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

Research Report 2009

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

Laboratory of Medical Microbiology
Head: Professor Andrzej Gamian, Ph.D.
Studies on the pathogenesis of some diseases of bacterial etiology and the role of bacterial surface glycoconjugates and protein antigens in immune response

The main topics of study in our laboratory are the mechanisms of pathogenicity of diseases with bacterial etiology, the role of molecular mimicry, bacterial proteins and glycolipids in pathogenicity, and the structures and functions of bacterial capsular antigens and endotoxins. Studies of molecular markers of infectious diseases and biochemical factors specific for inflammatory processes allowed developing a method for the quantitative determination of endotoxin, muramine, and sialic acid in the same sample. These parameters are important for diagnostic and prognostic purposes in clinical practice. The determination with high sensitivity of 3-deoxyoctulosonic acid specific to Gram-negative bacteria and muramic acid specific to Gram-positive strains in the presence of sialic acid as internal marker allows monitoring the clinical pattern of sepsis and septic shock. A description of the protective tools against invading bacteria, also for understanding the biological activities of microorganisms involved in probiotic processes, comprises determining the structures of the molecules involved in infection, protection, and immune processes. The structure of neutral exopolysaccharide produced by strain of *Lactobacillus johnsonii* has been determined. The strain was isolated from mice with experimentally induced inflammatory bowel disease. It was also found that this exopolysaccharide is biologically active in terms of inducing human dendritic cells to produce immunomodulatory mediators. In the framework of our studies on advanced glycation processes, the experiments involved the preparation of monoclonal antibodies against advanced glycation end-products (AGEs). A substrate for the AGE antigen for the immunization of mice was prepared using a microwave reactor. These antibodies will allow studying glycation in the organism, a physiological process which, however, is pathological when there is a high level of carbohydrates and products of carbonyl and oxidation stress.

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

The aim of our study was to examine the frequency of prevalence of JC and BK polyomaviruses in various body fluids and the pathogenicity connected with these viruses in patients infected with HIV-1. We tested 108 people of both sexes in whom HIV-1 infection was diagnosed and 23 healthy individuals with no HIV-1 infection history. JCV and BKV infection was diagnosed in 27 (25%) of the HIV-1-infected patients, where JCV constituted 18 (16.7%) cases and BKV 9 (8.3%). The infections were proven on the basis of the presence of viral genetic material in urine. There was no JCV or BKV detected in the blood and cerebrospinal fluid of any patient. In the patient group with a CD4+ T-lymphocyte counts <200 cell/µl, JCV DNA was detected in urine in 5 (11.4%), and BKV in 7 (15.9%) cases; in turn, in the group of people with neurological conditions, JCV was found in 3 (15%) and BKV also in 3 (15%) cases. Among the tested people with CD4+ T-cell counts >500 cell/µl, JCV DNA was detected in urine in 9 (20.5%) cases and BKV DNA in 1 (2.3%) case. These results indicate that JCV and BKV infection may be asymptomatic in HIV-1-infected people and involves patients with various HIV-1 infection stages. The results were published in *HIV & AIDS Review 2008; 7(2): 15-18 (published in 2009)*.

The aim of another study was to evaluate the association between the occurrence of calcifying nanoparticies (CNPs) and extraskeletal calcification in patients diagnosed with atherosclerosis and nephrolithiasis. A total of 134 clinical specimens, including serum samples (n=90), carotid artery plaques (n=20), and kidney stones (n=19), were cultured for CNPs. All the specimens were collected during a three-year period (2005-2007) from patients hospitalized in the Regional Specialist Hospital in Wrocław, Poland. The serum samples and carotid artery plaques were from patients with atherosclerosis and the kidney stones from patients with nephrolithiasis. Serum samples (n=25) from healthy volunteer donors were used as a control. All the samples were cultured in Dulbecco's Eagle's medium (DMEM) supplemented with 10% γ-irradiated fetal serum under cell culture conditions. The presence of CNPs was visualized by scanning electron microscopy (SEM). The cytotoxic effect of CNPs on human and mouse cells was evaluated by the MTT colorimetric assay. SEM analysis of the biofilm adhering to the culture flask revealed coccoid-shaped and/or rod structures in 73 (54.5%) of the 134 specimens subjected to this study. The most positive results (65.3%) were obtained in the culture derived from serum samples. Positive results were obtained in 25% of the carotid artery plaque and 31.6% of the kidney stone culture. None of the 25 control serum

were positive for CNPs. Attempts to propagate CNPs in fresh culture medium were unsuccessful. No cytotoxic effect of CNPs on human or mouse cell lines was shown. The findings demonstrate that CNPs cannot be considered living entities although they may be involved in pathological processes leading to extra skeletal calcification in humans. The results were published in *Adv Clin Exp Med 2009; 18(3): 269-275.*

Another paper presents results of an analysis of polycyclic aromatic hydrocarbon (PAH) concentration and toxicity emitted from 1,9 TDI self-ignition because of unstable parameters of engine self-ignition (pressure and temperature jumps). PHAs were extracted from the gas phase and solid phase (particle matter-PM). Because of their low level of concentration in exhaust gases, a chromatographic method (capillary gas chromatography) of polycyclic aromatic hydrocarbon identification and analysis needed to be supported by sample purification and enrichment stages. Calibration of the chromatograph was made by an attested mixture of 16 model samples (according to EPA, USA). Two different methods for determining toxicity were used in this study. The authors used relative carcinogenic coefficients (RCCs) which were determined by Nisbet and LaGoy for individual polycyclic hydrocarbons in relation to benzo(a)pirene. Samples consisting PAHs were also tested for cytotoxicity in a standardized cell-culture system (human cell line A549, mouse fibroblasts line cell L929). Cell growth, morphology, and viability were used as parameters to determine the cytotoxicity of the materials. The measure the lethal effect on cells was determined spectrophotometrically with the use of a mitochondrial enzyme activity assay for mitochondrial succinct dehydrogenase activity by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). The cells were exposed to the materials for 24,72, and 120 h. The results of *in vitro* tests are discussed. A lack of correlation between the toxicity measurement methods which were used in these studies was observed. The results were published in *Journal of KONES Powertrain and Transport 2009; 16(4): 195-199.*

A salmonella assay in the presence of TA98 and YG1041 strains, and also when using human lung adenocarcinoma cells A549 line, certified the mutagenic and cytotoxic properties of organic pollutants and fractions thereof adsorbed on suspended PM10 collected in winter and summer in the Wrocław urban area. The particulates were sampled using a high-performance Staplex air aspirator. Their extraction by dichloromethane was performed in a Soxhlet apparatus. The particulates were separated into three fractions: PAH, nitro-PAH, and dinitro-PAH, by a column chromatography method. The samples of particulates collected in winter showed higher mutagenic and cytotoxic effect than those collected in summer. Pollutants capable of directly and indirectly affecting genetic material, classified as mutagens

of the reading frame-shift type, were found in the samples tested. The mutation ratios (MRs) obtained in the majority of the experiments conducted in the presence of a fraction of the pollutants tested were lower compared with the MRs obtained for the whole (unfractionated) extracts. No mutagenic effect was found in the case of fractions derived from the samples of particulates collected in the summer when the experiment was conducted with metabolic activation. The greatest amount of compounds responsible for a cytotoxic effect was found in the nitro-PAH winter fraction and also in the nitro-PAH and dinitro-PAH summer fractions. The results were published in *Environment Protection Engineering 2009; 35(1): 37- 48.*

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

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

The role of molecules involved in mutual recognition of cells of the immune system and factors influencing the expressions of these molecules

a) Associations of KIR genotypes with spontaneous abortion

Natural killer (NK) cells are the most abundant lymphocyte population in the decidua. These cells express killer immunoglobulin-like receptors (KIRs), which upon recognition of HLA class I molecules on trophoblasts may either stimulate NK cells (activating KIRs) or inhibit them (inhibitory KIRs) to produce soluble factors necessary for the maintenance of pregnancy. KIR genes exhibit extensive haplotype polymorphism; individuals differ in both the number and kind (activating vs. inhibitory) of KIR genes. This polymorphism affects NK cell reactivity and susceptibility to diseases, including gynecological disorders. Therefore we KIR-genotyped 149 spontaneously aborting women and 117 control multiparae (at least 2 healthy-born children). Several genotypes (i.e. combinations of various KIR genes) were differently distributed among the patients and control subjects. Differences were observed in the numbers and the ratios of activating to inhibitory KIRs between patients and healthy women: (i) genotypes containing six activating KIR genes were less frequent and those containing six inhibitory KIR genes were more frequent in patients than in control subjects, and (ii) an excess of inhibitory KIRs (activating-to-inhibitory KIR gene ratios of 0.33 to 0.83) was associated with miscarriage, whereas ratios close to equilibrium (0.86-1.25) seemed to be protective. In addition, the results suggest for the first time that sporadic and recurrent spontaneous abortions as well as miscarriage in the presence or absence of autoantibodies may have different KIR genotypic backgrounds.

This work was done in collaboration with several clinical institutions in Poland and the City of Hope National Medical Center, Duarte, CA, U.S.A.

b) ***Incompatibilities in activating KIR genes affect the outcome of allogeneic hematopoietic stem cell transplantation***

Killer immunoglobulin-like receptors (KIRs) regulate the function of natural killer (NK) cells and a subset of T cells. In this study we prospectively evaluated the impact of donor and recipient activating KIR genes on the outcome of allogeneic hematopoietic stem cell transplantation (alloHSCT) in patients with hematological malignancies. One hundred consecutive recipients of myeloablative transplantation and their donors were tested for KIR genotype as well as for immune reconstitution, including activating KIR expression on NK cells and T cells. In a multivariate analysis, mismatches of particular activating KIRs such that the patient was negative and the donor was positive (P-D+) resulted in increased risk of acute (KIR2DS1) and chronic (KIR2DS3) graft-versus-host disease (GvHD) as well as relapse (KIR2DS5). KIR2DS1 incompatibility in the same direction in the presence of HLA-C-group 2 ligand in the recipient was associated with reduced overall (risk ratio, *RR*=3.01, *p*=0.01) and disease-free survival (*RR*=2.92, *p*=0.03). Activating mismatches in the P-D+ direction resulted in a decreased CD4+/CD8+ T-cell ratio up to 1 yr after alloHSCT as a consequence of decreased CD3+CD4+ number within the first 100 d and increased CD3+CD8+ number in later time points. Among six evaluated patients, the expression of activating KIRs on NK cells and T cells was particularly prominent for those developing intestinal GvHD. Our findings indicate that the presence of particular activating KIRs in the donor and their absence in the recipient enhances GvHD, which is not accompanied by a graft-versus-leukemia effect. Evaluation of activating KIR genotype may allow optimization of both donor selection and the transplantation procedure in order to avoid GvHD.

This work was done in collaboration with the Silesian Medical University, Katowice, and the Institute of Oncology, Gliwice.

c) ***p53 Tetramerization domain mutations in pediatric neoplasms***

Germline p53 mutations are associated with Li-Fraumeni syndrome (LFS) and other familial cancer phenotypes not fulfilling the definition for LFS. The majority of germline p53 mutations cluster in exons 5-8, corresponding to a DNA binding domain. We report the identification of two germline mutations and a somatic mutation in a tetramerization domain (TD), a rare site for mutations. The germline mutation R342X (16915C>T) and the novel mutation R342P (16916G>C) were found in a child with adrenocortical carcinoma and in a

LFS pediatric patient with multiple primaries. The novel somatic mutation R337G (16900C>G) was discovered in myelodysplastic syndrome with transformation to acute myeloblastic leukemia, developing as the third primary in the LFS child. These findings add further information on p53 TD mutations and TD contribution to tumorigenesis.

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

Laboratory of the Molecular Biology of Microorganisms
Head: Professor Jolanta Zakrzewska-Czerwińska, Ph.D.
The molecular basis of replication and segregation of bacterial chromosomes
Home page: www.iitd.pan.wroc.pl/dept/mic/index.htm

Functional analysis of Helicobacter pylori HobA-DnaA interactions

Replication of the bacterial chromosome is initiated by the binding of the DnaA protein to a unique DNA region called *oriC*. Many regulatory factors in numerous species act by controlling the ability of DnaA to bind and unwind DNA. The *Helicobacter pylori* genome contains only one homologue of the bacterial regulatory factors so far described, namely HobA. HobA, a structural homologue of *Escherichia coli* DiaA, is the only known protein involved in and absolutely necessary for orisome formation in *H. pylori*. It interacts specifically via DnaA with the *oriC*-DnaA complex and is essential for the correct formation and stabilization of the orisome by facilitating the spatial positioning of DnaA at *oriC*.

Our recent studies, conducted in close collaboration with Dr. Laurent Terradot of the European Synchrotron Radiation Facility, revealed the detailed structural and functional bases of DnaA-HobA interactions. The amino acids important for their interaction were determined and HobA with mutated crucial amino acids were further analyzed *in vivo* in *H. pylori*. The introduction of *hobA* encoding the mutated amino acids L80, Y175, and the E(76)R(77)P(78) triad to *H. pylori* was lethal to the cells, proving that these amino acids are required for DnaA-HobA interactions *in vivo*. What is interesting, the interaction surfaces and the particular amino acids involved in HobA-HpDnaA complex formation were also conserved in *E. coli* DnaA and DiaA, suggesting a similar inter-protein binding mode and possible regulatory mechanisms of orisome formation and function.

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

In studies conducted in 2009 we continued issues on the role of cAMP- and cGMP-dependent pathways in the course of inflammation. Their impact on the activity of NF-κB and/or AP-1 was analyzed. Experiments were performed on the human monocytic cell line THP-1 and rat peripheral blood mononuclear cells (PBMCs). As shown previously, the protein kinase PKG-I was the major enzyme responsible for the activation of NF-κB by nitric oxide in freshly isolated rat PBMCs. Therefore we examined whether this protein also participates in the inhibitory effect of cGMP on the activation of AP-1 and NF-κB induced by LPS in these cells. Our studies excluded the role of PKG-I but, based on earlier results, it was possible that phosphodiesterases belonging to the PDE3 family might be involved. Hydrolytic activities of PDE3 against cAMP are competitively inhibited in the presence of cGMP. We found that in rat PBMCs stimulated to synthesize cAMP, the atrial natriuretic peptide (ANP) that activates particulate guanylyl cyclase GC-A, further increases the cAMP level. This effect was not observed in the presence of a nonselective PDE inhibitor (IBMX) as well as specific inhibitors of PDE3 (cilostamid, milrinon) because then ANP did not affect the accumulation of cAMP. Based on the above observations, one can assume that cGMP produced in response to ANP inhibits PDE3 hydrolytic activity against cAMP and thus contributes to the increase in the level of cAMP in cells, leading to a reduction in the activity of NF-κB and AP-1. A similar effect was observed in the case of THP-1 cells. The results obtained support the potential role of the PDE3 family in the anti-inflammatory action of ANP.

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

Laboratory of Transgenesis and Lymphocyte Biology
Head: Professor Paweł Kisielow, Ph.D.
RAG/NWC locus and LAT gene studied in genetically modified mice

NWC is a third evolutionarily conserved gene within the *RAG* locus. Unlike *RAG* genes, *NWC* is ubiquitously expressed and its function, in contrast to *RAG* genes, is unknown. To study the possible role of *NWC* transcription in regulation of the expression of *RAG* genes, we generated two lines of transgenic mice. Transgenes in the form of an artificial bacterial

chromosome contained the entire *RAG/NWC* locus in which *RAG2* was fused with *GFP* and *NWC* was fused with *YFP* reporter genes encoding green and yellow fluorescent proteins, respectively, thus enabling cytofluorometric monitoring of their expression in different tissues. The first line (BAC-NY) contained a transgene which did not have any additional modifications and served as a reference line. The second line (BAC-M1) carried modified the BAC-NY transgene from which the *NWC* promoter region (active in nonlymphocytes but inactive in lymphocytes) was deleted. Analysis of these mice allowed making the following observations: (i) the expression of genes in the transgenic *RAG/NWC* locus in the BAC-NY line parallels the expression of genes in the endogenous locus, (ii) testis and myeloid cells in the bone marrow express the highest levels of NWC protein, (iii) transcription of transgenic *NWC* in BAC-M1 mice is undetectable in all examined tissues, except the testis, where it is reduced, and (iv) in nonlymphoid tissues of BAC-M1 mice, downregulation of the transcription of transgenic *NWC* does not result in the expression of *RAG* genes in the transgenic *RAG/NWC* locus.

From these observations we conclude that: (i) besides the previously identified *NWC* promoter, which was deleted in the BAC-M1 transgene, there must be an additional promoter responsible for *NWC* transcription remaining in the testis and (ii) the downregulation of *NWC* transcription in nonlymphoid cells is not sufficient to release *RAG* genes from suppression.

LAT is a gene that encodes an adaptor molecule playing a critical role in the T cell receptor (TCR)-mediated signaling pathway. Until recently it has been generally accepted that *LAT* deficiency results in complete blockade of T lymphocyte development. Studying a novel model of *LAT*-deficient mice we identified a previously undescribed population of γδ T lymphocytes at the early stage of development which accumulates in peripheral lymphoid tissue under conditions of lymphopenia.

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

Research on the biology of bacteriophages and their use in the treatment of bacterial infections

A bacterial profile of microbiological samples taken from patients with chronic infections treated at the Phage Therapy Unit in Wrocław was studied. The results of the identification and quantitative cultures of the bacterial strains isolated from 11 patients with wound infection before, during, and after completion of the phage therapy were analyzed. Phage formulations were applied locally as wet compresses (two times daily), for irrigation of a fistula (1-3 times daily), and in two cases both locally and orally (one 10-ml ampoule three

times daily after neutralization of gastric juice with dihydroxyaluminum sodium carbonate). Samples for the determination of bacteria were taken using a cotton swab or a small cotton compress and diluted in saline for quantitative culture. In nine patients the infection was caused by *S. aureus*, in one case by *P. mirabilis*, and in another by *P. aeruginosa*. In six of them a decrease in the amount of colonizing pathogens accompanied by clinical improvement was observed. We showed a reduction in the number (by 1-4 log) of bacterial colonies cultured form 1 ml of diluted samples between days 7 and 106 of the phage therapy.

The influence of phage lysates on carrageenan-induced paw edema in rat was investigated. The activity of *S. aureus* phage A5/80 and *E. coli* phage T4 was tested after intraperitoneal and local (paw soaking in the lysate) administration. A reduction of edema was observed both in the cases of intraperitoneal A5/80 and T4 phage lysates as well as sonicates of *S. aureus* and *E. coli*, which were used for the propagation of the phages. No influence of intraperitoneal injection of broth was observed. Local application of T4 lysate and *E. coli* sonicate caused comparable decreases in paw edema. However, phage A5/80 applied locally showed stronger anti-inflammatory activity than the *S. aureus* sonicate. These results suggest that the observed anti-inflammatory activity of phage preparations observed in patients with bacterial infections may result not only from their antibacterial properties.

Results of grant activities

Work on restoration of the Institute's phage bank and on isolating new phages were conducted with the use of over 1400 bacterial strains and 128 environmental samples. Forty-three phages were recovered, including 22 *K. pneumoniae* phages, 15 *P. aeruginosa* phages, and 6 *E. coli* phages. In total, 138 phages enriched the phage collection. Their most effective source proved to be incubated and condensed samples of crude sewages as well as hospital sewage. Phage occurrence varied depending on the examined sample and time of its storage. It was demonstrated that full phage lytic activity may be retained in environmental samples after five-year storage. Studies on the biological activity of newly isolated phages showed the unfavorable influence of temperature and chloroform on the lytic activity of phages of *E. coli*, *E. faecalis*, and *S. maltophilia*. Morphological and ultrastructural analysis of new *Stenotrophomonas* phages enabled classifying them into the *Siphoviridae* family, morphotype B1, and the *Myoviridae* family, morphotype A1.

A retrospective analysis was conducted of 22 men with chronic bacterial prostatitis (CBP) for whom the results of bacterial culture of expressed prostatic fluid (EPS) before and during or after phage therapy (PT) were available. The treatment was conducted at the Phage

Therapy Unit under the experimental protocol ”Experimental phage therapy of drug-resistant bacterial infections, including MRSA infections.” In all cases, previous antibiotic therapy was ineffective and chronic infection with the target pathogen was confirmed (*E. faecalis*: n=16, *E. coli*: n=5, *K. pneumoniae*: n=2, *P. aeruginosa*: n=1, and *S. haemoliticus*: n=1; 4 patients had a mixed infection). Specific phage lysates active against the isolated bacteria were applied rectally (n=20), orally (n=5), and/or topically on the glans penis (n=2). PT duration was 22-99 days (average: 47 days). Eradication of the target bacteria, as confirmed by two consecutive EPS cultures (an interval of at least two weeks) during or after PT, was observed in 50% of the cases. In six patients, bacterial eradication was confirmed in one EPS culture. In some patients we observed a substantial reduction of prostatitis symptoms, decreases in EPS leukocyte count, prostate volume, and the NIH chronic prostatitis symptom index, and improvement in maximum urinary flow rate. These results suggests that PT may be of interest as an alternative in the treatment of CBP patients, especially those for whom antibiotic therapy was inefficient.

Expression vectors containing genes encoding bacteriophage T4 capsid protein were constructed. The genes were *hoc*, *soc*, *24*, *24* (the cleaved form), *23*, *23* (the cleaved form), *11*, *18*, *35*, *36*, *37*, and *wac*. Final plasmid constructions allow an effective expression of recombinant proteins with amino-acid/protein motifs able to bind specific slurries and to perform chromatographic purification of the proteins. The individual conditions of expression, including the desired chaperons, the method of lysis, fraction separation, and chromatography were determined (native, non-denaturizing conditions). Proteolysis, which allows removing binding the motifs and obtaining the purified phage protein, was effectively developed as an “in-slurry” reaction. Then the HPLC conditions were carried out in two steps: carboxymethylocelulose and crosslink of agarose and dextran were used. Additionally, bacterial endotoxins were removed from the protein preparations using EndoTrap. The final purity and adequacy of the preparations were examined electrophoretically with the Lowry Protein Assay and Limulus Amebocyte Lysate Assay. The final bacteriophage protein preparations were highly purified; they contained more than 200 ng/ml gp24, gp24 cleaved, gp23, gp23 cleaved, gpHoc, and gp36 and less than 200 ng/ml gpSoc, gp18, gp35, and gp37. The range of final endotoxin content was 5-200 U/ml. The procedure of bacteriophage protein expression and purification was reproducible and stable. These preparations were tested for their immunological activities as well as in experimental cancer assays. The procedures will be used to prepare proteins for further investigations.

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

Laboratory of Tumor Molecular Immunobiology
Head: Professor Leon Strządała, Ph.D.
In vitro photodynamic therapy with chlorin e6 leads to apoptosis of human vascular smooth muscle cells

Percutaneous coronary intervention has become the most common and widely implemented method of heart revascularization. However, the development of restenosis remains the major limitation of this method. Photodynamic therapy (PDT) recently emerged as a new and promising method for the prevention of arterial restenosis. The efficacy of chlorin e6 in PDT was investigated *in vitro* using human vascular smooth muscle cells (TG/HA-VSMCs) as one of the cell types crucial in the development of restenosis. Photosensitization of TG/HA-VSMCs with a chlorin e6 and subsequent illumination with the light of a diode laser resulted in the generation of ROS, a decrease in cell membrane polarization, caspase-3 activation, as well as DNA fragmentation. Interestingly, the latter two apoptotic events could not be observed in photosensitized and illuminated NIH3T3 fibroblasts, suggesting different outcomes of the model of PDT in various types of cells. Our results obtained with human VSMCs show that chlorin e6 may be useful in the PDT of aerial restenosis, but its efficacy still needs to be established in an animal model.

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

Mechanism of antitumor activity of new genistein analogs

In our previous studies we showed that genistein analogs (IFG-27 and IFG-43) and complexes with polysaccharides (xyloglucan and schisophilan) revealing anticancer activity have differential influence on tumor cell death. We also showed that the analogs IFG-27 and IFG-43 reduced the expression of β3 integrin on cancer cell lines, indicating a possible anti-invasive and antimetastatic activity of these analogs. Taking into consideration the obtained results, the adhesive properties of A498 renal cancer cells to fibrinogen and fibronectin were examined. Both analogs of genistein inhibited the adhesion of A498 cells to fibrinogen and, to a lower degree, to fibronectin. However, complexes of genistein with polysaccharides did not reduce the expression of β3 integrin or diminish the adhesion of cancer cells.

Studies on an efficient carrier for siRNA delivery

In 2009, studies on an efficient carrier for siRNA delivery were continued. Polyethylenimines, both linear, and branched, were of special interest. Contrary to literature data, the efficiency of branched PEIs in siRNA delivery to B16 mouse melanoma and A498 human renal cancer cells was not confirmed in our experiments. For linear PEIs the relation between the length of the polymer and its efficiency in transfection as well as the most effective N:P ratio were determined. The kinetics of siRNA:PEI 22-kDa complex formation was defined. Finally, as a result of the chemical modification of the PEI chains, a variety of PEIs were obtained and in some cases an increase in the efficiency of transfection was observed compared with the commercially available ones.

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

Studies on methotrexate-fibrinogen conjugates

The Laboratory of Biomedical Chemistry is focused on the development of drug-carrier conjugates for the treatment of experimental cancer and immunological diseases. We investigate the biochemical properties and biological activities of protein (fibrinogen, albumin, antibodies) and carbohydrate (glucose or mannose polymers) methotrexate and raltitrexed conjugates.

Physiochemical studies of bacteriophages

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

DEPARTMENT OF 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

Hafnia alvei, a Gram-negative bacterium, is an opportunistic pathogen associated with mixed hospital infections, bacteremia, septicemia, and respiratory diseases. Kdo-containing fragments other than the known structures of core oligosaccharides were previously found among fractions obtained by mild acid hydrolysis of lipopolysaccharides (LPS, endotoxin)

isolated from some strains of *H.* alvei, but the position of such fragments in the LPS structure was not known to date. Analyses of de-*N*,*O*-acylated LPSs with the use of NMR spectroscopy and mass spectrometry allowed determining for the first time the location of Kdo-containing trisaccharide in structures of *H. alvei* 32 and 1192 LPSs. Trisaccharide [L-α-D-Hep*p*-(1→4)-[α-D-Gal*p*6OAc-(1→7)]-α-Kdo-(2→] has been found to be an integral but acid-labile part of the outer core oligosaccharides of these LPSs. The screening for Kdo-containing trisaccharides was performed on the group of 37 O-serotypes of *H. alvei* LPSs using monospecific antibodies recognizing this structure. The trisaccharide is a characteristic feature of the outer core oligosaccharide of *H. alvei* 2, 32, 600, 1192, 1206, and 1211 LPSs, but six weaker cross-reactions suggest the presence of similar structures also in the LPSs of strains 974, 1188, 1198, 1204, and 1214. Thus we defined a new example of enterobaterial endotoxins among those elucidated so far. This type of core oligosaccharide deviates from the classical scheme by the presence of such a structural motif with Kdo in the outer core. This finding demonstrates how important it is to use complementary instrumental techniques and chemical analytic procedures in LPS structure determination to avoid the loss of important structural information. Interesting cases of acid-labile interlinking LPS segments among *K. pneumonie, R. etli,* and *H. alvei* could prompt researchers to look for similar motifs during their structural analyses of LPSs.

Lipid A isolated from *H. alvei* endotoxins was analyzed for the first time with the use of ESI MS^n and MALDI-TOF mass spectrometry. Both lipids A contained a glucosamine backbone phosphorylated at the 1 and 4' positions. The disaccharide backbone was acylated by 14:0(3-OH) at positions 2 and 3. Positions 2' and 3' are substituted by 14:0(3-O-12:0) and 14:0(3-O-14:0), respectively.

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

Proline-rich polypeptide complex (PRP) isolated from ovine colostrum exerts immunoregulatory and procognitive activities. The immune system plays an important role in the pathogenesis of neurodegenerative processes. PRP in the form of orally administered tablets called Colostrinin™, containing 100 μg of polypeptide complex, improves the outcome of patients with Alzheimer's disease. It was previously shown that the beneficial effect of PRP may involve modification of cytokine release, functional and/or phenotypic differentiation of cells, effect on neurite outgrowth, and reduction of fibril formation and Aβ

aggregation. PRP as well as one of its components, nonapeptide VESYLPLFP (NP), inhibits the release if nitric oxide and affects iNOS activity. In the presence of PRP, the production of H_2O_2 induced by PMA was lowered as was SOD activity. PRP can enhance glutathione peroxidase activity, an enzyme playing a role in the first line of antioxidant defense.

Glutathione plays a key role in maintaining the physiological balance between prooxidants and antioxidants. Reduced glutathione (GSH) and oxidized glutathione (GSSG) are the most important. Each of these forms of glutathione can be beneficial or harmful to the organism depending on the cell type and its metabolic status. Under physiological conditions, the GSSG concentration in neurons and astrocytes is very low, but in conditions of oxidative stress it is significantly increased by even up to 40% of the whole glutathione pool. It was shown that the activation of glutathione reductase is involved in the regulation of oxidative stress by PRP and NP. In the presence of PRP and NP, the activity of glutathione reductase was significantly increased in a range comparable to a positive LPS control (PRP_{10}: 1459±1121 U/L, PRP_{100}: 1634±634 U/L, NP_{100}: 1355±1016 U/L, LPS: 1624±868 U/L). No influence on oxidized glutathione was observed. However, in the presence of both PRP and NP, a statistically significant increase in the reduced glutathione (GSH) level was noted (PRP_{10}: 997±243 μM, PRP_{100}: 995±234 μM, NP_{100}: 983±226 μM, LPS: 850±266 μM).

GSH deficiency or disturbance of its metabolism could be involved in central nervous system pathologies. The results suggest that the effect of PRP and NP on glutathione reductase activity and GSH level might at least in part contribute to the beneficial therapeutic effects in the case of AD.

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

The central roles that hFcRn plays in the protection and transportation of IgG under normal or inflammatory situations have led to an increased interest in the mechanism that controls the expression of the hFcRn gene. A more complete understanding of the transcriptional regulation of this physiologically important gene will create the possibility of modulating the biological functions of hFcRn. It may find future application, for example, in the therapy of IgG-mediated autoimmune diseases. Knowledge of the structure of the gene encoding hFcRn and identification of the hFcRn promoter provides a starting point for examining the transcriptional regulation of the human FcRn gene. In an earlier report it was demonstrated that the cell lines THP1, Caco-2, Lu 106, and HUVEC would be good candidates for studies on the regulation of hFcRn transcription.

The interaction of the nuclear proteins from the selected cell lines with the promoter region of the hFcRn gene was examined. The electrophoretic mobility shift assay (EMSA) was used to assess the binding of nuclear proteins to the hFcRn promoter. The double-stranded DNA probes for EMSA were prepared by annealing the complementary single-stranded oligonucleotides that are the sequences of the hFcRn promoter region containing the putative transcription factor binding motifs. DNA probes were labeled at the 5'-terminal with [γ-^{32}P] ATP by T4 polynucleotide kinase. EMSA analyses revealed the specific binding of nuclear protein extracts to the potential protein binding motifs: an AP1 site at –276, Pu1 site at –191, NFY/NF1 site at -357, CF1/YY1 sites at –584 and –353, AP2 site at +56, Ets1/E1AF site at +127, and a series of Sp1 binding sites at -643, -635, -316, +82, and +250. The specificity of these 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 a 100-fold molar excess of unlabeled DNA probes with a mutation in the core binding motif did not suppress complex formation. Small differences were observed with respect to the nuclear proteins binding to the hFcRn promoter in THP1, Caco-2, Lu-106, and HUVEC cells, which may point to subtle cell-type specific differences in hFcRn gene regulation. The results also strongly suggest that in the specific interactions with the hFcRn promoter, the transcription factors Sp1, Sp2, Sp3, the Ets family (Ets1/E1AF, Pu1), AP1, AP2, CF1, and NFY are involved. The nature of the nuclear proteins specifically binding to the identified *cis* regulatory elements within the hFcRn promoter will be confirmed by Supershift reactions using antibodies against the given anti-transcriptional factor.

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

Carcinoembryonic antigen (CEA) is a member of the immunoglobulin superfamily of cell adhesion molecules (IgCAMs). CEA was identified in the mid-1960s as a prominent tumor-associated antigen in human colon cancer. The antigen is characterized by having seven extracellular Ig domains and a glycosylphosphatidylinositol (GPI) anchor. 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 spp*.). Our main task was to characterize first the CEA regions and second the individual amino-acid residues responsible

for CEA homo- and heterophilic interactions. The first step of the study was to obtain the two first N-terminal domains of CEA (domains N and A1) in *E. coli* cells and their biophysical characteristics. According to literature, the N and A1 domains are probably mostly involved in CEA interactions. In future experiments we would like to create NA1 single and multiple mutants of decreased binding ability and test them by SPR analysis. When we discover the proper mutant, we will test it in a cell culture to confirm the biophysical results.

A contribution of B cells and autoantibodies has been demonstrated in MS; this leads to interest in the use of such autoantibodies as diagnostic or prognostic markers and as a basis for imunomodulatory therapy. ELISA and Western blotting fail to detect reactivity against epitopes displayed by native antigens expressed on myelin sheets. We described a cell-based assay that specifically identifies serum antibodies directed against the myelin autoantigens MBP, PLP, and MOG. The method detects antibody binding to recombinant antigens in their native conformation on MBP, PLP, or MOG transfected mammalian (hamster ovary) cells. Thirty-six patients with relapsing-remitting MS diagnosed according to the criteria of McDonald were recruited; the mean age was 38.2 and duration of the disease 7.1 years. Serum anti-MBP, anti-PLP, and anti-MOG IgG autoantibodies were detected in the MS patients and 35 healthy donors by FACS analysis. Compared with the healthy controls, the titers of IgG autoantibodies directed against membrane-bound recombinant myelin antigens were most significantly increased for PLP ($p<0.0001$) and MOG ($p<0.0003$), but not quite significant for MBP ($p=0.05$). The titers of anti-MBP antibodies in both groups were low in contrast to the high titers of anti-PLP and anti-MOG antibodies. The cell-based assay detection of autoantibodies directed against recombinant myelin antigens could be a useful tool providing serological markers in the diagnosis and progression of MS. Indeed, it could allow obtaining the molecular characteristics of disease in each patient in terms of antibody response against certain myelin and non-myelin antigens. We have shown that in RRMS patients, an elevated level of serum antibodies against PLP and MOG were significant, which should be considered in the search for a specific immunomodulatory therapy in MS.

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

Our previous studies revealed that lactoferrin (LF) significantly increases the mobilization of the myelocytic lineage in mice. Therefore we attempted to determine whether activation of the hypothalamic-pituitary-adrenal axis contributes to this phenomenon. We found that intravenous injection of LF caused an about 50% increase in the circulating blood leukocyte count and increased the proportion of the myelocytic lineage (band forms 10-fold and neutrophils 2-fold) 24 h post injection. The content of the myelocytic lineage (myelocytes, metamyelocytes, bands, and neutrophils) in the bone marrow rose from 51.6 to 63.4%. In addition, the administration of LF led to a decrease in total thymocyte number by 41.6%. Analogous changes in cell types and numbers in adrenalectomized mice following LF injection were minor. Mifepristone, a blocker of steroid receptors, reversed the effects of LF on leukocyte cell number and bone marrow cell composition. Finally, we showed that LF induced a rise in the serum levels of corticosterone in control but not in adrenalectomized mice. We conclude that the LF-induced upregulation of endogenous steroid levels is responsible for the stimulation of myelopoiesis.

In a collaborative project with Wroclaw University and the University of Ioannina, Greece, we studied the activities of HLA-DQ7 beta (1)- and beta (2)-derived peptides as immunomodulators. Modulation of the protein-protein interactions involved in the immune system by using small molecular mimics of the contact interfaces may lead to the blockage of the autoimmune response and the development of drugs for immunotherapy. The polymorphic beta regions, exposed to the microenvironment, of the modeled HLA-DQ7, which is genetically linked to autoimmune diseases, were determined. Peptides 132-141 and 58-67, located at the beta (1) and beta (2) domains of HLA-DQ7, respectively, were tested for their involvement in the interactions with CD4+ T lymphocytes. Linear, cyclic, and dimeric analogs that mimic the exposed surfaces of HLA-DQ7 were designed and synthesized. Their immunosuppressive activities, found in the secondary, humoral immune response to sheep erythrocytes in mice *in vitro*, ranged from 11% to 53%. The significance of the total change of the peptide, the pattern of the hydrogen bonding, and the presence of secondary structures

were investigated in relation to the immunomodulatory effect of the peptides. Two dimeric analogs of the HLA-DQ7 58-67 fragment, consisting of the two monomers covalently linked by a polyethylene glycol (PEG) spacer, able to mimic the superdimers, were also synthesized and studied. As the 58-67 segment is located at the beta (1) region of HLA-DQ7, close to the major histocompatibility complex (MHC) groove, one can assume that the 58-67 peptide could accommodate the association between T-cell receptor (TCR) and human leukocyte antigen (HLA) by activating a co-stimulatory molecule of the TCR/HLA interaction. The hypothesis is supported by the confocal laser image of the fluorescein-labeled 58-67 peptide and by the fact that it is an immunostimulator at low concentration.

Recently, ubiquitin was suggested as a promising therapeutic anti-inflammatory protein. In a collaboration with Wroclaw University we found that a peptide fragment corresponding to the ubiquitin (50-59) sequence (LEDGRTLSDY) possessed immunosuppressive activity comparable to that of ubiquitin. CD and NMR spectra were used to determine the conformational preferences of LEDGRTLSDY in solution. The peptide mixture, obtained by pepsin digestion of ubiquitin, was even more potent than the intact protein. Although the peptide exhibited a well-defined conformation in methanol, its structure was distinct from the corresponding 50-59 fragment in the native ubiquitin molecule.

Five types of cells are responsible for bone growth, remodeling, and regenerative processes. These are 1) osteoblasts, i.e. bone forming cells. 2) osteocytes, the resting form of osteoblasts possessing the ability to differentiate into osteoblasts, 3) osteoclasts, bone resorbing cells, 4) chondrocytes, cartilage tissue-forming cells, and 5) fibroblasts, cells present in the synovium.

There are the two main forms of destructive periodontal disease caused by imbalanced homeostasis between bone-associated cells: chronic and aggressive periodontitis. It is well known that severe forms of periodontal disease are clustered in the minority of individuals in a given population. By reason of serious after effects, these high-risk individuals should be identified at the earliest stage of disease so that preventive measures and treatment procedures can be provided efficiently. These studies were performed to confirm the diagnostic value of objective factors routinely assessed during clinical examination with the aim to distinguish patients with chronic and aggressive periodontitis. Moreover, these factors were verified with the subjective description of symptoms declared by the patients. To obtain the designated targets, multinominal logistic regression analysis was used.

Osteoblasts are responsible for the synthesis, deposition, and mineralization of the bone matrix. They are found mainly at the surfaces of a mature bone, where they form a monolayer.

Within the bone matrix, osteoblasts are found in regions that undergo remodeling. In the process of bone matrix formation, the osteoblasts become embedded in the matrix and transform into osteocytes. Chondrocytes are the only cells found in cartilage. They produce and maintain the cartilaginous matrix. Progenitor cells of chondrocytes are able to differentiate into osteoblasts. Fibroblasts are morphologically heterogeneous, with diverse appearances depending on their location and activity. Fibroblasts and chondrocytes form a fibrocartilage callus at the area of a bone fracture. All these cells influence each other and some of them are under immunological system control. Fibroblasts and chondrocytes are mostly important due to their plasticity. This means that these cells are able to change their phenotype and reveal features representative of other bone-associated cells. Moreover, they can transfer signals between bone and the immune systems. The described properties allowed using these cells in regenerative medicine, including prosthesis assimilation as well as bone or cartilage reconstruction with autologic implants. Contemporary biomaterials constructed for regenerative medicine, especially for bone or joint prosthesis, differing in surface roughness. This feature, apart from chemical content, is the most important for successful cell adhesion and growth. Consequence to the cells' growth, tissue formation and its connection with the surrounding graft tissues are possible. The main point of each biocompatibility investigation is to count the total cell number present on the studied surface. The total cell number present on a surface was routinely determined by cell counting under a light microscope after removing them from the surface with trypsin solution. Unfortunately, this method proved to be insufficient for rough surfaces and enabling the removal of cells growing inside the surface's pockets. Therefore we developed a new sensitive and specific method for the simultaneous measurement of total cell number as well as live cell number. There are no limits in surface roughness in our method, which is based on spectrophotometric measurements. This new method combines spectrophotometric protein measurements with the MTT assay, which is a well-known method for measuring cellular activity and proliferation. The results were verified with the conventional method used so far for cell counting and proved to be repeatable and specific. The present paper describes not only a modified extraction method using a lauryl sulfate mixture acidified to pH 5, but also the procedure for determining the total cell number using protein concentration measurements as well as the number of live cells. The modified method enables an optimal, easy to handle, and less time- and work-consuming assay for (i) the determination of cell adhesion kinetics, (ii) determination of the time needed for cells' successful biomaterial colonization, and (iii) a

quantitative measurement of live cell number on the surface of biomaterial. The method enables an authentic rating of the suitability of biomaterial for prosthesis.

Laboratory of Immunopathology

Head: Professor Irena Frydecka, M.D.

Studies on the mechanisms of immune deficiency in neoplastic and autoimmunological diseases

Polymorphism of KIR genes and their HLA-C ligands in B-cell chronic lymphocytic leukemia

Dysfunction in cellular and humoral immunity entails an increased risk of B-cell chronic lymphocytic leukemia (B-CLL). It has been suggested that innate immunity, especially natural killer cells, plays a key role in antitumor cytotoxity regulated by interaction between killer cell immunoglobulin-like receptors (KIRs) on NK cells and their HLA-class I ligands on target cells. 2DL2, 2DL3, and 2DS2 bind to the HLA-C1 allotype (carrying Asp at position 80), while 2DL1 and 2DS1 KIRs bind to the HLA-C2 allotype (carrying Lys at position 80). Many studies have been devoted to the contribution of genes encoding KIRs and their HLA ligands in the pathogenesis of autoimmune and neoplastic diseases. The present study was undertaken to determine the association between polymorphism of *KIR* genes and *HLA-C* allotypes and susceptibility to B-CLL.

Eighty nine individuals with B-CLL and 97 healthy subjects from the same region were enrolled in this study. Genotyping of the *KIR* genes *2DL1*, *2DL2*, *2DL3*, *3DL1*, *2DS1*, *2DS2*, *2DS3*, *2DS4fl*, *2DS4del*, *2DS5*, and *3DS1* was performed by the PCR-SSP method. the *KIR* ligands genes *HLA-C1* and *HLA-C2* were typed using Olerup SSP typing kits. We found a statistically significant decrease in the frequency of *KIR 2DS3* gene in B-CLL patients compared with healthy individuals (21.35 vs. 35.05%). The distribution of the other *KIR* genes did not differ between the two groups. The frequency of the *HLA-C2* allotype was lower in the group of B-CLL patients than in the controls (60.67 vs. 71.13%) and, as a consequence, the *2DL1+/C2+* combination was less common in the B-CLL patients than in the controls (56.18 vs. 70.10%). Our study could indicate that activating the *KIR 2DS3 gene* is associated with a decreased risk of developing B-cell chronic lymphocytic leukemia. The individuals possessing the *HLA-C2* allotype, especially coupled with its inhibitory *KIR 2DL1* receptor, are less prone to disease. Nevertheless, the observations require confirmation on a larger number of patients and controls.

CTLA-4, CD28, and ICOS gene polymorphisms in non-small-cell lung carcinoma in a Polish population

Lung cancer is one of the most common cancers and has become a predominant cause of cancer-related death throughout the world. As T cells play a key role in anti-tumor immunity, the expression of the co-stimulatory molecules CTLA-4, CD28, and ICOS, which mediate the regulation of T-cell activity, could influence cancer susceptibility. Several reports indicated that *CD28*, *CTLA-4*, and *ICOS* gene polymorphisms are associated with susceptibility to malignancies. To the best of our knowledge, no large cohort-based study on gene polymorphisms of co-stimulatory and down-regulatory molecules has been performed in non-small-cell lung carcinoma **(**NSCLC) in a Caucasian population.

A case-control study of 622 individuals including 296 NSCLC patients was conducted on five polymorphisms in the *CTLA-4* gene (*CTLA-4*c.49A>G, *CTLA-4*g.319C>T, *CTLA-4*g.*642AT(8_33), *CTLA-4*g.*6230G>A (CT60), *CTLA-4*g.*10223G>T (Jo31)), one in the *CD28* gene (*CD28*c.17+3T>C), and one in the *ICOS* gene *ICOS*c.1554+4GT(8_15). There were no statistically significant differences in the allele and genotype distributions between NSCLC patients and healthy controls for all the investigated polymorphic markers in the *CTLA-4*, *CD28*, and *ICOS* genes. The lack of association may show that the investigated polymorphisms do not modulate the risk of NSCLC.

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

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 on the potential influence of ER alpha on the function of antigen-presenting cells and the course of immune response were continued. We showed that orally administered estrogens and IFN-tau changed the level of ERalpha in spleen macrophages and dendritic cells of mice immunized with testicular germ cells (TGCs) as autoantigen. Experimental male mice were immunized s.c. with TGC and BM-DC (at a ratio of 10:1). The relative level of ER-alpha was significantly decreased in immature and mature macrophages and in mature dendritic cells in the immunized mice treated with INF-tau and 17-beta estradiol simultaneously compared with mice with IFN-tau or 17-beta estradiol alone. Moreover, the DTH reaction was decreased in the mice treated with estradiol; on the other hand, the concentration of IgG3 and IgG2 antibodies specific to TGC was significantly increased

compared with the immunized and IFN-tau-treated mice. The experiments indicated interactions between estrogens and IFN-tau in modulation of the autoimmune response. We also investigated the influence of estrogens on the expression of genes stimulated by I type of interferons. For this purpose we stimulated bone marrow-derived macrophages with IFN-tau and IFN-tau with 17-beta estradiol (30pg/ml). Gene expression was examined using an RT2 Profiler TM PCR Array (SA Bioscence). Eight (Ifna4, Ifnb1, Irf3,Oas1a, Hoxb2, H2-M10.2, H2-M10.4, H2-M10.6) of the 87 analyzed genes were significantly increased after stimulation with estrogens.

Laboratory of Glycobiology
Head: Professor Maciej Ugorski, Ph.D., D.V.M.
Ceramide galactosyltransferase (UGT8) is a molecular marker of breast cancer malignancy and metastases

Among the six genes which are highly over-expressed in lung metastases compared with other breast cancer metastases, *UGT8* gene, which encodes an enzyme responsible for the synthesis of galactosylceramide (GalCer), was found. As all the available information on the presence of UGT8 in breast cancer tissues was obtained only at the mRNA expression level and there are no data available on the presence of GalCer in cancerous cells, primary tumors and their lung metastases were analyzed for UGT8 expression at the protein level and the presence of UGT8 and GalCer were determined in breast cancer cell lines representing different tumor phenotypes. Significantly stronger staining with rabbit polyclonal antibodies directed against UGT8 was observed in the specimens from lung metastases than in paired primary tumors. It was found further that the amounts of UGT8 protein and mRNA increased with tumor malignancy grade and highly significant differences in UGT8 expression were found in G3 tumors vs. G2 tumors. Interestingly, highly increased expression of UGT8 was also observed in primary tumors forming lymph node metastases compared with non-metastatic primary tumors. Therefore our data suggest that UGT8 is a significant index of tumor aggressiveness and a potential marker for the prognostic evaluation of metastases in breast cancer. The expression of UGT8 at the mRNA and protein level in the established breast cancer cell lines correlated well with the results obtained for the clinical samples. The cell lines MCF-7, T47D, SKBR-3, and BT-474, which do not form metastases in nude mice model, did not express UGT8, in contrast to the metastasizing MCF10CA1a.c11, MDA MB 231, and BO2 cells. We also showed that the presence of GalCer is limited only to breast cancer cell lines forming metastases in nude mice.

In summary, we showed for the first time that 1) the expression of UGT8 is higher in breast cancer metastases to the lung than in primary tumors and that increased amounts of the enzyme in cancerous tissues are associated with progression to a more malignant phenotype and 2) in established *in vitro* breast cancer cell lines the expression of UGT8 and GalCer is limited to metastatic cells.

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

An original model of organo-specific, immortalized, and stabilized human endothelial cell lines was designed to evaluate tumor cell-endothelial cell interactions taking place during the metastatic process. EC lines established from human lymph node, appendix, lung, skin, and intestine microvessels were developed previously on the basis of collaboration with the group of Dr C. Kieda*.

Endothelial cells are critical in the recruitment and migration of circulating effector cells to sites of inflammation and necrosis as well as in tumor cell extravasation from blood vessel, involving close adhesive interactions of cancer cells with endothelial cells at the site of cancer cell extravasation. The association of chemokines with endothelial cells and extracellular matrices is required for the pro-migratory *in vivo* activity of these molecules. Chemokine receptor expression and chemokine presentation were investigated on organo-specific human endothelial cell lines. Experiments with CCL21 on peripheral lymph node endothelial cells demonstrated that the chemokine did not co-localize with its receptor, but was associated with extracellular matrix components. The specific activity of chemokines was clearly shown to be related to the endothelial cell origin. CX3CL1 and CCL21 promoted lymphocyte recruitment by endothelial cells from the appendix and peripheral lymph nodes, respectively, while CX3CL1 activity was restricted to endothelial cells from the appendix and skin. This unique cellular model demonstrated a fundamental role for chemokines in conferring to the endothelium its organo-specificity and its potential for tissue targeting through the selective binding, presentation, and activation properties of chemokines [Crola da Silva, Lamerant-Fayel, Paprocka, Mitterrand, Gosset, Duś, Kieda; Immunology 2009;126:394-404].

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

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