-2 and IFN-gamma, remained at lower concentrations in the iTNF group compared to the MTX group and controls. Increased IL-2 levels over control values were found in the MTX group. Neither MTX nor iTNF changed TNF-alpha and IL-17 in patients.

In conclusions, therapy of RA does not result in normalization of proinflammatory factors. The MTX group exhibits higher potential for reversion of an imbalanced cytokine network. Progression of disease leads to irreversible systemic Th1 cytokine defects. Restoration of Th1 protective responses would be of interest as a target for treatment of progressive RA. Our study shows that patients with progressive rheumatoid arthritis exhibit irreversible systemic Th1 cytokine defects, which may contribute to persistent imbalance in PB Th cells differentiation.

CTLA-4 gene polymorphisms influence susceptibility to acute graft versus host disease after allogeneic hematopoietic stem cell transplantation

Donor T lymphocytes play a crucial role in alloimmune recognition and their ability to detect non-self antigens can differentiate predisposition to acute graft versus host disease (aGvHD). The effective recognition and activation of naïve T-cells requires two independent signals. The first is antigen-specific and is sent via the TCR on T-cells. The second signal is critical for allowing full activation. Cytotoxic T-cell antigen (CTLA-4) is a co-inhibitory molecule which downregulates T-cell activation. Polymorphisms in the *CTLA-4* gene result in abnormal expression function as well as dysregulated trafficking of that molecule within cellular compartments. Moreover, the human *CTLA-4* gene is known as a susceptibility region for autoimmune diseases.

Therefore we postulate that polymorphism in the *CTLA-4* gene in donors after hematopoietic stem cell transplantation (HSCT) might be associated with predisposition to aGvHD. Our attention has focused on four functional polymorphic sites: *CTLA-4*c.49A>G, *CTLA-4*g.319C>T, CT60, and Jo31, which have been reported as associated with altered immune responses.

Altogether 175 patients (74 female/101 male), 67 transplanted from related donors (RD-HSCT) and 108 unrelated donors (URD-HSCT) in the Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice, Poland in 2006-2009 were included in this study. Median age of patients was 33 (range 18-57). In 86 patients there were no aGvHD symptoms, while in 87 patients aGvHD incidences were observed.

The single-nucleotide polymorphisms (SNPs) were genotyped using allelic discrimination methods with the following TaqMan SNP Genotyping Assays: C__*2415786_10* for c.49A>G, *C__27834180_10* for g.319C>T, C__*3296043_10* for CT60, C__*30981406_10* for Jo31.

The increased frequency of CT60 [G] allele among patients with aGvHD I-IV was observed in the whole group of patients (pts.) and in pts. after RD-HSCT, while it was not present in URD-HSCT pts. A similar trend was noted for Jo31 SNP. Associations between *CTLA-4*c.49A>G[GG] genotype and aGvHD were observed in all HSCT patients and in pts. after URD-HSCT. The donor haplotype *CTLA-4*c.49A>G[A], *CTLA-4*g.319C>T[C], CT60[A], Jo31[T] was protective against aGvHD grade I-IV in the whole studied group of patients (OR=0.60, p=0.025) and in the group of pts. transplanted from related donors (OR=0.39, p=0.02).

Our study indicated that *CTLA-4* gene polymorphism might be associated with occurrence of aGvHD, especially in recipients transplanted from HLA-identical sibling donors.

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

Bacterial infections are considered to be involved in the development of acute anterior uveitis (AAU). According to epidemiological and clinical data, infections with such enterobacteria as *Escherichia coli*, *Klebsiella*, and *Campylobacter* could play a significant role in the aetiopathogenesis of AAU. One of the common features of these bacteria is the presence of endotoxin (lipopolysaccharide, LPS), which is the main surface antigen of Gram-negative bacteria. Although its role in AAU remains unclear, endotoxins have been proved to be responsible for AAU in animals. In an experimental animal model of endotoxin-induced uveitis (EIU), symptoms of AAU have been successfully induced by LPS isolated from different bacterial species, e.g. *Escherichia*, *Shigella*, and *Salmonella*.

Most of the results related to bacterial etiology of AU are based on investigations of the presence of specific antibodies against bacterial antigens because it is difficult to identify bacteria directly among patients with AU without reactive arthritis. The purpose of our study was to detect antibodies against endotoxins of selected enterobacteria in the sera of patients with idiopathic AAU and search for correlations between the levels of these antibodies and the presence of HLA-B27 antigen accompanied by characteristic symptoms of EIU such as bilaterality and the absence of spontaneous recurrences of the disease. In our study, we selected the lipopolysaccharides (LPSs) isolated from five bacterial species to search for the presence of antibodies against the O-antigen, in the sera of patients and healthy volunteers.

We observed that the sera of patients with a first attack of AAU reacted more strongly with the LPS of *K. pneumoniae* O3 than the sera of patients with relapse of the disease. Patients with bilateral AAU had markedly higher levels of antibodies against four of the five used LPSs than patients with one eye involved. A statistically significant comparison showed higher levels of IgG reacting with LPS of *E. coli* O111 in patients with bilateral eye inflammation admitted with the first attack of AAU compared to controls. The incidence of recurrent form of AAU was significantly increased in HLA-B27-positive patients compared to HLA-B27-negative patients. However, we found in HLA-B27 carriers that those with the

bilateral form of AAU had over three times smaller risk of recurrence and showed a stronger anti-endotoxin immune response than patients with unilateral inflammation.

Our results suggest a potential role of endotoxins in the etiology of the nonrecurrent bilateral form of AAU. We conclude that not only HLA-B27 status but also determination of the number of involved eyes may be useful to assess the risk of recurrence of idiopathic AAU.

Laboratory of General Immunochemistry
Head: Professor Maria Janusz, Ph.D.

Studies on the mechanism of action of proline-rich polypeptide complex (PRP)

Proline-rich polypeptide complex (PRP) provides a combination of immunoregulatory and antioxidative effects. It shows multidirectional activity, concomitantly affecting the immune and nervous system. Some PRP complex properties are reflected by its nona- and hexapeptide constituent components.

The properties of PRP, its role in the development of the immune system and cognitive function indicated its potential use in the treatment of neurodegenerative disorders. The positive clinical effect of the polypeptide complex in the form of sublingual tablets Colostrinin® was demonstrated in the case of Alzheimer's disease (AD), especially when given to patients in the early stages of the disease.

After analysis of the pathogenic phenomena in the case of AD and the properties of PRP/Colostrinin known so far, we can assume that the beneficial clinical effects besides the direct effect on Aβ aggregation could also be connected with the modification of cytokines, reactive oxygen species release as well as the functional/phenotypic differentiation of cells.

In the pathogenesis of aging disease, e.g. AD, inflammatory processes – so-called inflammageing – play a role. Inflammageing can be regulated by transforming growth factor – TGF-β. The suggested role of TGF-β in the early stages of AD indicated the possible regulatory role of PRP and NP in TGF-β induction. It was shown in whole blood cell cultures that neither PRP nor NP are TGF-β inducers. Also no statistically significant modulatory effect of peptides studied at the level of TGF-β induced by LPS+PHA was observed.

The results obtained show that modulation of TGF-β secretion is not a component of positive effects of PRP/Colostrinin shown in the case of AD.

Results of grant activities

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

PC12 is a cell line derived from a pheochromocytoma of the rat adrenal medulla. PC12 cells stop dividing and terminally differentiate when treated with nerve growth factor. This makes them useful as a model system for neuronal differentiation.

In PC12 cells, treated and not treated with Aβ peptides, the influence of proline-rich polypeptide complex (PRP) and its constituent nonapeptide (NP) on expression and activity of neuronal nitric oxide synthase (nNOS) was described. In 80% of PC12 cells cultured in the presence of nerve growth factor (NGF) (10–100 ng/ml) neurite formation was observed. When PRP and NP were used instead of NGF neuritogenesis was also observed, but at lower intensity (30% of cells with shorter neuritis compared with NGF). The optimal effective dose of PRP and NP, 0.1 μg/ml, was selected for the next experiments. Much higher nNOS expression and activity was observed with the use of RT-PCR, Western blot and confocal microscopy. In these cells in the presence of PRP and NP increase of NO release was observed in 77% and 95%, respectively.

Currently, the protective role of PRP and NP against toxic effects of aggregated Aβ peptides (survival of cells, nNOS expression, intracellular NO and cGMP level) is being investigated.

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

In 2011, studies of the interaction of the nuclear proteins from interleukin-6 (IL-6), lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA)-stimulated THP-1, Caco-2, Lu 106, HUVEC cell lines with the promoter region of the hFcRn gene were carried out. The aim of these studies was: to indicate the specificity of the observed protein/DNA interactions; identify the transcription factors that interacted specifically with the hFcRn promoter and the binding sites for these nuclear proteins activated by agents such as IL-6, LPS or PMA. The electrophoretic mobility shift assays and Supershift-EMSA analysis revealed specific binding of nuclear proteins from IL-6, LPS or PMA-stimulated cell lines THP-1, Caco-2, Lu 106, HUVEC to the sequence at -497 and -233 of the hFcRn promoter. Specificity of the protein-DNA interactions was studied by competitive binding assays, which showed complete inhibition of complex formation by a 100-fold molar excess of unlabeled DNA probes. Specificity was further confirmed by the observation that 100-fold molar excess

of unlabeled DNA probes with a mutation in the core binding motif did not suppress complex formation. In the specific interaction with the regulatory element at position -497 of the hFcRn promoter there participate the heterodimer transcription factors of the C/EBP family NF-IL6 and NF-IL6β (also called C/EBPβ and C/EBPδ, respectively), activated in THP-1, Caco-2, Lu 106 cell lines, but in HUVEC, most likely, there participates a heterodimer transcription factor from the C/EBP family and nuclear protein from the other family. The regulatory sequence at position -233 of the hFcRn promoter binds an NF-IL6-NF-IL6β heterodimer from the stimulated THP-1, Caco-2 cell lines and a homodimer NF-IL6 activated in the Lu 106 and HUVEC cell lines. The specific DNA-protein complexes possessed distinct EMSA mobilities, suggesting that the different isoforms of NF-IL6 protein participate in the specific interaction with the regulatory sequences within the hFcRn promoter. The DNA -433/-409 probe representing the sequence of the hFcRn promoter containing the putative binding site for transcription factor STAT3/APRF at position -424 produced the specific DNA/protein complex only with the nuclear protein from the stimulated THP1-cell line. This complex was disrupted by the excess of unlabeled DNA -433/-409 probe and unlabeled DNA-433/-409 probes with mutations in the motif at -424 position, but remained unchanged in the presence of an antibody against STAT3. These data taken together suggest that it is possible that other unrecognized transcription factors from the stimulated THP-1 cell line may bind to the fragment between -433 and -409 bp of the hFcRn promoter.

The observations concerning the small differences in respect to the nature of IL-6, LPS or PMA activated nuclear proteins, binding to the hFcRn promoter, may point to subtle, cell-type specific differences in the regulation of hFcRn gene expression under pathophysiological conditions.

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

The purpose of this project was to produce the recombinant form of Region II of EBA-140 *Plasmodium falciparum* protein. Region II was cloned from genomic DNA of *P. falciparum* clone Dd2. To elucidate the most appropriate overproduction system for the recombinant form of EBA-140, two bacterial expression systems have been examined using the following vectors: pET28a$^+$ (Novagen) and pMALc2x (New England BioLabs). The His-tagged form of Region II of EBA-140, obtained in the pET expression system, was purified from cytoplasm,

periplasm and inclusion bodies of *E. coli* BL21 RIL on Ni-NTA agarose. The resulting protein was poorly soluble and degraded into two regions, F1 and F2, due to proteolysis. The full-length and soluble form of RII of EBA-140 ligand was generated as maltose-binding protein (MBP) fusion in the SHuffle strain of *E. coli* using pMAL-c2x vector. The fusion protein MBP-EBA-140 RII with added C-terminal His-tag and c-myc-tag was purified from cytoplasm of *E. coli* Shuffle by affinity chromatography on amylose and cleaved with Factor Xa. To remove a free form of MBP protein the recombinant form of RII ligand was next purified using Ni-NTA agarose resin. Fermentation culture resulted in average yields per 7 liters of 1 mg recombinant form of Region II of EBA-140.

Carcinoembryonic antigen (CEA) is a member of the immunoglobulin superfamily (IgSF). CEA interactions are involved in colon cancer metastasis and, according to recent studies, the antigen is a receptor for some pathogens (*E. coli* and some *Neisseria sp*.). Our main task was characterization of CEA interactions with several *Enterobacteriaceae* species (*Escherichia coli*, *Plesiomonas shigelloides*, *Shigella flexneri*, *Salmonella enteritidis*). Because of extensive glycosylation of CEA molecule, its N-terminal domain which is devoid of oligosaccharide chains is the most probable interaction site. In the first step we obtained domain N in *E. coli* cells, purified, and determined its biophysical characteristics. Next we performed dot-blot experiments to check the N-domain and lipopolysaccharide (LPS) binding. We found that CEA N-domain binds to LPS molecules, so we extended the dot-blot analysis using 96 O-serotypes of *P. shigelloides* and chose five strains with diverse affinity against the N-domain. We confirmed the results via ELISA. In order to compare the binding affinity we tested selected LPS as an analyte and N-domain as a ligand in SPR analysis. The biosensor analysis confirmed the N-domain – LPS binding specificity and enabled us to establish that lipid A is a part of the LPS molecule responsible for the interactions. The analysis showed that there is no binding between N-domain and polysaccharide molecules (LPS without lipid A) or LPS-OH (lipopolysaccharide with damaged lipid A).

The inheritable NOR polyagglutination was identified in 1982 in the USA, and the second case was found in a Polish family in 1999. The NOR red blood cells are agglutinated by most ABO blood group-matched human sera. It was shown before that the NOR antigens are two globoside elongation products with a terminal Galα1→4GalNAc unit. Reactivity of naturally occurring human antibodies with synthetic oligosaccharides showed that they recognize the terminal di- or trisaccharide unit of NOR glycolipids and cross-react weakly with Galα1→4Gal. We found that all available NOR-positive donors (13 members of the Polish

family and one member of the American family) were heterozygous for the mutation C→G at the nucleotide position 631 of the Pk transferase gene (*A4GALT*), which catalyzes synthesis of the Pk antigen (Galα1→4Gal). All tested NOR-negative members of the Polish family and non-related NOR-negative probands were homozygous for C at this position. The C→G mutation causes a Q→E substitution at position 211 of the polypeptide chain in the vicinity of the DXD motif, which is necessary for activity of transferases (amino acid position 193-195). In order to confirm that the C631G mutation causes change of specificity, the 2102Ep cells were transfected with the pCAG vectors containing cDNA encoding the wild-type Pk transferase or the enzyme with the Q211E mutation. Expression of Pk and NOR antigens was evaluated using anti-Pk and anti-NOR antibodies and measured by flow cytometry. We suggest that the substitution of an amide group by a carboxyl group at position 211 results in a change in specificity of the enzyme, which acquires the ability to add galactose not only to galactose, but also to N-acetylgalactosamine. As a result, the Pk transferase with Q211E mutation can synthesize both Pk antigen (Galα1→4Gal) and NOR antigen (Galα1→4GalNAc). To find the frequency of NOR antigen in the Polish population, samples from blood donors were evaluated using monoclonal anti-NOR antibody. In the year 2011, 200 blood donors were evaluated and no NOR-positive individuals were found.

The Duffy blood group system consists of two major antigens, Fy^a and Fy^b, encoded by two codominant alleles designated *FY*A* and *FY*B*. Three phenotypes Fy(a+b-), Fy(a-b+) and Fy(a+b+) are identified in the Caucasian population. The absence of both Fy^a and Fy^b antigens on erythrocytes, which is frequent in West Africans and those of African descent, is designated as the Duffy-negative phenotype Fy(a-b-). It is defined by homozygous $FY*B^{SE}/FY*B^{SE}$ (SE-silent erythroid) allele. The Duffy-negative phenotype is associated with -33TC mutation in the promoter region of the *FY* gene disrupting a binding site for the erythroid transcription factor GATA-1. The Duffy allele, *FY*X*, encodes an altered form of Fy^b antigen poorly expressed on the erythrocyte surface. Fy^x antigen differs from the native Fy^b by the Arg89Cys and Ala100Thr mutations due to C265T and G298A nucleotide substitutions in the *FYB* gene. In our study we describe a Polish family with three Duffy-negative siblings. These individuals are heterozygous $FY*X/FY^{ES}$. Their mother is heterozygous for FY^{ES} and their father is heterozygous for *FY*X*. To our knowledge this is the first report on occurrence of $FY*X/FY^{ES}$ genotype in the Polish population. The aim of another project was to evaluate 2C3 antibody that recognizes DARC and construct recombinant fragments that are able to inhibit binding of *P. vivax* to erythrocytes. We found that the

epitope recognized by 2C3 antibody is a linear sequence, ^{22}FEDVW^{26}, with some minor effect on binding that can be attributed also to sulfation at Tyr-30 that occurs close to the recognizing sequence. The Fab fragment of 2C3 antibody has been prepared, and it was shown that it binds strongly to its target and can inhibit DARC functions. As an alternative to the mouse monoclonal antibody, two libraries of variable domains of camel heavy chain antibodies (VHH) have been obtained. The libraries were prepared from dromedaries immunized by variants of the first extracellular domain of DARC expressed in bacteria. Several VHH fragments recognizing DARC have been obtained, but almost all of them recognized the same epitope, ^{22}FEDVW^{26}. One of the VHHs, named CA52, was selected for further studies because of its high affinity to the antigen. It was established that CA52 inhibits some DARC functions, such as chemokine binding and invasion of erythrocytes by *P. vivax* merozoites. It was also demonstrated that immobilized CA52 can be used for purification of DARC from K562 cells transfected with cDNA encoding DARC or from red blood cell membranes. Thus, it was shown that fragments Fab and VHH can be useful tools in DARC studies, as both of them bind DARC with a high affinity and can inhibit its functions.

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

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

Deregulation of BNIP3 by oncogenic ras – implications for oncogene-regulated autophagy

Autophagy, regulated cellular self-digestion, represents one of the fundamental mechanisms regulating cell survival. During cancer progression autophagy can be triggered by ionizing radiation, chemotherapeutics and hypoxia, the latter of which activates several responses mediated by HIF-1. High levels of HIF-1 are usually associated with poor prognosis and may signify tumor promoting changes in metabolism, angiogenesis, drug responses and survival.

BNIP3 protein is among notable HIF-1 targets implicated in cell survival. BNIP3 regulation in cancer cells has been mainly subject to external influences, such as hypoxia-dependent activation of HIF-1, but we reasoned that similar effects are often exerted intrinsically, by the activation of oncogenic pathways within cancer cells. This circumstance could render BNIP3 expression constitutive, and alter its molecular context and activity.

Indeed, we observed marked upregulation of BNIP3 in cancer cells harboring activated ras. Thus, we screened a set of human cancer cell lines for Ras activity and BNIP3 status, and found a consistent and positive correlation – the highest Ras activity, the highest BNIP3 expression. Transformation of mouse dermal fibroblasts with H-ras expression vector led to a robust increase in BNIP3 level.

Our results shed new light on the regulation of BNIP3 expression in cancer cells. In addition, they point to a new aspect of the oncogenic transformation, where a single genetic event (e.g. ras mutation) may significantly influence the ability of cells to undergo autophagy.

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

The comparison of two vitamin D analogs' antitumor activity in combined treatment with imatinib in mice bearing A549 tumors

Our previous *in vitro* studies have shown that the proliferation inhibition caused by imatinib can be potentiated/enhanced by PRI-2191 or PRI-2205 calcitriol analogs.

In the present studies, mice receiving imatinib alone had lower tumor weight than animals from the control group, but this effect was not statistically significant. There was also a tumor growth decrease observed in groups treated with imatinib in combination with vitamin D analogs. Moreover, in almost all days of the experiment, the A549 tumor volume of mice treated with imatinib combined with PRI-2191 or PRI-2205 was statistically significant as compared to control mice.

All mice receiving imatinib had lower average body weight during the course of the experiment and this effect was even more pronounced in mice receiving combined chemotherapy with the addition of vitamin D analogs. The highest decrease in body weight (15%) was observed on the 22^{nd} day of the experiment in mice treated with imatinib combined with PRI-2191; after this day mice started to recover.

Our previous results of the individual PRI-2191 s.c. and p.o. toxicity evaluations, showed that the LD_{50} values after s.c. injection varied from 13.6 to 32.4 μg/kg/day in 3 independent determinations, whereas those after p.o. administration were a little higher (lower toxicity) and ranged from 20.0 to 47.1 μg/kg/day. The aim of this experiment was to compare the antitumor effect of PRI-2191 administered p.o. or s.c. in mice bearing A549 tumors and treated with imatinib. Imatinib administered alone in the dose and schedule used

(75 mg/kg/day) did not affect tumor growth. The antitumor effect of PRI-2191 used alone or in combined treatment with imatinib depends on the route of administration. In the case of oral administration, PRI-2191 used alone exhibits a better effect than after s.c. injections. However, in combined treatment with imatinib, synergistic interaction was observed when PRI-2191 was administered subcutaneously. Comparing two days of the experiment, 14 and 21, when both schedules of treatment led to statistically significant tumor growth inhibition when compared to control or imatinib alone, analysis of interactions shows a synergistic effect only in the case of s.c. administration.

Moreover, we observed a decrease of body weight of mice treated with imatinib and PRI-2191. The highest body weight decrease was observed in mice administered orally. However, even in this schedule of the experiment body weight decrease did not exceed 12%.

Studies on a new carrier for effective delivery of siRNA into cancer cells

Studies were carried in a B16 melanoma model. siRNA against integrin β3 were applied. Attention was focused on the chemical modification of side chain of polyethyleneimines (PEIs). N-methylated derivatives of linear PEIs were obtained. This modification aimed at increasing the basicity of a carrier and, in consequence, the strength of a bond between siRNA and a carrier. The relationship between the degree of methylation and efficiency of transfection was examined. Moreover, additional basic groups (e.g. imidazole ring) were introduced into the lateral chain of PEIs. These modified PEIs resemble polylysine, used for transfection. In contrast to polylysine, PEI possess basic centers both in main and lateral chains. In both cases, no enhancement of the efficacy in transfection was observed in comparison to non-modified PEI.

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

Studies on drug-carrier 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 chemical and physical properties of drug conjugates with proteins (for example fibrinogen, albumin, antibodies) and oligosaccharides. Moreover, we developed original procedures for constructing conjugates of boron with carriers potentially applicable to neutron capture therapy.

Physicochemical studies of bacteriophages

Besides the chemical modification of macromolecules, we investigate the physicochemical properties of bacterial viruses (bacteriophages). In particular, we have developed an effective procedure for the purification of bacterial viruses.

Collaboration with industry.

We collaborate with the pharmaceutical companies Adamed and Finepharm.

The Laboratory of Biomedical Chemistry is a part of the Integrated Laboratory of Experimental Oncology and Innovative Technologies - "NEOLEK"

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

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

Genetic background and pathomorphological evaluation of allogeneic reaction after transplantation of hematopoietic stem cells

The research activity of the laboratory is devoted to elucidation of factors influencing the outcome of allogeneic hematopoietic stem cell transplantation (HSCT). The study consists of research on the genetic background and phenotypically described immune system network. In addition, we are carrying out a study on reactivation of Herpes and Polyoma viruses to determine the immune system potential in patients post HSCT. The outcome is as follows:

1. In continuation of our previous study on CXCL12 (SDF-1) we looked at the polymorphism of CXCR4, which is a receptor of SDF-1. It appeared that CXCR4 (rs2228014) – T allele was present in similar proportions in donors and recipients (30.5 vs. 47.5%). The polymorphism did not influence the mobilization potential of donors but the presence of T allele favored platelet recovery post transplantation.
2. The immune system network involves a recently described population of Th17 lymphocytes which produce IL-17, a very potent proinflammatory cytokine. Th17 activity may be controlled by T regulatory cells furnished with suppressor cells machinery visualized by staining for FoxP3. Immunocytochemical analysis documented that the presence of IL-17 producing cells is closely associated with the severity of mucous injury in the course of graft-versus-host disease (GvHD). Unfortunately, these Th17 FoxP3-

positive cells associated with inflammation are rarely present at the tissue site. However, FoxP3-positive cell proportions in GvHD lesions follow the extent of infiltration of the tissue by CD3+ cells not being IL-17+.

3. Survival post HSCT largely depends on the potential of the immune system originated/stemmed by transplanted CD34+ progenitors. We found that the percentages of naive T cells significantly correlate with numbers of CD34+ in transplanted material (CD4+, CCL7+, CD45RA+). Also it was apparent from our study that anti-thymocyte antibodies hampered T cell recovery but only during the first week post transplant. In contrast, anti-CD52 antibody negatively influenced lymphocyte recovery at least for one month post HSCT.
4. In our recent study, we defined the pace of CD4+ cells reconstitution. We found that early post transplant HHV6 reactivation exerted an advert effect of CD4+ cells recovery. In contrast, cytomegalovirus (CMV) reactivation and Epstein-Barr virus (EBV) reactivation followed poor CD4+ cell reconstitution. Survivors post CMV reconstitution showed an increase in proportions of CD8+ cells (sign of cytotoxic cell response to the virus) but those having EBV reactivation also showed in a period of 1 month an increase in CD20+ lymphocytes (sign of B lymphocytes expansion and humoral response). This observation coined a standard of CD4+ cell reconstitutions in patients with event-free post-transplant outcome and in those with Herpes viruses reactivation.

The above activity was undertaken as a preliminary work performed prior to grant application. Considering the activity supported by grants the following achievements have been gained so far.

1. Dynamics of Th17 changes in a population of peripheral blood stem cells (PBSC) were elucidated. It was documented that the increase of IL-17+ cells may be seen prior to acute GvHD (aGvHD) but they promptly disappeared from the blood when aGvHD began. Immunopatho-morphology of aGvHD was evaluated to find out whether homing of Th17 cells at the site of aGvHD was associated with clinical symptoms and tissue pathology.
2. Our group plays a leading role in the study on "*Implementation and harmonization of national immunogenetic studies supporting a final decision of recipient - alternative donor pair selection of allogeneic hematopoietic cell transplantation*". This study was based on 370 donor-recipient pairs that gave an opportunity to discuss a number of

concordant and discrepant results to provide a hint as to the clinical significance of selected immunogenetic factors. The outcome brought information on the association between IFNγ, TNFα, IL-6, CCR5, CXCL12 and NOD2/CARD15 genotypes and aGvHD and infections post transplant. Also the study on IL-10 genotype gave a new insight as to the association between IL-10 genotype and aGvHD. ATA/ATA genotype was found to be associated with aGvHD, independently of whether present in donor or recipient. ATA carriers were at higher risk of CMV reactivation during one year post HSCT observation. Recipients having ACC haplotype or transplanted with ACC-positive donors less frequently developed aGvHD. Patients with ACC haplotype had higher proportions of CD4+FoxP3+ lymphocytes in blood in two longitudinal observations post HSCT, at the time of hematological recovery and two weeks later, while those that were ACC/ACC homozygotes were characterized with better 5-year overall survival.

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

Immunogenetics of human diseases

We are presently carrying out 12 projects granted by the Polish Ministry of Science and Higher Education or by the National Centre of Science, and one project (Wrovasc) from the Programme Innovative Economy co-sponsored by the European Fund for Regional Development of the European Union. Some of these projects are at their beginnings. The most important results of others are as follows:

a) ***Associations of HLA-DR and HLA-DQ alleles with the presence of antisperm autoantibodies in cryptorchidism***

This work was done in collaboration with Professor Maciej Kurpisz from the Institute of Human Genetics, Polish Academy of Sciences, Department of Reproductive Biology and Stem Cells, Poznań, Poland.

Cryptorchidism is a frequent syndrome occurring in 1-2% of males within the first year of age. Autoimmune reactions, particularly directed to testicular elements and/or spermatozoa, have been found to be often associated with cryptorchidism. Therefore we investigated in this study the frequency of HLA class II alleles in order to recognize possible genetic predisposition for antisperm antibodies development in prepubertal boys with diagnosed cryptorchidism in a Caucasoid population. Sixty prepubertal boys with cryptorchidism and sixty healthy boys were examined for anti-sperm antibodies by indirect immunobead test as

well as for their HLA-DRB1 and -DQB1 alleles using DNA obtained from peripheral blood leukocytes. The typing of HLA-DRB1 and -DQB1 was performed by using the PCR-SSP low resolution method.

Allele frequencies of HLA-DRB1 and HLA-DQB1 did not differ between boys with cryptorchidism and control boys. However, weakly significant differences in DRB1*04 (p corrected = 0.0475) and DQB1*06 (p corrected = 0.0385) were seen between cryptorchid patients with and without AsA, but neither of these two patient groups differed significantly in HLA class II frequencies from controls except for AsA-negatives and HLA-DQB1*06 (p corrected = 0.0247). On the other hand, comparison of cryptorchid boys with familial cryptorchidism and/or infertility to control boys revealed a highly significant (p corrected = 0.0006) difference in HLA-DRB*11 frequency, whereas boys with sporadic cryptorchidism did not differ from controls. A much weaker, but still significant difference in DRB*11 frequency was also observed between boys with bilateral cryptorchidism and controls (p corrected = 0.037), whereas patients with unilateral cryptorchidism were not different from controls in frequency of any HLA-DRB1 or -DQB1 allele tested.

Thus, predisposition to produce anti-sperm antibodies seems to be only weakly associated with HLA class II genes, although this question requires further study on a much larger population sample. It is plausible that familial and sporadic cryptorchidism may present a distinct genetic background. The same may, to a lower extent, apply to bilateral and unilateral cryptorchidism. (*publication*)

b) ***Genetics of kidney allograft rejection: role of KIR and HLA genotype***

Recipient NK cells may detect the lack of recipient's HLA antigens on donor renal tissue using their killer cell immunoglobulin-like receptors (KIRs). *KIR* genes are differently distributed in individuals, possibly contributing to differences in response to allogeneic graft. We compared frequencies of 10 *KIR* genes by PCR-SSP in 89 kidney graft recipients rejecting allogeneic renal transplants with those in 194 recipients accepting grafts and 690 healthy control individuals. HLA matching results were drawn from medical records. We observed higher frequency of both a full-length *KIR2DS4* gene and its variant with 22-bp deletion in rejectors than in non-rejectors. This effect was modulated by the *HLA-B,-DR* matching, particularly in recipients who did not have glomerulonephritis but had both forms of the *KIR2DS4* gene. In contrast, in recipients with glomerulonephritis, *HLA* compatibility seemed to be much less important for graft rejection than presence of the *KIR2DS4* gene. Simultaneous presence of both gene variants strongly decreased the probability of free-from-

rejection course. Interestingly, *KIR2DS5* seemed to protect the graft in the presence of *KIR2DS4fl* but in the absence of *KIR2DS4del*. Our results suggest a protective role of *KIR2DS5* in graft rejection and a possible association of *KIR2DS4* with kidney rejection, particularly in recipients with glomerulonephritis. (*publication under consideration by editorial office*)

c) *Genes of killer cell immunoglobulin-like receptors and their HLA ligands associated with susceptibility to non-small-cell lung cancer and with response to treatment*

Non-small-cell lung carcinoma (NSCLC) is a multifactorial disease influenced by both environmental and genetic factors. Here, we examined whether the repertoire of killer immunoglobulin-like receptor (*KIR*) genes and genes for their ligands, C1 and C2 (on HLA-C) and Bw4 (on HLA-B and some HLA-A), may affect susceptibility to non-small-cell lung cancer and response to treatment. We typed 269 NSCLC patients and 690 healthy control individuals for *KIR* genes and for C1/C2 and Bw4 markers. Neither individual *KIR* genes nor numbers of activating or inhibitory *KIR* genes were associated with NSCLC. C1C2 genotype was less frequent in patients than in controls, whereas both C1C1 and C2C2 homozygotes were more frequent in patients ($\chi^2 = 7.73$; df = 2; p=0.021). Patients positive for *KIR2DL2* and *KIR2DS2* genes and homozygous for the C1 *HLA-C* ligand were 6 times more likely to respond to treatment than those with other genotypes (p = 0.034). In accordance with this, patients with the *KIR2DL2*+/*KIR2DS2*+, C1C1 genotype survived longer than others (p=0.0094). Median survival was 23 months for KIR2DL2/2DS2-positive, C2-negative patients, but only 10 months for other genotypes.

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

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

Biology of endothelial precursor cells

Our main topic was to prepare model endothelial cell lines, representing early endothelial cell differentiation stages. These model cell lines may be applied for studies on regenerative potential of endothelium, but also serve for elaboration of a tumor neoangiogenesis inhibition strategy. In collaboration with Dr Claudine Kieda from Centre Biophysique Moleculaire (CNRS, Orleans, France) we have established two unique cell lines of human early

endothelial precursor cells ($CD133^+$, $CD34^-$, $VEGFR2^+$) with retained high proliferative potential. The phenotypic and functional analysis of these cell lines was published in an original paper (Cytometry part A, 2011, 79(8): 594-602). Both these cell lines were also patented (patent No: PTC/FR2011/050045).

Another study, performed in collaboration with Wrocław Medical University clinics, concerned the levels of circulating endothelial precursor cells (EPCs) and circulating endothelial cells (CECs) in patients with pregnancy-induced hypertension (PIH) and in psoriasis. Both EPCs and CECs are described as cell populations reflecting the state of patients' vasculature, in the context of its regenerative potential (EPCs) and/or vascular dysfunction (CECs). In PIH patients both EPC and CEC numbers were found to be diminished, as compared with the controls, which may suggest severe endothelial dysfunction. In contrast, in psoriatic patients EPC levels were decreased, whereas those of CECs were significantly increased. Moreover, plasma levels of factors connected with endothelial injury, such as sICAM-1, sE-selectin, von Willebrand factor and VEGF, were found to be significantly increased in psoriatic patients as compared with the controls. The levels of these factors are presently considered, in the context of clinical parameters of the patients, as potential prognostic disease markers.

Expression of MUC1 in human and murine mammary carcinoma cells change their glycosylation profile and carbohydrate-dependent adhesive properties

MUC1 mucin is one of the most studied molecular markers associated with breast cancer. This mucin is the carrier of many O-glycans which in normal breast epithelium are synthesized as extended carbohydrate chains. However, during neoplastic transformation O-glycans undergo characteristic changes and are present as short core 1-based structures represented, among others, by T antigen (Galβ1-3GalNAc), another well-established marker of breast cancer. The T antigen is synthesized by C1GalT1, which adds a Gal residue to Tn antigen (GalNAc). In normal breast tissues, the core 1 T antigen is usually converted to the branched core 2 structures by the action of C2GnT, resulting in elongation of the carbohydrate chain. However, in breast cancer cells the expression of this enzyme is highly decreased, giving T antigen. This structure in breast tumors is usually cryptic and present as sialyl T antigen, because of high activity of ST3GalI. In breast cancer the major carrier of T antigen is MUC1. Despite this fact there is not much known about the relations between the expression of this mucin and the occurrence of this carbohydrate antigen in breast carcinoma. Also, essentially nothing is known about the mechanisms which control the expression of

C1GalT1, C2GnT and ST3 GalI in normal and cancerous breast tissues. In the current studies, we present evidence that overexpression of MUC1 mucin in breast cancer cells can affect their O-glycosylation pattern by down-regulation of C2GnT and ST3GalTI. As T antigen, the product of such changes, is mostly carried by MUC1, it is the first example of a glycoprotein which directs its own glycosylation. We also show that changes in the expression of tumor-associated carbohydrate structures such as the loss of sialyl LewisX structures and appearance of T antigen could strongly affect the adhesive properties of cancer cells and in this way their metastatic properties.

Publications – 2011

Articles published in the journals from Thomson Reuters Master Journal List

1. Boryczka S, Mól W, Milczarek M, Wietrzyk J, Bębenek E.: Synthesis and *in vitro* antiproliferative activity of novel (4-chloro- and 4-acyloxy-2-butynyl)thioquinolines. *Med Chem Res,* 2011, 20, 1402–1410 IF - 1,058
2. Borysowski J, Łobocka M, Międzybrodzki R, Weber-Dąbrowska B, Górski A.: Potential of bacteriophages and their lysins in the treatment of MRSA. *BioDrugs,* 2011, 25(6), 347-355 IF – 4,192
3. Brzozowska E, Bazan J, Gamian A.: The functions of bacteriophage proteins. *Post Hig Med Dośw,* (online), 2011, 65, 167-176 IF - 0,511
4. Bugla-Płoskońska G, Korzeniowska-Kowal A, Guz-Regner K.: Reptiles as a source of *Salmonella* O48 – clinically important bacteria for children: The relationship between resistance to normal cord serum and outer membrane protein patterns. *Microb Ecol*, 2011, 61(1), 41-51 IF - 2,875
5. Całkosiński I, Rosińczuk-Tonderys J, Dzierzba K, Bronowicka-Szydełko A, Seweryn E, Majda J, Całkosińska M, Gamian A.: Estimation of the action of three different mechlorethamine doses on biochemical parameters during experimentally induced pleuritis in rats. *Pharmacol Rep*, 2011, 63(2), 501-517 IF - 2,50
6. Całkosiński I, Rosińczuk-Tonderys J, Szopa M, Dobrzyński M, Gamian A.: High doses of tocopherol in the prevention and potentiation of dioxin in experimental inflammation-potential application. *Post Hig Med Dośw,* (online), 2011, 65, 143-157 IF - 0,511
7. Ciszek-Lenda M, Nowak B, Sróttek M, Gamian A, Marcinkiewicz J.: Immunoregulatory potential of exopolysaccharide from *Lactobacillus rhamnosus* KL37. Effects on the production of inflammatory mediators by mouse macrophages. *Int J Exp Pathol*, 2011, 92(6), 382-391 IF - 2,127
8. Ciszek-Lenda M., Strus M., Górska-Frączek S., Targosz-Korecka M., Śróttek M., Heczko P.B., Gamian A., Szymoński M., Marcinkiewicz J.: Strain specific immunostimulatory potential of lactobacilli-derived exopolysaccharides. *Cent. Eur J Immunol*, 2011, 36(3), 121-129 IF - 0,451
9. Crowther-Swanepoel D, Di Bernardo MC, Jamroziak K, Karabon L, Frydecka I, Deaglio S, D'Arena G, Rossi D, Gaidano G, Olver B, Lloyd A, Broderick P, Laurenti L, Szemraj-Rogucka Z, Robak T, Catovsky D, Houlston RS.: Common genetic variation at 15q25.2 impacts on chronic lymphocytic leukaemia risk. *Br J Haematol,* 2011, 154(2), 229-33 IF - 4,942

10. Czepczyńska-Krężel H, Czerwiński M, Krężel A, Krop-Wątorek A.: Isolation of carcinoembryonic antigen N-terminal domains (N-A1) from soluble aggregates. *Prot Exp Purif,* 2011, 78, 78-85 IF - 1,644
11. Dobrzyńska D, Jerzykiewicz L.B, Bejczak J, Wietrzyk J, Świtalska M.: Synthesis and characterization of chloro-bis(quinoline-2-carboxylato-κ2N,O)gallium(III). *J Coord Chem,* 2011, 64(6), 1082–1090 IF – 1,932
12. Donczew M, Ginda K, Zakrzewska-Czerwińska J, Jakimowicz D.: Solving the mysteries of the bacterial cell - application of novel techniques in fluorescence microscopy. *Post Hig Med Dośw,* (online), 2011, 65, 114-123 IF – 0,511
13. Drulis-Kawa Z, Mackiewicz P, Kęsik-Szeloch A, Maciaszczyk-Dziubinska E, Weber-Dąbrowska B, Dorotkiewicz-Jach A, Augustyniak D, Majkowska-Skrobek G, Bocer T, Empel J, Kropinski AM.: Isolation and characterization of KP34-a novel φKMV-like bacteriophage for *Klebsiella pneumoniae*. *Appl Microbiol Biot*, 2011, 90(4), 1333-45 IF – 3,280
14. Gamian A, Mieszała M, Lipiński T, Zielińska-Kuźniarz K, Gawlik-Jędrysiak M, Dzierzba K, Pietkiewicz J, Szeja W.: O-Aminoacylation of bacterial glycoconjugates: from native structure to vaccine design. *Curr Pharm Biotechnol*, 2011 12(11), 1781-91 IF - 4,744
15. Gąsiorowski K, Lamer-Zarawska E, Leszek J, Parvathaneni K, Yendluri B.B, Błach-Olszewska Z, Aliev G.: Flavones from root of *Scutellaria baicalensis* Georgi: drugs of the future in neurodegeneration? *CNS Neurol Disord Drug Targets*, 2011, 10, 184-191 IF - 3,618
16. Gliszczyńska A, Lysek A, Janeczko T, Switalska M, Wietrzyk J, Wawrzeńczyk C.: Microbial transformation of (+)-nootkatone and the antiproliferative activity of its metabolites. *Bioorg Med Chem,* 2011, 19, 2464–2469 IF – 2,978
17. Gliszczyńska A, Switalska M, Wietrzyk J, Wawrzeńczyk C.: Synthesis of a natural γ-butyrolactone from nerylacetone by *Acremonium roseum* and *Fusarium oxysporum* cultures. *Nat Prod Commun,* 2011, 6(3), 367-370 IF - 0,894
18. Gorczyca D, Paściak M, Szponar B, Gamian A, Jankowski A.: An impact of the diet on serum fatty acid and lipid profiles in Polish vegetarian children and children with allergy. *Eur J Clin Nutr,* 2011; 65(2), 191-195 IF – 2,563
19. Górska-Frączek S, Sandström C, Kenne L, Rybka J, Strus M, Heczko P, Gamian A.: Structural studies of the exopolysaccharide consisting of a nonasaccharide repeating unit isolated from *Lactobacillus rhamnosus* KL37B. *Carbohydr Res*, 2011, 346(18) 2926-2932 IF - 1,898
20. Hańczyc P, Matczyszyn K, Pawlik K, Olesiak-Bańska J, Leh H, Buckle M.: Spontaneous formation of liquid crystalline phases in highly concentrated plasmid DNA. *Liquid Crystals*, 2011, 38, 461–468 IF – 1,653
21. Hojka A, Rapak A.: Receptory aktywowane proliferatorami peroksysomów (PPAR). Właściwości antyproliferacyjne. *Post Hig Med Dośw*, 2011, 65, 404-413 IF – 0,511
22. Hryncewicz-Gwóźdź A, Jagielski T, Sadakierska-Chudy A, Dyląg M, Pawlik K, Baran E, Szepietowski J.C.: Molecular typing of *Trichophyton rubrum* clinical isolates from Poland. *Mycoses,* 2011, 54 e726-e736 IF – 1,667
23. Iwaszko M, Bogunia-Kubik K.: Clinical significance of the HLA-E and CD94/NKG2 interaction. *Arch Immunol Ther Exp,* 2011, 59, 353-367 IF – 2,385
24. Iwaszko M, Bogunia-Kubik K.: Znaczenie polimorfizmu HLA-E w odpowiedzi immunologicznej. *Post Hig Med Dośw*, 2011, 65, 616-662 IF – 0,511

25. Jagielski T, Augustynowicz-Kopeć E, Pawlik K, Dziadek J, Zwolska Z, Bielecki J.: A two-step strategy for molecular typing of multidrug-resistant *Mycobacterium tuberculosis* clinical isolates from Poland. *Pol J Microbiol*, 2011, 60(3), 233–241 IF – 0,660
26. Jarząb A, Górska-Frączek S, Rybka J, Witkowska D.. Enterobacteriaceae infection – diagnosis, antibioticresistance and prevention. (Zakażenia pałeczkami jelitowymi – diagnostyka, oporność na antybiotyki i profilaktyka). *Post Hig Med Dośw,* (online) 2011; 65, 55-72 IF - 0,511
27. Jończyk E, Kłak M, Międzybrodzki R, Górski A.: The influence of external factors on bacteriophages-review. *Folia Microbiol,* 2011, 56(3), 191-200 IF – 0,977
28. Kałas W, Świderek E, Rapak A, Kopij M, Rak J, Strządala L.: H-ras up-regulates expression of BNIP3. *Anticancer Res*, 2011, 31, 2869-2875 IF – 1,656
29. Karabon L, Jedynak A, Giebe.: *KIR/HLA* gene combinations influence susceptibility to B-cell chronic lymphocytic leukemia and the clinical course of disease. *Tissue Antigens,* 2011, 78(2), 129-38 IF - 3,024
30. Karabon L, Jedynak A, Tomkiewicz A, Wolowiec D, Kielbinski M, Woszczyk D, Kuliczkowski K, Frydecka I.: ICOS gene polymorphisms in B-cell chronic lymphocytic leukemia in the Polish population. *Folia Histochem Cytobiol,* 2011, 49(1), 49-54 IF - 0,902
31. Karabon L, Pawlak E, Tomkiewicz A, Jedynak A, Passowicz-Muszynska E, Zajda K, Jonkisz A, Jankowska R, Krzakowski M, Frydecka I.: *CTLA-4*, *CD28,* and *ICOS* gene polymorphisms associations with non-small cell lung cancer (NSCLC). *Hum Immunol*, 2011, 72(10), 947-54 IF - 2,872
32. Katarzyńska J, Mazur A, Rudzińska E, Zimecki M, Artym J, Jankowski S, Zabrocki J.: Cyclolinopeptide derivatives reverse methotrexate-induced suppression of the humoral immune response in mice. *Eur J Med Chem*, 2011, 46, 4608-4617 IF – 3,193
33. Kiełbowicz Z, Kuryszko J, Strządała L.: Experimental allogenic transplantation of cornea endothelial cell in cats. *Pol J Vet Sci*, 2010, 13(4), 731-741 IF – 0,507
34. Kisielow P.: Lymphocyte development, tolerance and autoimmunity: a personal perspective. *Arch Immunol Ther Exp,* 2011, 59(5), 327-30 IF -2,385
35. Kolesinska B, Barszcz K, Kaminski ZJ, Drozdowska D, Wietrzyk J, Switalska M.: Synthesis and cytotoxicity studies of bifunctional hybrids of nitrogen mustards with potential enzymes inhibitors based on melamine framework. *J Enzyme Inhib Med Chem,* 2011 Sep 8. IF - 1,574
36. Kosmaczewska A, Świerkot J, Ciszak L, Wiland P.: Rola subpopulacji limfocytów pomocniczych Th1, Th17 i Treg w patogenezie reumatoidalnego zapalenia stawów z uwzględnieniem przeciwzapalnego działania cytokin Th1. The role of Th1, Th17, and treg cells in the pathogenesis of rheumatoid arthritis including anti-inflammatory action of Th1 cytokines. *Post Hig Med Dośw*, 2011, 65, 397-403 IF - 0,511
37. Krawczyk P, Matuszyk J.: Mechanizm aktywacji fosfolipazy Cγ1. *Post Hig Med Dośw,* (online), 2011, 65, 470-477 IF – 0,511
38. Kryczek I, Zhao E, Liu Y, Wang Y, Vatan L, Szeliga W, Moyer J, Klimczak A, Lange A, Zou W.: Human Th17 cells are long-lived effector memory cells. *Science Translational Medicine*, 2011, 3(104), 104ra100 IF – 3, 292
39. Krzystek-Korpacka M, Mierzchala M, Neubauer K, Durek G, Gamian A. Midkine, a multifunctional cytokine, in patients with severe sepsis and septic shock: a pilot study. *Shock,* 2011, 35(5), 471-477 IF - 3,203

40. Krzystek-Korpacka M, Patryn E, Kustrzeba-Wojcicka I, Chrzanowska J, Gamian A, Noczynska A.: The effect of a one-year weight reduction program on serum uric acid in overweight/obese children and adolescents. *Clin. Chem. Lab. Med*. 2011; 49(5): 915-921. IF - 2,069
41. Krzystek-Korpacka M, Patryn E, Kustrzeba-Wojcicka I, Chrzanowska J, Gamian A, Noczynska A.: Gender-specific association of serum uric acid with metabolic syndrome and its components in juvenile obesity. *Clin Chem Lab Med*,. 2011, 49(1), 129-136 IF - 2,069
42. Krzystek-Korpacka M, Patryn E, Bednarz-Misa I, Mierzchała M, Hotowy K, Czapinska E, Kustrzeba-Wojcicka I, Gamian A, Noczynska A.: Circulating adipocyte fatty acid-binding protein, juvenile obesity, and metabolic syndrome. *J Pediatr Endocr Met*, 2011, 24 (11-12), 921-928 IF – 0,887
43. Kurpisz M, Nakonechny A, Niepieklo-Miniewska W, Havrylyuk A, Kamieniczna M, Nowakowska B, Chopyak V, Kuśnierczyk P.: Weak association of anti-sperm antibodies and strong association of familial cryptorchidism/infertility with HLA-DRB1 polymorphisms in prepubertal Ukrainian boys. *Reprod Biol Endocrinol,* 2011, 9(1), 129-134 IF- 1,695
44. Kuśnierczyk P, Giebel S, Nowak I.: Association of KIR2DS4 gene with susceptibility to leukemia: Chinese-Polish discrepancy. *Leukemia Res,* 2011, 35(11), 1540 IF – 2,555
45. Laszkiewicz A, Cebrat M, Miazek A, Kisielow P.: Complexity of transcriptional regulation within the Rag locus: identification of a second Nwc promoter region within the Rag2 intron. *Immunogenetics,* 2011, 63(3), 183-7 IF – 2,942
46. Lisowska E, Duk M.: Diversity of natural anti-α-galactosyl antibodies in human serum. In: „Immunology of complex carbohydrates 3", red. Wu AM. *Adv Exp Med Biol*, 2011, 705, 571-583 IF - 1,379
47. Liszkiewicz H, Nawrocka W.P, Sztuba B, Wietrzyk J, Jaroszewicz J, Nasulewicz A, Pełczyńska M.: Synthesis and antiproliferative activity *in vitro* of new pyrido[1,4-b]diazepine derivatives and imidazo[4,5-b]pyridine. *Acta Pol Pharm,* 2011, 68(3), 349-355 IF – 0,465
48. Maj T, Switała-Jelen K, Miazek A, Szafarowicz-Basta B, Kiczak L, Slawek A, Chelmonska-Soyta A.: Effects of tamoxifen on estrogen receptor-α level in immune cells and humoral specific response after immunization of C3H/He male mice with syngeneic testicular germ cells (TGC). *Autoimmunity,* 2011, 44(6), 520-30 IF – 2,138
49. Maj T, Sławek A, Kuratewicz-Kaleta K, Chełmońska-Soyta A.: 17-β estradiol and interferon tau interact in the regulation of the immune response in a model of experimental autoimmune orchitis. *J Interferon Cytokine Res*, 2011, 31(11), 825-37 IF - 2,576
50. Mazur G, Jaskuła E, Kryczek I, Dłubek D, Butrym A, Wróbel T, Lange A, Kuliczkowski K.: Proinflammatory chemokine gene expression influence survival of patients with non-Hodgkin's lymphoma. *Folia Histochem Cytobiol*, 2011, 49(2), 240-247 IF – 0,902
51. Mazur L, Kozioł AE, Modzelewska-Banachiewicz B, Ucherek M, Zimecki M, Artym J.: 2-(4-phenyl-5-pyridin-2-yl-4H-1,2,4-triazol-3-yl) cyclohexanecarboxylic acid and its DMSO solvate: synthesis, crystal structure and biological activity. *J Chem Crystallogr*, (online), 2011, 41(6), 880-885 IF – 0,666

52. Mierzchala M, Krzystek-Korpacka M, Gamian A, Durek G:. Quantitative indices of dynamics in concentrations of lipopolysaccharide-binding protein (LBP) as prognostic factors in severe sepsis/septic shock patients--comparison with CRP and procalcitonin. *Clin Biochem*, 2011;,44(5-6), 357-363 IF - 2,043
53. Misiuk-Hojło M, Międzybrodzki R, Grzybowski A, Lugowski C, Niedziela T, Turno-Kręcicka A, Szymaniec S.: Elevated levels of anti-endotoxin antibodies in patients with bilateral idiopathic acute anterior uveitis. *Acta Ophthalmol*, 2011, 89, 283-288 IF – 2,809
54. Mitkiewicz M, Kuropatwa M, Kurowska E, Gorczyca WA: Different effects of soluble and particulate guanylyl cyclases on expression of inflammatory cytokines in rat peripheral blood mononuclear cells. *Immunobiology*, 2011, 216(3), 423-30 **IF – 4,114**
55. Myc LA, Gamian A, Myc A.: Cancer vaccines. Any future? *Arch Immunol Ther Exp*, 2011, 59(4), 249-259 IF - 2,385
56. Naumova E, Ivanova M, Pawelec G, Constantinescu I, Bogunia-Kubik K, Lange A, Qguz F, Carin M, Franceschi C, Caruso C, Middleton D.: Immunogenetics of Aging': report on the activities of the 15th International HLA and Immunogenetics Working Group and 15th International HLA and Immunogenetics Workshop. *Tissue Antigens*, 2011, 77(3), 187-192 IF - 3,024
57. Nawrot U, Zaczyńska E, Czarny A, Lubenets V.I, Karpenko E, Gosciniak G.: New synthetic analogs of garlic biocides with antifungal activity. *Mycoses,* 2011, 54, suppl.s2, 64 IF – 1,667
58. Nowak I, Majorczyk E, Płoski R, Senitzer D, Sun JY, Kuśnierczyk P.: Lack of KIR2DL4 gene in a fertile Caucasian woman. *Tissue Antigens*, 2011, 78(2), 115-119 IF - 3,024
59. Nowak I, Malinowski A, Tchórzewski H, Barcz E, Wilczyński JR, Banasik M, Gryboś M, Kurpisz M, Łuszczek W, Majorczyk E, Wiśniewski A, Senitzer D, Sun JY, Kuśnierczyk P.: HLA-C C1C2 heterozygosity may protect women bearing the killer immunoglobulin-like receptor AA genotype from spontaneous abortion. *J Reprod Immunol*, 2011, 88(1), 32-37 IF - 2,204
60. Olesiak-Bańska J, Nyk M, Kaczmarek D, Matczyszyn K, Pawlik K, Samoć M.: Synthesis and optical properties of water-soluble fluoride nanocrystals co-doped with Eu3+ and Tb3+. *Optical Materials*, 2011, 33, 1419-1423 IF – 1,679
61. Oslizlo A, Miernikiewicz P, Piotrowicz A, Owczarek B, Kopciuch A, Figura G, Dabrowska K.: Purification of phage display-modified bacteriophage T4 by affinity chromatography. *BMC Biotechnology,* 2011, 11, 5-14 IF – 2,859
62. Paprocka M, Krawczenko A, Dus D, Kantor A, Carreau A, Grillon C, Kieda C.: CD133 positive progenitor endothelial cell lines from human cord blood. *Cytometry A*. 2011, 79(8), 594-602 IF – 3,753
63. Pasikowski P, Goździewicz T, Stefanowicz P, Artym J, Zimecki M, Szewczuk Z.: A novel immunosuppressory peptide originating from ubiquitin sequence. *Peptides,* 2011, 32(2), 2418-27 IF – 2,654
64. Piekarska K, Zaciera M, Czarny A, Zaczyńska E.: Application of short-term tests in assessment of atmospheric air pollution. *Environ Protect Engin*, 2011, 2(37), 85-98 IF – 0,427
65. Pietkiewicz J, Bronowicka-Szydełko A, Dzierzba K, Danielewicz R, Gamian A.: Glycation of the muscle-specific enolase by reactive carbonyls: effect of temperature and the protection role of carnosine, pyridoxamine and phosphatidylserine. *Protein J*. 2011, 30(3), 149-158 IF - 1,101

66. Podbielska M, Levery SB, Hogan EL.: The structural and functional role of myelin fast-migrating cerebrosides: pathological importance in multiple sclerosis. *Clin Lipidol*, 2011, 6, 159-179 IF - 0,556
67. Psurski M, Błażewska K, Gajda A, Gajda T, Wietrzyk J, Oleksyszyn J.: Synthesis and antiproliferative activity of novel alpha- and beta-dialkoxyphosphoryl isothiocyanates. *Bioorg Med Chem Lett,* 2011, 21(15), 4572-6 IF – 2,661
68. Pula B, Jethon A, Piotrkowska A, Gomulkiewicz A, Owczarek T, Calik J, Wojna A, Witkiewicz W, Rys J, Ugorski M, Dziegiel P, Podhorska-Okolow M.: Podoplanin expression by cancer-associated fibroblasts predicts poor outcome in invasive ductal breast carcinoma. *Histopathology*, 2011, 59(6), 1249-1260 IF – 3,569
69. Puszko A, Brzuszkiewicz A, Jezierska J, Adach A, Wietrzyk J, Filip B, Pelczynska M, Cieslak-Golonka M.: Systematic coordination chemistry and cytotoxicity of copper(II) complexes with methyl substituted 4-nitropyridine N-oxides. *J Inorg Biochem,* 2011, 105(8), 1109-14 IF – 3,317
70. Puszyńska-Tuszkanow M, Grabowski T, Daszkiewicz M, Wietrzyk J, Filip B, Maciejewska G, Cieślak-Golonka M.: Silver(I)complexes with hydantoins and allantoin. Synthesis, crystal and molecular structure, cytotoxicity and pharmacokinetics. *J Inorg Biochem,* 2011, 105(1), 17-22 IF – 3,317
71. Rossowska J, Pajtasz-Piasecka E, Ryśnik O, Wojas J, Krawczenko A, Szyda A, Duś D.: Generation of antitumor response by IL-2-transduced JAWS II dendritic cells. *Immunobiology*, 2011, 216, 1074-1084 IF - 4,114
72. Rusin A, Zawisza-Puchałka J, Kujawa K, Gogler-Pigłowska A, Wietrzyk J, Switalska M, Głowala-Kosińska M, Gruca A, Szeja W, Krawczyk Z, Grynkiewicz G.: Synthetic conjugates of genistein affecting proliferation and mitosis of cancer cells. *Bioorg Med Chem*, 2011, 19(1), 295-305 IF – 2,978
73. Ryng S, Zimecki M, Jezierska-Mazzarello A, Panek J.J, Mączyński M, Głowiak T, Dobrowolska-Sawka W, Koll A.: A complex study of 5-amino-3-methyl-4-[2-(5-amino-1,3,4-oxadiazolo)] – isoxazole monohydrate: a new low molecular weight immune response modifier. *J Mol Struct*, 2011, 999, 60-67 IF – 1,599
74. Siednienko J, Maratha A, Yang S, Mitkiewicz M, Miggin S.M, Moynagh P.N.: The NF-κB subunits RelB and cRel negatively regulate TLR3-mediated IFN-β production via induction of the transcriptional repressor protein YY1. *J Biol Chem,* 2011, 286(52) 44750-63 IF - 5,328
75. Siednienko J, Nowak J, Moynagh P.N, Gorczyca W.A.: Nitric oxide affects IL-6 expression in human peripheral blood mononuclear cells involving cGMP-dependent modulation of NF-κB activity. *Cytokine,* 2011, 54, 282-288 IF- 3,537
76. Sieńczyk M, Winiarski Ł, Kasperkiewicz, P, Psurski M, Wietrzyk J, Oleksyszyn J.: Simple phosphonic inhibitors of human neutrophil elastase, *Bioorg Med Chem Lett,* 2011, 21(5), 1310-4 IF – 2,661
77. Sochocka M, Boratyński J.: Osmoregulacja - ważny parametr rozwoju bakterii. *Post Hig Med Dośw*, (online), 2011, 65, 714-724 IF - 0,511
78. Swierzko A, Lukasiewicz J, Maciejewska A, Jachymek W, Niedziela T, Matshusita M, Cedzynski M, Lugowski C.: New ligands for H-ficolin among lipopolysaccharides of *Hafnia alvei* – structure, activity and practical applications. *Glycobiology,* 2011 Sep 2. doi: 10.1093/glycob/cwr119. IF – 3,791

79. Szajerka T, Klimczak A, Jabłecki J.: Chimerism in hand transplantation. *Ann Transplant,* 2011, 23, 16(1), 83-89 IF – 0,97
80. Świst K, Pajtasz-Piasecka E.: The influence of transcription factors on CD4+ T cell differentiation. *Post Hig Med Dośw, (online),* 2011, 65, 414-26 IF – 0,511
81. Tylińska B, Jasztold-Howorko R, Mastalarz H, Szczaurska-Nowak K, Materek P, Wietrzyk J.: Synthesis of new 1-phenyl-6H-pyrido[4,3-b]carbazole derivatives with potential cytostatic activity. *Acta Pol Pharm,* 2011, 68(1), 31-7 IF – 0,465
82. Walczak R, Śniadek P, Dziuban J.A, Kluger J, Chełmońska-Soyta A.: Supravital fluorometric apoptosis detection in a single mouse embryo using lab-on-a-chip. *Lab Chip,* 2011, 11, 3263-8 IF - 6,260
83. Wojciechowski D, Sroka Z, Gamian A.: Investigation of antiradical potential of different kinds of teas and extracts from these teas using antiradical activity units (TAU). *Post Hig Med Dośw,* (online), 2011, 65, 796-803 IF – 0,511
84. Wolański M, Donczew R, Kois-Ostrowska A, Masiewicz P, Jakimowicz D, Zakrzewska-Czerwinska J.: The level of AdpA directly affects expression of developmental genes in *Streptomyces coelicolor*. *J Bacteriol*, 2011, 193(22), 6358-6365 IF – 3,726
85. Wolański M, Wali R, Tilley E, Jakimowicz D, Zakrzewska-Czerwinska J, Herron P.: Replisome trafficking in growing vegetative hyphae of *Streptomyces coelicolor* A3(2). *J Bacteriol*, 2011, 193(5), 1273-1275 IF – 3,726
86. Zawilak-Pawlik A, Donczew R, Szafrański S, Mackiewicz P, Terradot L, Zakrzewska-Czerwińska J.: DiaA/HobA and DnaA: a pair of proteins co-evolved to cooperate during bacterial orisome assembly. *J Mol Biol*, 2011, 408(2), 238-251 IF – 4,008

Articles published in Polish journals:

1. Czarny A, Zaczyńska E, Nawrot U, Włodarczyk K, Janicka A, Walkowiak W.: Aktywacja jądrowego czynnika transkrypcyjnego i produkcja TNF-α przez ludzkie komórki płuc pod wpływem wielopierścieniowych węglowodorów aromatycznych i zarodników grzybów w warunkach *in vitro*. *Mikologia Lekarska*, 2011, 18, (1),15-19
2. Donesz A., Krzak-Roś J., Kochanowska I., Będziński R.: The structure optimization of implants materials of the surface layers by sol-gel coating. *Aktualne Problemy Biomechaniki,* 2011, 5, 25-28
3. Frydecka I, Kosmaczewska A, Ciszak L, Masternak A, Woszczyk D.: A blocade of the inhibitory CTLA-4 molecule – a novel strategy for the immunotherapy of neoplastic diseases. *Acta Haematol Pol*, 2011, 42(2), 181-191
4. Gieryng A.: Report from the EFI Education and training bursary. Study of ligands of the adaptor molecule 3BP2 in haematopoietic cells. *EFI Newsletter*, 2011, 64, 2527
5. Jarząb A, Witkowska D, Szostko B, Hirnle L, Gamian A.: Potential carrier for conjugate vaccines: OMP-38 protein from *Shigella flexneri* 3a – isolation and purification strategies. *Sepsis*, 2011, 4(1), 39-45
6. Jaworska J, Gamian A.: Immunochemiczna charakterystyka egzopolisacharydów i kwasów tejchojowych z *Lactobacilluscasei*. Prace Naukowe Studentów Wydziału Chemicznego Politechniki Wrocławskiej, 2011, 9, 91-95
7. Krzystek-Korpacka M, Diakowska D, Gamian A, Matusiewicz M.: Increase in serum platelet-derived growth factor (PDGF)-BB reflects lymph node involvement in esophageal cancer patients independently from platelet count. *Exp Oncol*, 2011, 33(3), 140-144

8. Kutner A, Paszkowska-Reymer T, Wietrzyk J.: Aktywne metabolity i analogi witamin D - aktualne wskazania i potencjalne terapie. *Standardy Medyczne/Pediatria,* 2011, 8(2), 183-194
9. Lipiński T, Ejchart A, Bernatowicz P, Pastuch-Gawołek G, Gawlik-Jędrysiak M, Dzierzba K, Szeja W, Gamian A.: Synthesis of model aminoacylmethylglycosides and NMR spectroscopic analysis of site of esterification on sugar ring. *Sepsis*, 2011, 4(1), 85-90
10. Makuch E, Matuszyk J.: Rola fosfodiesteraz w aktywacji czynnika transkrypcyjnego CREB przez neurotrofiny. *Prace Naukowe Studentów Wydziału Chemicznego Politechniki Wrocławskiej,* 2011, 9, 149-153
11. Nawrot U, Zaczyńska E, Czarny A, Lubenets V, Karpenko E, Gościniak G.: Aktywność przeciwgrzybiczna nowych, syntetycznych analogów allicyny - badania wstępne. *Mikologia Lekarska,* 2011, 18 (4), 197-200
12. Wątorek E, Paprocka M, Duś D, Kopeć W, Klinger M.: Endostatin and vascular endothelial growth factor: potential regulators of endothelial progenitor cell number in chronic kidney disease. *Pol Arch Med Wewn*, 2011, 121(9), 296-301

Published chapters of books:

1. Błach-Olszewska Z, Leszek J.: Wrodzona odporność w chorobach otępiennych. Rozdział w książce: „*Choroby Otępienne, Teoria i Praktyka*" (wydanie II, poprawione, uzupełnione i rozszerzone). Wyd. Continuo, red: J. Leszek, 2011, 229-238
2. Choromańska A, Kulbacka J, Chwiłkowska A, Skołucka N, Gamian A, Saczko J.: Can photodynamic therapy be an alternative method in melanoma treatment? In: „*Treatment of Metastatic Melanoma"*, Edited by: Rachael Morton, Publ.: In Tech, ISBN 978-953-307-574-7, Chapter 12, 2011, 271-294
3. Dzierzba K, Pietkiewicz J, Pastuch-Gawołek G, Gamian A.: Zastosowanie modelowych glicylowanych i fosforylowanych struktur węglowodanowych w badaniach biologicznych dotyczących poszukiwania wspólnych epitopów występujących w lipopolisacharydach bakterii Gram-ujemnych. W: Interdyscyplinarność badań naukowych praca zbiorowa pod red. J. Szreka; 2011, Wrocław: Oficyna Wydaw. Politechniki Wrocławskiej, ISBN 978-83-7493-616-3, s. 44-49
4. Gawlik-Jędrysiak M., Gamian A.: Sepsa – patogeneza choroby i metody leczenia. Prace Naukowe – Monografie. Instytut Techniki Górniczej KOMAG, Gliwice. 2011.
5. Szponar B.: Endotoxins in environmental and clinical samples assessed by GC-Tandem MS. W: „*Detection of Biological Agents for the Prevention of Bioterrorism*". Ed. J. Banoub. NATO Science for Peace and Security Series A: *Chemistry and Biology*. Springer, 2011, 245-265.
6. Szymborska-Małek K, Czarny A, Nawrot U, Zaczyńska E, Karpenko E, Lubenets V.. Właściwości grzybobójcze syntetycznych analogów allicyny. W: „*Interdyscyplinarność badań naukowych 2011*". Praca zbiorowa pod redakcją J. Szreka. Oficyna Wydawnicza Politechniki Wrocławskiej, 2011, 96-101
7. Zabłocka A, Janusz M.: Hipoteza stresu oksydacyjnego w chorobie Alzheimera. Rozdział w książce: „*Choroby Otępienne, Teoria i Praktyka*" (wydanie II, poprawione, uzupełnione i rozszerzone).Wyd. Continuo, red. J. Leszek, 2011, 255-268
8. Karabon L, Frydecka I.: The role of polymorphisms in co-signalling molecules in susceptibility to B-cell chronic lymphocytic leukemia In: "*Chronic Lymphocytic Leukemia*" (ISBN 978-953-307-699-7). Intech-Open Access Publisher, Croatia, w druku

Articles without IITD affiliation, published by Institute employees working abroad:

1. Pozsgay V, Kubler-Kielb J, Coxon B, Marques A, Robbins JB, Schneerson R.: Synthesis and antigenicity of BBGL-2 glycolipids of *Borrelia burgdorferi*, the causative agent of Lyme disease. *Carbohydr Res*, 2011, 346, 1551-1563 IF - 1,898
2. Kubler-Kielb J, Vinogradov E, Lagergard T, Ginzberg A, King JD, Preston A, Maskell DJ, Pozsgay V, JD Keith, Robbins JB, Schneerson R.: Oligosaccharideconjugates of *Bordetella pertussis* and *bronchiseptica* induce bactericidal antibodies, an addition to the pertussis vaccine. *PNAS,* 2011, 108, 4087-4092 IF - 9,771
3. Kubler-Kielb J, LaiW-T, Schneerson R, VinogradovE.: The structure of the *E. coli* O148 lipopolysaccharide core region and its linkage to the O-specific polysaccharide. *Carbohydr Res*, 2011, 346, 150-152 IF - 1,898
4. Chen Z, Schneerson R, Lovchik J, Lyons CR, Zhao H, Dai Z, Kubler-Kielb J, Leppla SH.: Purcell R. Pre- and postexposure protection against virulent anthrax infection in mice by humanized monoclonal antibodies to *Bacillus anthracis* capsule. *PNAS,* 2011, 108, 739-744 IF - 9,771
5. Kubler-Kielb J.: Conjugation of LPS-derived Oligosaccharides to Proteins Using Oxime Chemistry. *Methods Mol Biol*, 2011, 751, 317-327 IF - 1,887
6. Brown JR, North EJ, Hurdle JG, Morisseau C, Scarborough JS, Sun D, Korduláková J, Scherman MS, Jones V, Grzegorzewicz A.: Crew RM, Jackson M, McNeil MR, Lee RE. The structure-activity relationship of urea derivatives as anti-tuberculosis agents. *Bioorg Med Chem, 2011,* 19(18), 5585-5595 IF - 2,978
7. Lipinski T, Kitov P, Szpacenko A, Paszkiewicz E, Bundle DR.: Synthesis and immunogenicity of a glycopolymer conjugate. *Bioconjug Chem*, 2011, 22, 274-281 IF - 5,002
8. Lipinski T, Luu T, Kitov P I, Szpacenko A, Bundle D.R.: A structurally diversified linker enhances the immune response to a small carbohydrate hapten. *Glycocon J.* 2011, 28, 149-164 IF - 2,700
9. Bundle D.R, Costello C, Nycholat C, Lipinski T, Rennie R.: Designing a *Candida albicans* conjugate vaccine by reverse engineering protective monoclonal antibodies, In: "Anticarbohydrate Antibodies" Edited by Paul Kosma and Sven Müller-Loennies. Springer-Verlag, 2011.
10. Wu X, Cui L, Lipinski T, Bundle DR.: Bundle. Synthesis of monomeric and dimeric repeating units of the Zwitterionic Type 1 Capsular Polysaccharide from *Streptoccuspneumoniae*. JACS, *Chemistry – Eur J,* 2010, 16(11), 3476-3488 IF - 5,476
11. Siemionow M, Duggan W, Brzezicki G, Klimczak A, Grykien C, Gatherwright J, Nair D: Peripheral nerve defect repair with epineural tubes supported with bone marrow stromal cells: a preliminary report. *Ann Plast Surgery*, 2011, 67(1), 73-84 IF – 1,274
12. Bienkowska-Haba M, Sapp M.: The cytoskeleton in papillomavirus infection. *Viruses*, 2011, 3, 260-271 IF - 1,000
13. Dasgupta J, Bienkowska-Haba M, Ortega M.E, Patel H.D, Bodevin S, Spillmann D, Bishop B, Sapp M, Chen X.S.: Structural basis of oligosaccharide receptor recognition by human papillomavirus. *J Biol Chem*, 2011, 286, 2617-2624 IF - 5,328

14. Estep R.D, Messaoudi I, O'Connor M.A, Li H, Sprague J, Barron A, Engelmann F, Yen B, Powers M.F, Jones J.M, Robinson B.A, Orzechowska B.U, Manoharan M, Legasse A, Planer S, Wilk J, Axthelm M.K, Wong S.W.: Deletion of the monkeypox virus inhibitor of complement enzymes locus impacts the adaptive immune response to monkeypox virus in a nonhuman primate model of infection. *J Virol*, 2011, 85, 9527-9542 IF - 5,189

15. Grzegorzewicz A.E, Pham H, Gundi V, Scherman M.S, North J, Hess T, Jones V, Gruppo V, Born S.E.M, Kordulakova J, Chavadi S.S, Morisseau C, Lenaerts A.J, Lee R.E, McNeil M.R, Jackson M.: Adamantyl urea inhibitor preventing the translocation of mycolic acid across the plasma membrane. *Nature Chem Biology*, manuscript NCHEMB-A110606681B **IF -** 15,808