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Research Report – 2003

DEPARTMENT OF INFECTIOUS DISEASE MICROBIOLOGY
Head: Associate Professor Andrzej Gamian, Ph.D.

Laboratory of Medical Microbiology

Head: Professor Andrzej Gamian, Ph.D.

Studies on the pathogenesis of some autoimmune diseases of bacterial etiology and n the role of sialic acid, glycolipids, endotoxins and bacterial proteins

The current activity of this laboratory is focused on studies of mechanisms of the pathogenicity of autoimmune diseases with bacterial etiology, the role of molecular mimicry, bacterial proteins in pathogenicity, and the structure and functions of bacterial capsular antigens and endotoxins.

The general strategy for elaborating the protective tools against invading bacteria involves determining the structures of the molecules involved in infection and immune processes and in probiotic mechanisms, their chemical and genetic manipulations, as well as understanding their biological activities. The structures of several such antigens have thus been established (*Eur. J. Biochem.*, 2003, 270, 2732-2738, *Carbohydr. Res.*, 2003, 338, 1389-1395, 2153-2158, *FEMS Immunol. Med. Microbiol.*, 2003, 36, 71-76). The interference of some bacterial components with the functions of tissue structures may contribute to the mechanisms of pathogenicity. Due to structural mimicry, care should be taken when antibacterial vaccines are designed to avoid the induction of autoantibodies. Thus epitopes specific only to the bacteria are desired for the construction of vaccines, as was the approach applied for the development of anti-meningococcal vaccine (*Carbohydr. Res.*, 2003, 338 167-175). As the protective role of probiotic bacteria can be better understood by structural study, the structure of an exopolysaccharide from such a microorganism has been established (*Carbohydr. Res.*, 2003, 338, 605-609). Structural studies of glycolipids from pathogenic actinomycetal microorganisms (*J. Biol. Chem.*, 2003, 278, 3948-3956) allow using glycolipids for identification and as chemotaxonomic and immunodiagnostic markers useful for the

classification and identification of clinical isolates and for recognizing opportunistic actinomycete as well as nocardiosis-like infections. The monitoring of specific markers for sepsis and septic shock could significantly facilitate the prognosis of these diseases and their treatment. Therefore, current work concerns the determination of endotoxins as markers for monitoring different stages of septic shock and patient status during treatment.

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 gene expression and designing compounds inhibiting these processes

Molecular basis of *Helicobacter pylori* chromosome replication

Helicobacter pylori is a Gram-negative, spiral-shaped pathogenic bacterium. This organism is a human gastric pathogen associated with peptic ulcer disease and chronic gastritis. The genome sequences of two unrelated isolates of *H. pylori*, 26,695 (1,667,867 bp) (1) and J99 (1,643,831 bp) (2), have been determined. Over the last 20 years, extensive studies of *H. pylori* biology have been carried out. However, experimental data concerning the replication of *H. pylori* are scarce.

Using *in silico* methods, the putative *oriC* region containing five DnaA boxes has been located upstream of the *dnaA* gene (3). Each of five DnaA boxes located in the *H. pylori oriC* region has at least one mismatch to the consensus sequence of the *E. coli* DnaA box (TTATNCACA).

The key protein in the initiation of *H. pylori* chromosome replication, DnaA, has been characterized (4). The amount of DnaA protein was estimated to be approximately 3000 molecules per single cell; a large part of the protein was found in the inner membrane. The *H. pylori* DnaA protein has been analyzed using *in vitro* (gel retardation assay and surface plasmon resonance (SPR)) as well as *in silico* (comparative computer modeling) studies. DnaA binds a single DnaA box as a monomer, while binding to the fragment containing several DnaA box motifs, the *oriC* region, leads to the formation of high-molecular-mass nucleoprotein complexes. In comparison with the *Escherichia coli* DnaA, the *H. pylori* DnaA protein exhibits lower DNA-binding specificity; however, it prefers *oriC* over non-box DNA

fragments. As determined by gel retardation techniques, the *H. pylori* DnaA binds with a moderate level of affinity to its origin of replication (4 nM). Comparative computer modeling showed that there are nine residues within the binding domain which are possible determinants of the reduced *H. pylori* DnaA specificity. Of these, the most interesting is probably the triad PTL; all three residues show significant divergence from the consensus, and Thr398 is the most divergent residue of all.

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2. Alm R. A., Ling L.-S. L., Moir D. T., King B. L., Brown E. D., Doig P. C. *et al.*, *Nature*, 1999, 397, 176–180
3. Zawilak A., Cebrat S., Mackiewicz P., Król-Hulewicz A., Jakimowicz D., Messer W, & Zakrzewska-Czerwińska J., *Nucl. Acids Res.*, 2001, 29, 2251–2259
4. Zawilak, A., Durrant, M.C., Jakimowicz, P., Backert, S. & Zakrzewska-Czerwińska J.: *J. Mol. Biol.*, 2003, 334, 933–947.

Laboratory of Signaling Proteins

Head: Associate Professor Wojciech Gorczyca, Ph.D.

Function and physicochemical properties of proteins involved in Ca²⁺-dependent signal transmission by cAMP and cGMP in cells of the immune system

The intracellular concentration of cGMP increases after activation of guanylyl cyclases (GCs) and is regulated by the activity of phosphodiesterases (PDEs). The cGMP signal is in turn transmitted to other proteins, of which the cGMP-dependent protein kinase 1 (PKG1) appears to be the most ubiquitous effector. Guanylyl cyclases exist as cytosolic (soluble, sGC) or membrane-bound (particulate, pGC) enzymes. Both forms differ in structure, mechanism of activation, subcellular localization, and the cellular effects of their activities are supposedly also distinct. The role and metabolism of cGMP in cells of the immune system, including macrophages, have not been extensively explored and are still poorly understood. However, it has been noted that the expression profile of enzymes which constitute the “cGMP pathway” changes during the maturation of monocytes to macrophages, indicating that the role of cGMP may also be variable. Thus, identification of these enzymes appears to be critical for understanding the cellular effects of cGMP. The aim of our studies was to establish which isoforms of GCs, PDEs, and PKGs may contribute to cGMP signaling in inflammatory macrophages isolated from the peritoneal cavity of the guinea pig and rat. We found that in guinea pig peritoneal macrophages (PMs), synthesis of the nucleotide was significantly enhanced in response to activators of sGC, and it was only slightly stimulated by specific activators of pGC. At the same time, rat PMs responded strongly to atrial natriuretic peptide (ANP), the activator of particulate GC type A (GC-A), and not to activators of sGC. The

activity of PDEs hydrolyzing cGMP was apparently regulated by cGMP itself in the PMs of both species. In contrast to the rat cells, guinea pig PMs revealed an activity of Ca²⁺/calmodulin-dependent PDE1. Using Western blotting and RT-PCR analysis we were unable to detect the presence of cGMP-dependent protein kinase 1 (PKG1) in PMs isolated from either species. In summary, our findings indicate that particulate GC-A is the main active form of GC in rat PMs, while in guinea pig macrophages the sGC activity dominates. Since the profiles of PDE activities in rat and guinea pig PMs are also different, we conclude that the mechanisms regulating cGMP metabolism in PMs are species specific. Moreover, our results suggest that targets for cGMP other than PKG1 should be present in the PMs of both species.

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

Laboratory of Clinical Immunology

Head: Professor Andrzej Lange, M.D.

Genetic background and pathomorphologic evaluation of the alloreactive reaction following hematopoietic stem cell transplantation (HSCT)

The effect of donor-recipient matching for HLA class III genes on the outcome of allogeneic HSCT from alternative (related-haplo-identical and unrelated) donors

Our recent studies documented that polymorphism of the HLA-DRB1, TNF-alpha (TNFA), and TNF-beta (TNFB) genes influences the risk of post-transplant complications, the development of aGvHD, and chemotherapy-related toxicity. Also, a strong linkage between DRB1 and TNFA and TNFB alleles was reported. In the present work, in addition to DRB1 and TNF, the heat shock protein (HSP70-hom) gene polymorphism at position +2763 (G/A) was investigated. This gene, as TNFA and TNFB, is located within the class III region of human MHC. HSP70-hom alleles were detected with the use of the ARMS technique in 105 patients and 50 donors.

- An analysis of the relationship between DRB1 and HSP70-hom alleles showed a positive linkage between DRB1*03 and HSP-G and between DRB1*11 and HSP-A in both patients and donors.
- It was also observed that HSP-AA homozygous patients were more prone to conditioning regimen-related toxicity (0.91 vs. 0.56, p=0.02), while transplants from alternative donors carrying the DRB1*03 and HSP-GG genotype were more likely to induce aGvHD (1.00

vs. 0.44, $p=0.035$) and severe (grades III and IV) toxic complications (0.75 vs. 0.22, $p=0.05$).

- No significant effect of donor-recipient matching for the analyzed HLA class III genes on the manifestation of aGvHD and toxic complications was observed. However, patients grafted from HLA-A, B, and DR matched, TNFA and/or TNFB mismatched donors had a stronger tendency to develop aGvHD than those transplanted with HLA-A, B, DR, TNFA, and TNFB matched donors (0.71 vs. 0.27, $p=0.088$).

These results imply that polymorphic features of the HLA class III genes of patients and donors of allogeneic HSCT influence the risk of post-transplant complications and that matching for HLA class III alleles may affect the development of aGvHD.

Hematological and immunological reconstitution after allogeneic HSCT with Reduced Intensity Conditioning (RIC)

We analyzed the phenotype of peripheral blood lymphocytes in the early post-transplant period, prior to overt manifestation of aGvHD (2 – 3 weeks post transplant), using flow cytometry. We found that after RIC, immunological reconstitution is different from standard myeloablative chemotherapy regimen with regards to:

- Lower fraction of CD4⁺ lymphocytes (16.3%±1.7 vs. 31.7%±2.1, $p=4.6\times 10^{-7}$)
- Lower fraction of CD45RO⁺ lymphocytes (41.1%±2.6 vs. 50.8%±2.8, $p=0.006$)
- Higher fraction of NK cells (CD56⁺: 41.0%±2.6 vs. 26.7%±2.4, $p=9.5\times 10^{-5}$)
- Higher fraction of CD57⁺ lymphocytes (30.0%±1.7 vs. 20.6%±1.5, $p=2.0\times 10^{-5}$)

In summary, immunological reconstitution following non-myeloablative Reduced-Intensity Conditioning is characterized by a lower proportion of CD4⁺CD45RO⁺ immuno-competent cells and higher proportions of CD56⁺ and CD57⁺ NK cells and T lymphocytes. The findings explain the differences observed in the clinical course: the lower aGvHD occurrence, the relatively low relapse rate (GvL effect by NK cells and CD57⁺T cells), and a higher susceptibility to opportunistic infections following RIC compared with standard myeloablative conditioning.

Post-transplant chimerism was evaluated with the use of STR microsatellite typing of DNA isolated from blood and marrow specimens. Out of 54 patients who had undergone RIC transplantation, 38 showed complete donor chimerism, 13 mixed chimerism, and 3 a lack of donor chimerism. The mixed chimerism was a transient state resolving after 53 days at the latest into complete donor chimerism in all 13 cases. In 4 cases the initial complete donor chimerism reversed to a lack of donor genotype, confirming the clinically observed relapse of

the primary malignancy. Monitoring post-transplant chimerism offers the best insight into post-transplant reconstitution, creating possibilities to adapt immunosuppressive treatment, apply Donor Lymphocytes Infusion (DLI), monitor Minimal Residual Disease, and detect relapsing leukemia at its earliest, enabling clinical intervention.

Immunopathomorphological assessment of trephine biopsies taken from patients in the course of hematological reconstitution after allogeneic HSCT

We used immunocytochemistry to evaluate the architecture of bone marrow tissue in the post-transplant period. The underlying disease dictated the set of markers used to detect residual disease and to differentiate relapse from lymphoproliferative syndrome, which is induced by EBV reactivation in an immunocompromised host after allogeneic hematopoietic stem cell transplantation. EBV reactivation was documented by measuring a number of EBV copies and by immunostaining lymph node biopsies by monoclonal antibodies specific to EBV-associated antigens.

Laboratory of Immunogenetics

Head: Associate Professor Piotr Kuśnierczyk, Ph.D.

Distribution of HLA-C and LILRA3 alleles in a healthy Polish population and in psoriasis vulgaris

The importance of HLA-C, a human class I “classical” major histocompatibility complex molecule, has long been neglected in the clinic. However, there has recently been growing interest in the immunogenetics and biological role of HLA-C due to the discovery of its contribution to a number of immune phenomena, including organ graft rejection, graft-versus-host disease, resistance to natural killer cells and infectious diseases such as AIDS, and its strong association with psoriasis vulgaris. Therefore, knowledge of HLA-C allele distribution in a given human population is highly desirable. We examined the distribution of HLA-C alleles in 207 unrelated healthy persons from the region of Lower Silesia in Poland and compared the results with those described for other human populations. Similarly to other Caucasoids, *HLA-Cw*07* was most frequent (0.3067), followed by *HLA-Cw*12* (0.1400), *HLA-Cw*04* (0.1376) and *HLA-Cw*03* (0.0918), whereas the least frequent alleles (frequencies < 0.02) were *HLA-Cw*14* and *HLA-Cw*15*, and *HLA-Cw*18* was absent. Comparison with *HLA-C* frequency data published for 23 other ethnic groups revealed the greatest similarities to Germans, Slovaks and the English, less similarity to other Europeans,

and, as expected, an even lower degree of similarity to non-Caucasoid populations. We have also recently described an extremely strong association of the *HLA-Cw*06* allele with psoriasis vulgaris. The *LILRA3* (*ILT6*) gene is localized on human chromosome 19 in the 19q13.4 region in the leukocyte receptor complex that encodes leukocyte receptors such as LILR (ILT/LIR), KIR, LAIR, Fc IgA receptor, and others. The biological role of the LILRA3 molecule and the nature of its ligand are not known. Comparison of the *LILRA3* gene sequence with those of other *LILRs* suggests that LILRA3 is a soluble molecule. If LILRA3 binds HLA class I molecules as do other LILRs whose ligands are known, then it might block the recognition of HLA by these receptors, influencing immune response and susceptibility to HLA class I-associated disease. A deletion of the *LILRA3* gene was found in a minority of the British population, but it was not known whether it is also present in non-British populations. Therefore, we typed genomic DNA samples derived from the blood of 108 healthy individuals from the Low Silesian region for the normal *LILRA3* gene and its deletion using polymerase chain reaction in order to find out whether the deletion is present in our population. A deleted *LILRA3* gene was found in 25% of Poles (allele frequency, 0.139) which gives a result similar to that of the British population. To see whether *LILRA3* gene deletion affects the susceptibility to psoriasis vulgaris (a disease associated with *HLA-Cw*06*), we typed 103 patients diagnosed with psoriasis for *LILRA3* to examine whether *LILRA3* deletion was distributed differently in persons affected with the disease. No differences in frequencies of the *LILRA3* deletion were found between controls and patients or between HLA-Cw6+ and HLA-Cw6- controls or patients, suggesting that LILRA3 has no role in psoriasis.

DEPARTMENT OF CANCER IMMUNOLOGY
Acting head to July 31 - Professor Leon Strz̄adala, Ph.D.
Head since August 1 - Professor Paweł Kisielow, Ph.D.

Laboratory of Cellular Immunology
Head: Professor Leon Strz̄adala, Ph.D.

Normal and pathological development and selection of lymphoid and neuronal cells

Using cDNA-Representational Difference Analysis it was found that the expression of the *Opg*, *Ctse*, *Krt2-4*, *Fut-2*, *24p3* and *Wif-1* genes was elevated in intestinal adenomas compared with normal epithelial cells of *Apc^{Min/+}* mutant mice. Expression of *Wif-1*, which encodes Wnt inhibitory factor-1, was also detected in a number of tumor cell lines of epithelial cell origin, including two human colon adenocarcinoma cell lines. A hypothetical

explanation of the role of *Wif-1* over-expression in the etiology of colorectal cancer was presented.

We showed that thymic lymphomas from anti HY-TCR transgenic mice were resistant to ionomycin-induced apoptosis but sensitive to etoposide-induced apoptosis. Furthermore, Fas ligand (FasL) induction is abrogated in thymic lymphomas treated with ionomycin, but not with etoposide, which still induced the expression of FasL and Fas receptor as well as apoptosis. Sensitivity to ionomycin-induced apoptosis could be restored by FK506 treatment, which also abolished the abrogation of the induction of FasL expression. Moreover, induction of FasL was always accompanied by an increase in the expression of Fas receptor. It appeared that the Fas/FasL system can successfully serve as a molecular target for tumor therapy, even for tumor cells initially lacking Fas expression. Results indicate that FK506 can sensitize resistant tumor cells to induction of apoptosis and it could be considered as a potential agent in the combined therapy of thymic lymphomas.

TrkC is a receptor for neurotrophin-3 that regulates the development of neuronal precursors. The transduction of signals into receptor-dependent signaling pathways is mainly due to the activation of the intrinsic tyrosine kinase of the TrkC receptor. Alternative splicing of the TrkC transcripts generates catalytic and non-catalytic isoforms. MB-G cells (a neural cell line derived in our laboratory from whole brain of embryos of transgenic tsA58-SV40 mice) and H19-7 cells (a rat hippocampal progenitor cell line) were tested for the expression of mRNA encoding catalytic (TrkC-K) and non-catalytic (TrkC-NC2) isoforms. H19-7 cells were found to express mRNAs encoding both the TrkC-K and TrkC-NC2 isoforms. In contrast, MB-G cells were shown to express mRNA only for TrkC-NC2. Results suggest that cells with the phenotype of the neuron-restricted precursors may express mRNA encoding TrkC-NC2 without concomitant expression of mRNA encoding catalytic isoforms of TrkC.

DEPARTMENT OF MEDICAL IMMUNOLOGY

Head: Professor Andrzej Górski, M.D.

Laboratory of Bacteriophages

Head: Professor Andrzej Górski, M.D.

Application of specific bacteriophages in the treatment of bacterial infections and their possible role in host defense and disease

Acute bacterial infection-induced sepsis with shock, metabolic acidosis, oliguria, or hypoxemia remains a major medical challenge in view of the increasing antibiotic resistance

among different bacterial strains.

We present our results based on 94 patients, with sepsis of various origin, who had been treated previously with antibiotics without apparent benefit. Successful phage therapy were achieved in 80 cases (85.1%). In 14 patients (14.9%) phage therapy was ineffective.

We present evidence that some coliphages (T4) may produce immunosuppressive effects extending transplant survival: bacteriophages significantly enhanced allogenic skin allograft survival in mice. This effect was paralleled by phage-mediated inhibition of alloantigen-induced human T4 and B-cell responses *in vitro* as well as specific antibody production in mice.

We showed that the sepharose 4B-purified *Staphylococcus aureus* phage A20/R exhibited costimulatory activity in splenocyte proliferation induced by suboptimal and optimal concentrations of ConA. It was also found that the phage preparation can elicit IL-6 production in splenocyte cultures and enhance ConA-induced production of that cytokine.

The knowledge of phage interactions with mammalian cells is very limited, and it is believed they have no intrinsic tropism for those cells. We postulate that, because some coliphages (T4) express KGD sequence which binds β_3 integrins, they could bind β_3^+ cells and downregulate activities of those cells by inhibiting integrin functions. If phages can modify some functions of β_3^+ cells, like, for example, cell migration. This opens novel perspectives in their potential use in the treatment of cardiovascular and autoimmune disease, graft rejection, and cancer.

Laboratory of Cellular Interactions

Head: Associate Professor Danuta Duś, Ph.D.

Phenotypic characteristics of cells which determine the organ specificity of metastatic secondary growth

The variable outcome of cancer patients with regard to known clinical parameters creates a need of searching for new, reliable prognostic indicators of nodal status, tumor recurrence, and survival. Therefore, the aim of the study is to search for new molecular markers of tumor progression.

New cancer progression markers in laryngeal cancer. The aim of the study was to evaluate a possible relationship between the presence of immunochemically detectable Bcl-Xl and c-myc proteins as well as DNA ploidy status and proliferation index (PI) versus clinicopathological data in laryngeal squamous cell carcinomas. The Bcl-2 family proteins take part in the

regulation of cellular apoptotic pathways. In Bcl-XI-positive tumors an inverse relationship between Bcl-XI protein level and the tumor grade and nodal status was noticed. Similarly, the presence of detectable levels of c-myc protein was characteristic for non-metastasizing tumors. The study was performed in cooperation with Dr. T. Kręcicki's group at the Department and Clinic of Otolaryngology; Medical University of Wrocław.

New cancer progression markers in urological cancers. The prognostic value of p53 protein expression level and ploidy status in transitional cell carcinoma (TCC) of the bladder was evaluated. Overexpression of p53 protein was related to the clinical course of the disease. The results suggest that p53 protein evaluation can add for prediction of disease progression, similarly as tumor DNA ploidy status. Retrospective flow cytometry analysis revealed that 5- and 9-year disease-specific survival rates of patients with aneuploid TCC tumors (n=53) were 30- 23%, respectively, versus a 95-82% survival rate for those with diploid tumors. DNA analysis was also applied to renal clear cell carcinoma patients (n=74). The preliminary results indicate a relatively higher percentage of aneuploidy in higher grade tumors. These results are a confirmation of the potential prognostic value of tumor DNA ploidy evaluation. The study was performed in cooperation with Prof. J. Lorenz's group at the Department and Clinic of Urology; Medical University of Wrocław.

Organ-specific phenotype of human vascular endothelial cells. Vascular endothelium is a site where, in response to specific signals, adhesion molecules are induced to recruit leukocytes as well as metastasizing tumor cells. The subject of the study was a panel of human endothelial cell lines we established with preserved organ specificity of endogenous lectins, cytokine receptors, addressins, and other adhesion molecules. We revealed, for the first time, the presence of IL-7 functional receptor on human endothelial cells. The studies on endothelial cell features, performed in collaboration with Dr. C. Kieda at CBM CNRS, Orleans, France (program POLONIUM 4304.II/2002/2003), are being continued due to a common grant sponsored by the Ministry of Scientific Research and Information Technology (former Committee for Scientific Research): PBZ-KBN-083/P05/11 (2003-2005) entitled “*Endothelial progenitor cells from cord blood – in vitro differentiation*”. In 2003 the isolation of endothelial progenitor cells and their *in vitro* growth and differentiation methods were elaborated, together with the phenotypic characteristics during the differentiation process.

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

The dependence of innate immunity on age and sex was studied. The immunity was measured by using the direct method of infection of peripheral blood leukocytes with the indicatory virus VSV and studying the kinetics of its replication for three days. The method was previously elaborated in our laboratory. The degree of immunity was evaluated according to the titer of virus. Lack of virus replication indicates complete innate immunity, a low level of VSV (2-3 log TCID₅₀) indicates partial immunity, and a high titer of VSV (>4 log) very weak immunity or, simply, deficiency. The kinetics of VSV replication was studied in leukocytes isolated from 127 individuals aged 0-89 years. The individual differentiation of the kinetics of VSV replication indicates the different degree of innate immunity even in newborns. The age-related differences in natural immunity were observed: low immunity in newborns, the best in the group of 31- to 40-year-olds, and reduced immunity at ages over 60. Sex-dependent innate immunity was shown in the group of aged persons, higher in woman than the men.

Conclusions: Innate immunity of leukocytes develops to the age 30-40, then a reduction of it is observed in older persons. A sex dependence was observed only in the group of aged persons, women expressing higher immunity, which is probably consistent with their longevity.

The different dialkyl and diaryl diselenides with carbamoyl and sulfamoyl moieties and other substitutes in the *ortho* position of the benzene ring as well as derivatives of 1,2,4-benzoselenadiazine were tested as antiviral agents. Some were found in an antiviral assay to be *in vitro* strong inhibitors of the cytopathic activity of encephalomyocarditis virus (EMCV).

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

Lymphocytes and endometrial cell function during endometriosis in women

Endometriosis is a common benign disease of women during the reproductive years. The pathogenesis of the disease involves the implantation and proliferation of endometrial tissue following retrograde menstrual blood and tissues. An immunological basis has been considered to be important in the pathogenesis of endometriosis. The aim of the research

conducted in this laboratory was to examine some functional and phenotype characteristics of peripheral blood lymphocytes (PBLs) and endometrial cells derived from patients with endometriosis.

Increased proliferative rates of PBLs and endometrial cells in primary cultures from biopsy specimens from women with endometriosis were shown. A significant increase in the percentage of T lymphocytes in S phase of the cell cycle after concomitant stimulation with anti-CD3 antibody and collagen IV in patients with endometriosis or leiomyoma (another benign gynecologic disorder in women at reproductive age) was also observed. In the peripheral blood of women with endometriosis we found an increased number of CD3+CD29+ lymphocytes in response to anti-CD3 and collagen IV, though these cells did not augment the expression of the T cell activation marker (CD69). Endometriosis is a reproductive failure in which resistance to apoptosis is recognized as one of the most important pathological mechanisms. Our experiments showed that endometrial cells of the ectopic endometrium in primary cultures are resistant to sphingosine-induced apoptosis. Moreover, PBLs of patients with endometriosis expressed CD95 antigen significantly less frequently.

THP-1 cell subline resistant to calcitriol-induced cell differentiation

Calcitriol (1,25-dihydroxyvitamin D₃) induces the differentiation and inhibits the proliferation of human promyelocytic leukemia cells, for example THP-1 cells. A subline of THP-1 cells resistant to calcitriol-induced differentiation was examined in search of the basis of this resistance. Resistance to PMA-induced cell differentiation and resistance to calcitriol-induced inhibition of proliferation were observed in these cells. The expression of the vitamin D receptor (VDR) was similar to the parental cell line, but its intracellular distribution was different.

Laboratory of Tissue Immunology

Acting head: Assistant Professor Beata Nowakowska, Ph.D.

HLA locus A and B homozygosity in the South-West Polish population

One of the hallmarks of the MHC complex is the high polymorphism of its loci. HLA class I molecules (HLA-A and -B) present foreign antigenic peptides to CTLs. Their advantage of diversity ensures that a wide range of antigenic peptides will elicit an immunogenic reaction. The observed deficiency of homozygotes in some human populations indicates that selection favors heterozygotes.

A large number of studies indicate that homozygosity is associated with some

diseases. For example, HLA-A2 homozygosity is associated with Alzheimer disease, HLA class I homozygosity accelerates disease progression in HIV-1 infection, and there is an association between human leukocyte antigen homozygosity and antibody levels to measles vaccine. The aim of our study was to establish the frequency of HLA locus A and B homozygosity in the South-West Polish population. We analyzed HLA 871 Polish individuals recruited among family members. Family data are most informative for defining homozygosity. The frequency of HLA-A homozygosity was 14.3%. Among these, the most frequent was HLA A2 (57.7%), HLA A1 (17.9%) and A3 (6.6%).

The frequency of HLA-B homozygosity was 6.6%, the most frequent being HLA B7 (29.3%), B44 (19.0%), B8 (13.8%) and B35 (12.1%). The homozygosity factor F, obtained by summing up the squared frequencies of all alleles occurring in a population at a given locus, was for locus A 0.1489 and for locus B 0.0879.

Genetic predisposition to cancer development in familial cancer aggregations

Our studies concern the molecular basis of familial cancer aggregations of the complex phenotype known as Li-Fraumeni syndrome (LFS) and, due to a significant overlap, also hereditary breast/ovarian cancer syndrome (HBOC). The spectrum of cancers in these families comprises mostly breast cancers, bone and soft tissue sarcomas, brain tumors, and leukemias. In such families the high risk of cancer is associated with germline mutations of the p53 and BRCA1/2 genes. However, in several families, variants of p53 or BRCA1/2 are found whose contribution to cancer development is not known at present. Therefore, our recent studies concerned polymorphisms of the p53 gene which are potentially associated with a risk of cancer, namely those in codon 72 (Arg/Pro), intron 3 (duplication of 16 nt) and intron 6 (G13494A, loss of MspI site). Studies were performed in several groups of high-risk patients and controls using the PCR-RFLP or PCR-SSCP method. The polymorphisms in introns 3 and 6 co-segregated; therefore, studies were limited to the one in intron 6 G13494A. Frequencies of the alleles A1/2 and the genotypes A1, A1A2 and A2 in the studied groups and controls were not significantly different. Comparison of the combination of the codon 72 and int6/MspI genotypes showed a protective effect of the genotype 2-1 2-2 in pediatric patients (rr 0.64; CI 0.44-0.94). Thus, in the studied groups of cancer patients, no association between p53 polymorphisms at codon 72 and int6/MspI or their genotype combinations and high-risk of cancer was found.

Microchimerism in some autoimmune diseases

The most important source of natural microchimerism is pregnancy. Traffic of cells occurs during normal human pregnancy between the fetus and mother, with quantitatively greater transfer in the direction from fetus to mother. Bianchi et al. reported that fetal cells persist in the woman's circulation for years after pregnancy. Persistent microchimerism was found in the lymphoid progenitor cell (CD34⁺CD38⁺) population. Maloney et al. demonstrated the presence of maternal cells in up to half of normal adults. Observations arising from transplantation biology (cGvH) and fetal-maternal medicine led to the hypothesis that microchimerism are involved in autoimmune diseases. First, we examined a control group composed of healthy individuals. We separated a subpopulation from blood cells by magnetic cell sorting using MACS technology, which is highly specific and ideal for the selection of rare cells. The presence of microchimeris was assayed using:

- examination of DNA from blood cells of women with son(s) by the detection of Y-specific fetal sequences (PCR reaction with suitable primers),
- examination of DNA from cells of men and women by modified PCR-SSP typing.

Cellular microchimerism (HLA) was found in the CD34⁺CD38⁺ cell subpopulation of 32% and in the peripheral blood cells of 18% of all persons (men and women).

Analysis of Y chromosome sequences in the sorted cells from women with son(s) demonstrated the presence of microchimerism in 60% of cases.

We are now studying whether microchimerism might be involved in the pathogenic processes of psoriasis, multiple sclerosis, and rheumatoid arthritis.

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

Our studies in the year 2003 were focused on the immunochemical characterization of endotoxins isolated from opportunistic pathogens such as *Plesiomonas shigelloides*, *Escherichia coli*, *Hafnia alvei* and *Citrobacter*. All these bacteria cause typical nosocomial infections, sometimes resulting in severe complications as bacteremia and endotoxic shock. Endotoxin (lipopolysaccharide) is a major component of the outer cell membrane of Gram-

negative bacteria and is essential for its physical organization and function. Lipopolysaccharide (LPS) covers up to 75% of the bacterial surface. LPS is thus a very important virulence factor is the most exposed antigen in non-capsulated bacteria and the target of specific antibodies. Endotoxins are responsible for the initiation of endotoxic shock. LPS induces the synthesis of pro-inflammatory cytokines, free radicals, and nitrogen oxide by target cells. In its free form or on bacterial cells, it is a toxic, reactogenic, T-independent antigen, unsuitable as a vaccine component. On the contrary, the sugar component of LPS linked to the protein can be a non-toxic, highly immunogenic, T-dependent antigen.

LPS is composed of a lipid part called lipid A and a heteropolysaccharide, which generally consists of a core oligosaccharide (OS) and an O-specific polysaccharide present in most bacterial families. The core OS, in contrast to the O-specific polysaccharide, is evolutionarily well conserved, so antibodies produced against this region have been considered to exhibit *in vitro* cross-reactivity and *in vivo* cross-protection against the harmful endotoxin effect. We examined the immunogenicity of the covalent conjugates of incomplete (*E. coli* J5) and complete (*E. coli* R4) core oligosaccharides with tetanus toxoid (TT). We also obtained polyclonal rabbit antibodies against these glycoconjugates. It was found that antibodies produced against complete core structures reacted strongly with smooth LPS of identical or related structure in a free form in the presence of serum proteins. Those antibodies were also able to recognize LPS present on the surface of smooth, live bacterial cells. Antibodies against R4 core OS conjugated with TT inhibited tumor necrosis factor (TNF) α and NO-stimulating activity of smooth *E. coli* LPS (FEMS Immunol. Med. Microbiol., 37, 59-67, 2003). *E. coli* J5 is a rough mutant which produces LPS that lacks an O-specific polysaccharide and possesses an incomplete core OS of Rc chemotype. These antibodies were able to react with the smooth LPS of *E. coli* in a free form or present on live bacterial cells but, in contrast to anti-R4 antibodies, they did not inhibit the TNF α , interleukin 6, and nitric oxide-stimulating activity of the endotoxin (*Acta Biochim. Pol.*, 49, 721-734, 2002). Immunogens containing an incomplete core OS share the common primary structure with different LPSs, but they differ from smooth endotoxin in secondary and tertiary structures. This is probably the reason for the lack of endotoxin neutralization activity of anti-incomplete core antibodies.

We also established novel structures of bacterial O-specific polysaccharides isolated from the *H. alvei* strain PCM 1546 and different *Citrobacter* strains through the use of NMR spectroscopy and mass spectrometry in conjunction with chemical and immunochemical methods. An acidic, partially O-acetylated O-specific polysaccharide of the *H. alvei* strain PCM 1546 is built of pentasaccharide repeating units (*Carbohydr. Res.*, 338, 2153-2158,

2003). *Citrobacter gillenii* strain PCM 1540 produces a branched, neutral O-specific polysaccharide composed of O-acetylated hexasaccharide repeating units (*Carbohydr. Res.*, 338, 1389-1395, 2003). We found that O-specific polysaccharide structures of *Citrobacter* strains from serogroups O2, O20 and O25 are identical. It was shown that structural differences in the lipopolysaccharide core region of these strains might substantiate their classification in different serogroups (*FEMS Immunol. Med. Microbiol.*, 36, 71-76, 2003).

We performed structural and serological studies on a new 4-deoxy-D-arabino-hexose-containing O-specific polysaccharide isolated from the LPS of *Citrobacter braakii* PCM 1531. It was demonstrated that this polysaccharide is not related serologically to other known 4-deoxy-D-arabino-hexose-containing O-polysaccharides isolated from different *Citrobacter* strains (*Eur. J. Biochem.*, 270, 2732-2738, 2003).

(In collaboration with the N.D. Zielinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, the Russian Federation).

Laboratory of Glycoconjugate Immunochemistry

Head: Associate Professor Hubert Krotkiewski, Ph.D.

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

Duffy determinants are located in a 338 aa glycoprotein, spanning 7 times the membrane of erythrocytes and some other cells. Its N-terminal domain contains Fy^a/Fy^b and Fy6 epitopes which are recognized by specific monoclonal antibodies. We investigated two new anti-Fy6 monoclonal antibodies (MIMA-107 and MIMA-108), looking for their entire epitopes in the Duffy molecule. To perform this we used a *pepscan* method, which is based on the manual synthesis of defined peptides coupled to the plastic sticks. These sticks are used to react with a monoclonal antibody in a 96-well plate and then an ELISA test is done. A series of octapeptides was synthesized covering the aa sequence from Ser-13 to Gly-31 of the Duffy molecule. MIMA-107 antibody was shown to react with the amino acid sequence LDFEDV and MIMA-108 antibody with the sequence LDF. Searching for epitopes of the Duffy protein is important because its remarkable function is its reaction with chemokines and malarial parasites.

Carbohydrate moiety of human serum IgG in rheumatoid arthritis (RA) has a characteristic modification, i.e. it lacks to different extent Gal residues from the conservative N-glycans (Asn-297) in the C_H2 domain of the heavy chains. Three independent methods were

used to analyze this galactosylation: 1) total sugar analysis using GLC-MS after acid hydrolysis, 2) ELISA test using two biotinylated lectins: RCA-I (*Ricinus communis*, it recognizes terminal galactoses) and GSL-II (*Griffonia simplicifolia*, which recognizes terminal GlcNAc residues), and 3) biosensor BIAcore using the same lectins, but unmodified. Before the ELISA test, the IgG samples were reduced using dithiothreitol directly on the plate; before applying the biosensor, the IgG samples were reduced with the same reagent in a standard way. Among others, we analyzed IgG samples from patients treated with Metotrexate and antibodies against IL-6 and TNF- α . It was shown that this treatment improved the condition of the patients, which was accompanied by an increasing amount of galactose residues in the conservative N-glycans.

Carcinoembryonic antigen (CEA) is an oncofetal cell surface glycoprotein which acts in several biological phenomena, including cell adhesion. We intended to determine which fragment of the CEA molecule is responsible for the homotypic cell adhesion. The idea was to obtain peptidic fragments covering the sequences 1-80, 1-100, and 1-120. cDNA for these fragments was subjected to PCR reaction, then expressed in *E. coli* periplasma. The peptidic fragments produced were isolated in affinity chromatography on a gel with Ni-cations. A soluble fragment of CEA 1-120 could not be obtained, so but three other fragments were obtained: 1-105, 1-110, 1-115. There is a possibility that the CEA fragment 115-120 is responsible for its oligomerization.

Very rare NOR erythrocytes are agglutinated by all sera except by that of the donor. Previously we isolated and analyzed two glycolipids: NOR1 and NOR2. This year we isolated two more: NOR_{int} and NOR3. The former contained non-reducing sequence GalNAc β 1-3Gal α 1-4GalNAc, which fits exactly between the sugar structure of NOR1 and NOR2. NOR3 is under investigation now, and most probably contains the non-reducing sequence of Gal α 1-4Gal α 1-. The carbohydrate structure of three out of the four glycolipids isolated from NOR erythrocyte membrane are terminated with an α -Gal residue. Therefore, Gal α 1-4 transferase seems to be the crucial enzyme in the biosynthesis of these carbohydrate structures. The question arises if this enzyme is also involved in the biosynthesis of antigens from the blood group P system, where a very similar sequence, Gal α 1-4Gal β 1-, exists.

Head: Associate Professor Maria Janusz, Ph.D.
Studies on the mechanism of action of proline-rich polypeptide (PRP).
Effect on phagocytosis.

A proline-rich polypeptide complex (PRP) isolated from ovine colostrum shows immunoregulatory properties. It was found that PRP has not only immunotropic, but also procognitive properties. In the form of orally administered tablets called Colostrinin[®], containing 100 µg of PRP, it improves the outcome of Alzheimer's disease (AD) patients. Recently, there has been considerable interest in the involvement of the immune system in the pathogenesis of AD. A very important role, both positive and negative, is played by microglial cells, macrophages of the central nervous system. These cells can, by phagocytosis, remove deposits of Aβ or damaged neuronal cells. However, when an overproduction of the deposits occurs, a permanent activation of microglial cells by Aβ aggregates is observed. This subsequently perturbs phagocytosis, e.g. by inhibition of processing inside the cells or damage of neurons by accumulated peptides. Therefore, any substance which can influence phagocytosis may exhibit a beneficial effect in AD. In our studies human HL-60 and THP-1 cells in the form of monocytes or differentiated to macrophages with vitamin D₃ were used as a model of microglial cells. In the case of HL-60 cells, functional as well as phenotypic maturation was inhibited by PRP simultaneously added to the cell cultures with vit. D₃. Expression of CD11b and CD14 markers was inhibited in 28% and 40%, respectively. No statistically significant influence of PRP on the expression of CD11b and CD 14 on differentiated THP-1 cells was found. The effect of PRP on the function of both differentiated and undifferentiated cells was measured using phagocytic activity of cells treated and untreated with PRP as a marker. PRP alone did not induce *Saccharomyces cerevisiae* phagocytosis, but it inhibited (60 – 65%) the phagocytic activity of HL-60 cells induced with vit. D₃. THP-1 cells showed phagocytic activity even without treatment with vit. D₃. An increase in this activity was observed after differentiation with vit. D₃ (48.6±10.56 vs 71.6±10.19 % of phagocytic cells). PRP did not affect the phagocytic activity of THP-1 cells, both treated and untreated with vit. D₃. The results obtained suggest that PRP can inhibit the early steps of both the phenotypic and functional differentiation of cells, and attenuate intracellular accumulation of βA peptides and hyperactivation of microglial cells which induce neurodegenerative processes.

Laboratory of Glycobiology**Head: Professor Maciej Ugorski, Ph.D., D.V.M.****Study on the structure and functions of cell adhesion molecules**

It was shown that carcinoembryonic antigen (CEA) can mediate cell-cell adhesion through homotypic and heterotypic interactions; however, its role in the expression of the malignant phenotype remains obscure. To study whether the formation of both primary tumors and metastases is directly related to the presence or absence of CEA, we applied the antisense RNA strategy. By transfecting human CX-1.1 colon carcinoma cells with CEA antisense-expressing vector or with the vector itself, cell variants with a highly decreased expression of CEA were obtained. Profound differences in proliferative abilities among parental and obtained subclones of CX-1.1 cells were revealed when the cells were implanted subcutaneously into nude mice. In contrast to their highly tumorigenic parental CX-1.1 cells (with high expression of membrane-bound and secreted CEA), two subclones with substantially decreased expression of membrane-bound and secreted CEA showed a considerably diminished growth rate. Even more striking results were obtained with subclone AS8Q, representing cells producing a residual amount of this glycoprotein. However, another clone, with cells producing a large amount of secreted CEA, did not differ significantly in its tumorigenic properties from CX-1.1 cells. Our experiments performed in nu/nu mice suggest that CEA supports the growth of primary tumors, but is not involved in the formation of metastases by colon cancer cells.

DEPARTMENT OF EXPERIMENTAL THERAPY**Head: Professor Michał Zimecki, Ph.D.****Laboratory of Immunobiology****Head: Professor Michał Zimecki, Ph.D.****Studies on the mechanism of action of synthetic and natural immunomodulators of potential application in prevention and therapy**

Studies were continued on reconstitution of the immune system function by lactoferrin (LF) in mice given a sublethal dose of cyclophosphamide (CP). The humoral immune response in these mice was strongly reduced even after 5 weeks following CP injection. However, mice given LF in drinking water (0.5% solution) showed a 7-fold elevation of the humoral immune response, although constituting still only 40% of the control value. We also analyzed the peripheral blood picture in mice treated with CP and LF. The results

demonstrated that CP caused deep leukopenia and altered neutrophil/lymphocyte ratio. LF treatment led to a rapid upregulation on the leukocytes and normalization of the proportions in the major blood cell types. In the same model we showed that mice treated with CP produced more IL-6 upon LPS challenge both *in vivo* and *in vitro*. This phenomenon could be due to enrichment of a macrophage subpopulation producing high IL-6 levels. Additional treatment of CP-immunocompromised mice with LF did not significantly change the enhanced IL-6 production. CP-treated mice demonstrated also enhanced clearance of *Escherichia coli* and *Staphylococcus aureus* in the livers and spleens. Increased neutrophil numbers could account for that phenomenon.

The effects of psychical stress on the immune response are well known. We showed that LF given orally to mice counteracted the inhibitory effects of a 5-day immobilization stress on delayed type hypersensitivity and the stimulatory effect of a short stress exposure.

Other studies revealed that a substrain of C₃H mice (C3H/HeCr), highly susceptible to infection, produces several times more TNF- α upon challenge with LPS than CBA mice. LF, which normally protects mice against lethal infection or LPS administration, did not protect C₃H/HeCr mice against *E. coli* or LPS-induced lethality. Moreover, pretreatment of these mice with LF enhanced, not lowered, LPS-induced TNF- α serum levels. IL-6 release, on the other hand, was not significantly changed. We conclude that unbalanced cytokine production upon LPS challenge may be the cause of the high susceptibility of C₃H/HeCr mice to infection.

Other investigations regarded the adjuvant action of LF in delayed type hypersensitivity to OVA and BCG in mice. We demonstrated that LF given *per os*, together with antigen, significantly enhanced the immune response. Coadministration of mannose, but not galactose, blocked the adjuvant effect, indicating involvement of a mannose receptor in this process.

We also carried out studies on peptides potentially useful in inhibiting penetration of *Mycobacterium kansasii* into cells. Investigation of a series of GRGDV analogs containing gradually elongating oligoglycine linkers between Arg and Asp showed that the distance between them in an active compound should be about 9Å. It is known that the penetration of *M. kansasii* is conditioned by the formation of a complex between bacterial Ag85 antigen and fibronectin in serum. We demonstrated that phagocytosis of *Mycobacterium* may be inhibited by anti-adhesion peptides such as RGDV and GRDG, which block cellular receptors for fibronectin. Such an observation opens new perspectives in the therapy of tuberculosis.

Continuing studies on the phagocytosis of the *M. kansasii* 3 series of peptides, based on RGDVY and GRGD sequences, were investigated. We showed their inhibitory activities depended on amino acid composition and sequence. We also found that thymopentin – RKDVY pentapeptide exhibited high inhibitory activity.

By application of a new method of determining the osteoclast activity of cells derived from peripheral blood mononuclear cells (PBMCs), we demonstrated that integrin receptors are responsible for this activity. They appear on immature PBMCs during culture on a bone plate. The presence of such receptors was proven in a test of ligand (integrin) binding inhibition by interaction with specific anti-integrin antibody. Studies on heterotropic osteoinduction by implantation of HeLa cells into mouse thigh muscle revealed the involvement of morphogenetic proteins secreted by the implanted cells. In parallel studies on an analogous potential of guinea pig uroepithelium, mRNA was determined and the products of RT-PCR reactions for two BMP-3 and BMP-4 isoforms were sequenced. It is possible that thanks to a very high (more than 90%) interspecies homology, bone morphogenetic proteins from guinea pig epithelium induce osteogenesis in the mouse muscle, similarly as HeLa cells.

Laboratory of Immunopathology

Head: Professor Irena Frydecka, M.D.

The mechanisms of immune deficiency in neoplastic and autoimmunological diseases

Several mechanisms have been suggested to account for the poor immune response of T lymphocytes in B chronic lymphocytic leukemia (B-CLL). Recently, there has been increasing interest in costimulatory and inhibitory regulators of immune activation. We examined the kinetics and the magnitude of costimulatory CD28 and down-regulatory CTLA-4 molecule expression on unstimulated as well as anti-CD3MoAb+rIL-2 stimulated peripheral blood T lymphocytes in patients with B-CLL and controls. The study was performed in 33 patients with B-CLL and 24 age- and sex-matched healthy subjects. CD28 and CTLA-4 expression were analyzed by the triple fluorescence method and expressed as proportions of CD3+/CD4+ and CD3+/CD8+ cells co-expressing CD28 or CTLA-4 antigen. In B-CLL patients we observed a significant decrease in CD28 expression and a significant increase in CTLA-4 expression on both CD3+/CD4+ and CD3+/CD8+ unstimulated cells compared with the control group. Additionally, we found abnormal kinetics and CD28 and CTLA-4 expression levels on both the studied subpopulations of T-cells in B-CLL after *ex vivo* stimulation. In controls, the lowest proportions of CD3+/CD4+/CD28+ and CD3+/CD8+/CD28+ cells were found after 24 h, and returned to pre-stimulation levels after

48 h of stimulation. In contrast, in patients with B-CLL the levels of CD28 expression on both T-cell subpopulations decreased rapidly after stimulation, reaching their lowest levels between 24 h and 48 h, and returned to basal levels after 72 h of culture. In case of CTLA-4 expression, the highest percentages of CD3⁺/CD4⁺/CTLA-4⁺ and CD3⁺/CD8⁺/CTLA-4⁺ cells were observed in normals after 72 h and in B-CLL after 24 h and remained statistically higher after 48, 72, and 96 h of stimulation. CTLA-4 molecule expression returned to pre-stimulation levels after 96 h of culture in controls and after 120 h in B-CLL. Moreover, the levels of CTLA-4 expression, expressed as the percentage of CTLA-4⁺ T-cells, as well as the mean fluorescence intensity (MFI) were significantly higher tested at the same time points than in controls. Our study provided evidence of different kinetics, increased CTLA-4 and decreased CD28 expression on peripheral blood T lymphocytes in B-CLL which, in consequence, may result in a stronger, earlier, and longer-lasting down-regulation of T-cell activation and may be one of the mechanisms of immunological impairment in this disease.

Recent data have shown that both transplanted organ rejection and tolerance are the active processes in which costimulatory and inhibitory pathways are involved. We evaluated the levels of surface CD28 and CTLA-4 as well as intracellular CTLA-4 expression on unstimulated and anti-CD3 MoAb+rIL-2-stimulated peripheral blood CD4⁺ lymphocytes in kidney graft recipients using the double immunofluorescence method. The study was performed in 32 patients with deteriorating graft function due to acute or chronic rejection (group I) and 82 patients with good graft function during post-transplant clinical course (group II). The control group consisted of 24 age- and sex-matched healthy subjects. We found that the percentages of CD4⁺ cells co-expressing CTLA-4 molecule on the surface as well as intracellularly were significantly higher in both groups of patients than the control group. Additionally, in group I we observed a significantly higher proportion of unstimulated CD4⁺ lymphocytes expressing CTLA-4 on the surface than intracellularly, while in group II this difference was not seen. In the case of CD28 expression, there was no difference in the percentage of CD28⁺/CD4⁺ unstimulated cells among all studied groups. Moreover, we found that *ex vivo* CD4⁺ lymphocyte stimulation significantly decreased surface CTLA-4 and increased intracellular CTLA-4 expression, but did not change the percentage of CD28⁺/CD4⁺ cells in group I. In contrast, in group II a significant decrease in the proportion of CD28⁺/CD4⁺ cells without any changes in either surface or intracellular CTLA-4 expression was observed. The results provide the first evidence of a different pattern of CD28 and CTLA-4 expression on unstimulated and stimulated peripheral blood CD4⁺ lymphocytes in renal graft recipients with episodes of graft rejection compared with patients with

uneventful post-transplant course.

Laboratory of Tumor Immunology

Head: Associate Professor Adam Opolski, Ph.D.

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

Chemoimmunotherapy in mice carrying HPV16-associated tumors. Effects of chemotherapy associated with cytokines and genetically modified tumor vaccines.

The effectiveness of chemoimmunotherapy with the ifosfamide derivative CBM-4A and recombinant IL-2, IL-12, GM-CSF, or genetically modified cytokine-producing tumor vaccines was examined in mice carrying HPV16-associated MHC class I+ (TC-1) and MHC class I- (MK16) tumors. Intraperitoneal treatment of TC-1 or MK16 tumor-bearing mice with CBM-4A produced a significant tumor-inhibitory effect. When the i.p. treatment of the MHC class I+ TC-1 tumor-bearing mice with CBM-4A was followed by peritumoral s.c. administration of IL-2, IL-12, or both cytokines, the growth of TC1 tumors was inhibited more vigorously than after the chemotherapy alone. In contrast, when the i.p. treatment of the MHC class I- MK16 tumor-bearing mice with CBM-4A was followed by peritumoral s.c. administration of IL-2 or IL-12, the cytokine therapy had no potentiating effect. The only potentiating effect of the MK16 tumor immunotherapy was obtained when the i.p. CBM-4A pretreatment was followed by peritumoral s.c. administration of IL-2 plus IL-12. In further experiments, the TC-1 and MK16 tumor-bearing mice were i.p. pretreated with CBM-4A and then injected s.c. peritumorally with genetically modified, IL-2 or GM-CSF-producing MK16 tumor vaccines. Whereas both genetically modified tumor vaccines produced a substantial tumor-inhibitory effect in mice carrying TC-1 tumors, no effect of the vaccines was observed in mice carrying MK16 tumor inocula. The systemic effects of local cytokine treatment were examined in mice carrying s.c. MK16 neoplasms, which were pretreated i.p. with CBM-4A and then injected peritumorally with IL-2 or GM-CSF. Peritumoral administration of GM-CSF had no antimetastatic effect, whereas peritumoral IL-2 administration produced substantial reduction of lung metastases. The systemic antimetastatic effect of IL-2 contrasted with the negligible effect of IL-2 on the s.c. MK16 tumor inoculum. Taken collectively, the results indicate that in mice carrying the MK16 (MHC class I-) tumor, the effects of the adjuvant cytokine therapy were substantially weaker than in mice carrying the TC-1 (MHC class I+) tumor inoculum.

Laboratory of Biomedical Chemistry (this laboratory was established in July 2003)
Head: Associate Professor Janusz Boratyński, Ph.D.

Research interests:

Chemical modification and purification of biological macromolecules.

Chemistry and biology of high temperature protein modification.

Conjugation of various agents with macromolecules as an approach to selective antitumor therapy.

Chemical properties of bacteriophages.

Purification of bacteriophages

Physical and chemical properties of bacteriophages

Publikacje – 2003 r.

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