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Research Report – 2002

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

Structural and immunological studies of lipopolysaccharides isolated from opportunistic Gram-negative bacteria were continued. The core oligosaccharide (OS) as well as O-specific polysaccharide (PS) – carbohydarate parts of endotoxin are important for the biological and physical properties of the entire lipopolysaccharide and play a significant role in interactions with the host. Thus we performed structural and immunochemical studies of the core OS isolated from *Plesiomonas shigelloides* serotype O54 LPS and O-specific PS of *Citrobacter gillenii* PCM 1542 LPS using NMR, FAB and MALDI-TOF mass spectrometry, monosaccharide and methylation analysis, and immunochemical methods.

The main core OS of *P. shigelloides* O54 is composed of a decasaccharide not substituted by phosphate residues and represents a novel core type of bacterial LPS that is characteristic for *P. shigelloides* O54 and three other O-serotypes of this species (J. Biol. Chem., 277, 11653-11663, 2002). The O-antigen biological repeating unit structure and its linkage to the core OS was also established. (In collaboration with Department of Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden).

We also determined the structure of O-specific PS of *C. gillenii* PCM 1542 LPS. It is composed of neutral pentasaccharide repeating units containing glucose, three N-acetylated hexosamins and one N-acylated with 3-hydroxybutyric acid residue (Carbohydr. Res., 337, 1541-1546, 2002). (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

During last years we have investigated the presence of the ABH blood group antigens in human glycophorin. This year we investigated the correlation between the presence of blood group A (in GPA-A) and B (in GPA-B) antigens and secretor status, using two methods: surface plasmon resonance, set as a detection system in biosensor BIAcore and ELISA. In both methods, we used mouse MoAb A008 as a reagent to detect A antigens. For the detection of B antigens we used mouse MoAb B006 – in ELISA and *Bandeiraea simplicifolia*-IB_4 lectin – in the biosensor method. The obtained results indicated that both for GPA-A and GPA-B there were no differences in the content of relevant blood group antigens when comparing secretors and nonsecretors.

We developed a system expressing recombinant bivalent Fab fragments in *E. coli*. Three modified pComb3H vectors were constructed, each containing cDNA sequences encoding a peptide linked to the C-terminus of a heavy chain CH1 region: an IgG1 hinge region (called the „Hinge"), a leucine zipper (called the „Zip") or a peptide containing the hinge and zipper sequences in tandem (called the „HingeZip"). The vectors were used to express two cloned Fab fragments recognizing human MN blood group antigens: NNA7 (anti-N) and 425/2B (anti-M.). The recombinant proteins were purified and evaluated by ELISA and hemagglutination. The dimeric Fab fragments directly agglutinated RBCs in concentrations similar to those of bivalent IgG antibodies. This approach may be useful in obtaining inexpensive, serologic reagents that may replace or complement conventional MoAbs.

Carcinoembryonic antigen (CEA) is an oncofetal cell surface glycoprotein, and functions in several biological phenomena, including homotypic and heterotypic cell adhesion. Cell-cell interactions can be modulated by post-translational modifications, such as glycosylation. We examined whether changes in carbohydrate moiety of CEA can influence homotypic CEA-CEA interactions. We used two different glycosylation mutants of CHO cells: Lec2 (producing nonsialylated N-glycans) and Lec8 (producing more truncated glycans), together with parent CHO cells (Pro5). The cell lines were transfected with CEA cDNA. All three CEA glycoforms showed an ability to mediate CEA-dependent cell adhesion. This indicates that interactions between CEA molecules depend solely on the polypeptide structure.

We investigated the specificity of the natural anti-NOR antibodies using several (Galα1-4)-containing synthetic oligosaccharides and oligosaccharide-conjugates to polyacrylamide. We used ELISA and hemagglutination inhibition. It was shown that these antibodies recognize three monosugar-containing, non-reducing sequences: Galα1-4 GalNAcβ1-3Gal. The first and third galactoses are very important, and the middle GalNAc residue can be substituted by another Gal. Concomitantly, we elaborated a simple test to identify polyagglutination NOR in blood group A and O erythrocytes, using GSL-IB_4 lectin.

It is known from the literature that carbohydrate moiety of human serum IgG in rheumatoid arthritis patients is effected in the conservative N-glycan (Asn-297), i.e. it decreases galactosylation of both antennas. To detect these changes we used ELISA and two lectins: one recognizing terminal Gal (RCA-I lectin) and the other recognizing terminal GlcNAc (GSL-II lectin). The degree of N-glycan degalactosylation was determined by the increasing proportion of the GSL/RCA reaction. Concomitantly, we performed sugar analysis of the IgG samples and analysis in biosensor using PVL lectin. All results indicated a correlation between disease progression and degalactosylation degree of IgG N-glycans.

Laboratory of General Immunochemistry
Head: Associate Professor Maria Janusz, Ph.D.
Studies on the effect of PRP and NP on nitric oxide (NO) and superoxide anion (O_2^-) production induced in THP-1 cells

A proline-rich polypeptide complex (PRP) isolated from ovine colostrum shows immunoregulatory activity. Similar activity was observed when PRP was replaced by a nonapeptide (NP) isolated from the chymotryptic digest of PRP. The polypeptide complex also shows procognitive activity. In the form of orally administered tablets, called Colostrinin®, containing 100 μg of PRP, it may improve the outcome of Alzheimer's disease (AD) patients. The mechanism of action of PRP/Colostrinin® in AD is not yet clarified. Microglial cells involvement in AD has been related to amyloid β (Aβ) internalization, the release of inflammatory cytokines, overproduction of nitrogen oxide (NO) and superoxide anion (O_2^-), and the development of neuritic plaques. It was previously found in our laboratory that PRP regulates the secretion of an array of cytokines. It was also shown, in preliminary experiments using human blood cells and murine macrophages, that PRP inhibits the production of NO and O_2^- induced by LPS. Now, the effect of PRP and NP on the release of nitrogen oxide and superoxide anion induced by LPS was investigated on THP-1 cells. The human monocyte/macrophage THP-1 cell line has been widely used as a model of human

microglial cells. The results obtained showed that THP-1 cells release NO when activated with LPS. However, neither PRP nor NP induced production of NO. Although the nonapeptide, at higher concentration (100 μg/ml), showed an inhibitory activity on the release of NO induced by LPS, no inhibition was observed when PRP was used. THP-1 cells treated with LPS, PRP or NP did not release O_2^-.

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

In 2002 we continued our work on the isolation and characterization of mucin ligands for selectin E from human colon cancer cells. These studies were performed with the use of gel filtration and affinity chromatography of radioactively labeled cell lysates. In collaboration with the Department of Biochemistry, Pharmacology and Toxicology Faculty of Veterinary Medicine, Wrocław Agriculture University, we cloned and sequenced the bacterial adhesin FimH from *Salmonella enteritidis.* The protein was expressed in *E. coli* and purified by affinity chromatography on Ni-agarose column. Recombinant FimH binds to the surface of different human cell lines, as was shown by c-ELISA as well as glycoproteins with high-mannose N-glycans, based on the results obtained by ELISA and Western-bloting analysis.

Our work on new carriers for gene transfer, (Grant PBZ-KBN 04/P04/98), were continued in the following three major areas: (i) the effect of surface charge on the association of liposomes with human colon cancer cells and the construction of immunoliposome, (ii) cloning of a α1,3/4-fucosyltransferase promotor, and (iii) analysis of CEA promoter cell-specificity.

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 on the role of lactoferrin (LF) in the immune response and in some patholological states were continued. It was shown, applying BSA-mannose and BSA-galactose complexes, that a receptor bearing mannose specifity plays a crucial role in the

induction of the adjuvant action of LF in the generation of delayed type hypersensitivity in mice.

Two T cell lines, specific for bovine and human lactoferrin, were generated (BLFK1 and HLFK1) in CBA mice. These cell lines exhibited different characteristics. HLFK1 was specific only for human LF, while BLFK1 proliferated in the presence of both human and bovine LF. It was suggested that HLFK1 is of TH2 type and BLFK1 bears the TH1/TH0 phenotype. Bovine LF inhibited, in addition, antigen-specific proliferation of HLFK1 cells.

Studies were also conducted on the reconstitution, by LF, of the impaired function of the immune system following administration of a sublethal dose of cyclophosphamide (CY) to mice. It was shown that LF, given *per os* for the period of 2-4 weeks restored the levels of $CD3^+$, $CD4^+$, $CD8^+$ and partially Ig^+ cells. The results were obtained by applying both the panning technique and fluorocytometry. The treatment of mice with LF also resulted in restoration of the levels of alveolar and peritoneal macrophages. LF partly reversed, in addition, a diminished proliferative response of splenocytes to concanavalin A after CY administration.

LF given orally to rats, with induced obstructive jaundice, regulated the production of TNF-α and IL-6 in splenocyte cultures derived from the animals on days 7 and 14 after bile duct ligation. These effects also correlated with decreased pathological changes in the livers.

In the human model (patients with cervical cancer), incubation of peripheral mononuclear blood cells from these patients with human LF significantly elevated the percentage of T cells expressing zeta chain of the CD3 complex. The effect of LF was stronger than that of anti CD3 antibodies.

In studies on the effects on synthetic peptides on the immune response, two cyclic and two linear analogs of cyclic linopeptide were synthesized, where two-peptide segments: Pro^6-Pro^7 and Val^5-Pro^6, were substituted by their tetrazolic analogs. It was shown that the immunosuppressive activity of the cyclic analogs was the same as for cyclosporine A and native CLA.

Protein fragments of the interleukin 1 receptor inhibitor (IL-1Ra) were studied taking into account their potential abilities to inhibit IL-1 – IL-1Ra interactions. Analogous fragments (in sequence and activity) were found in the C10L of the smallpox virus. On that basis a hypothesis was formulated on the existence of a yet unknown molecular interaction of that virus with the human immune system.

The phenomenon of osteoinduction was studied in the model of induction of heterotropic bone (ossicle). Ossicle developed in a mouse thigh muscle following injection of

Hela cells. By application of immunohistochemical and Western-blot methods, it was shown that in the process of ossicle formation the morphogenic proteins (BMP) are involved, secreted by Hela cells. Various Hela sublines exhibited different osteoinductive potentials. That heterogeneity was revealed in the histologic preparations and confirmed by determination of the mineral ossicle mass and semiquantitive RT-PCR assay for morphogenic proteins.

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

Monitoring of disease activity and effectiveness of treatment plays an important role in the management of patients with non-Hodgkin's lymphomas (n-HL). In the search for markers useful for the biochemical monitoring of cancer patients, a group of proteins called adhesion molecules have attracted special attention. sICAM-1 and selectin E levels were determined in the sera of n-HL patients in different phases of the disease and compared with those of normal subjects. sICAM-1 levels were similar in healthy controls and in patients in remission and significantly lower in patients in active disease and relapses. Serum soluble selectin E levels, however, demonstrated no differences among patients groups nor from those of controls. The presented results indicate the utility of serum sICAM-1 level determination in the assessment of activity of intermediate and high malignancy grade non-Hodgkin's lymphomas.

Additionally, we performed a comparative analysis of CA125, tissue polypeptide specific antigen (TPS) and sIL-2 alpha levels in sera, cyst and ascitic fluids of patients with ovarian carcinoma. CA125, TPS and sIL-2 alpha levels were significantly elevated in the sera of patients with ovarian cancer compared with benign neoplasms. Patients in FIGO (International Federation of Gynecology and Obstetrics) stages III/IV had significantly higher serum levels of the studied markers than in the early stages FIGO I/II. Concurrent measurement of serum CA125 and sIL-2 alpha identified 100% early stages ovarian cancer. All carcinoma patients demonstrated markedly higher levels of CA125 and TPS in both cyst and ascitic fluids compared with the corresponding sera. The level of sIL-2 alpha was statistically higher in the ascitic fluid than in sera; however, its values in sera and cyst fluid were comparable. In the ascitic fluid, the CA125 level was significantly higher in patients in FIGO III/IV than in the I/II stages, while such correlations were not found for TPS and sIL-2 alpha. It was found that the assessment of the serum sIL-2 alpha level has a potential

significance complementary to that of CA125 for the detection of ovarian cancer in the early FIGO stages.

The measurement of serum levels of soluble activation and differentiation antigens released by activated cells might be useful for the indirect assessment of the activation of certain mechanisms responsible for the development of demyelinization foci in multiple sclerosis (MS). We studied serum s-TNFR-1 and sFas levels in patients with relapsing-remitting MS during disease relapse and remission and the correlation among the serum levels of these molecules, disease duration and number of MS relapses and EDSS scale. We found that the measurement of sFas levels in serum seems to be of significant clinical value as it enables one to monitor the course of the disease and the results of treatment. The same cannot be said of sTNFR-1 measurements as serum levels of this molecule did not differ significantly from those of the control group.

Laboratory of Immunopharmacology
Head: Associate Professor Stanisław Szymaniec, M.D.
New synthetic and natural compounds of potential antiinflammatory and immunotropic activity

In previous studies of the isothiazole derivatives synthesized by Prof. Zdzisław Machoń (Department of Organic Chemistry Medical University of Wrocław) we have shown that they have antiinflammatory activity in vivo. In the present studies, the six most potent *in vivo* isothiazole derivatives were investigated in tests *in vitro* for their mechanism of action and biological activity. Their influence on erythrocyte membrane stabilization, chemiluminescence of PMA stimulated leukocytes, production of nitric oxide by peritoneal macrophages stimulated by LPS, and pro-inflammatory cytokines (TNF-α and IL-6) production by LPS stimulated peripheral blood mononuclear cells (PBMC) were measured.

It was found that the diamides MS-13/97 and MR-17/95W (derivatives of 5-(benzoyl-amino)-3-methyl-isothiazole-4-carboxylic acid) were active and that MR-17/95W had the highest activity of all the compounds tested. The amides M-2/99 and M-3/93 (derivatives of 3-methyl-4-isothiazole carboxylic acid) were less active than the diamides. The amidines M-II and M-5/96 (derivatives of N-phenyl-N'-(4-carboxy-3-methyl-isothiazol-5-yl)-benzamidine) inhibited only the chemiluminescence of PMA stimulated leukocytes.

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 the designing of compounds inhibiting these processes

Molecular biology of *Streptomyces coelicolor* A3(2)

Streptomyces coelicolor A3(2), a representative of a genus of Gram-positive mycelial soil bacteria, known for their ability to differentiate and to produce many valuable antibiotics and other secondary metabolites, possesses a large (8.67 Mb) linear and GC-rich (~ 72 %) chromosome with a centrally located *oriC* region.

The molecular basis of replication and segregation of *S. coelicolor* chromosome

Recently, chromosome partitioning genes (*parA* and *parB*) and putative ParB binding sites (*parS* sequences) were identified in its genome. The *S. coelicolor* chromosome contains more *parS* sequences than any other bacterial chromosome characterized so far. Twenty of the 24 *parS* sequences are densely packed within a relatively short distance (approximately 200 kb) around *oriC*. A series of in vitro and in vivo experiments showed that *S. coelicolor* ParB protein interacts specifically with the *parS* sequences, albeit with a rather low affinity. Our results suggested that the binding of ParB is not only determined by the *parS* sequence, but also by the location of the target DNA close to *oriC*. The unusually high number and close proximity to each other of the *parS* sites, together with *in vivo* and in *vitro* evidence that multiple ParB molecules may assemble along the DNA from an initial ParB-*parS* complex, suggest that a large DNA segment around the replication origin may form a massive nucleoprotein complex as part of the replication-partitioning cycle.

Characterization of a gene cluster for the new polyketide synthases type I in *S. coelicolor*

Type I polyketide synthases (PKSs) are complexes of large, multimodular enzymes that catalyze the biosynthesis of polyketide compounds via repetitive reaction sequences, during which each step is catalyzed by a separate enzymic domain. Many type I PKSs, and also non-ribosomal peptide synthetase clusters, contain additional thioesterase genes located adjacent to PKS genes. These are discrete proteins called type II thioesterases (TE IIs) to distinguish them from the chain-terminating thioesterase (TE I) domains that are usually fused to the terminal PKS module. A gene of a new TE II, *scoT*, associated with the cluster of putative type I PKS genes from *S. coelicolor* A3(2), was found. The deduced amino acid

sequence of the gene product shows extensive similarity to other authentic thioesterase enzymes, including the conservation of characteristic motifs and residues involved in catalysis. When expressed in the heterologous host *S. fradiae*, *scoT* successfully complemented the resident TE II gene (*tylO*), and, by restoring a significant level of macrolide production, proved to be catalytically equivalent to the TylO protein. S1 nuclease mapping of *scoT* revealed a single potential transcription starting point, with expression being switched on for a short period of time during a transitional phase of growth.

Laboratory of Signaling Proteins
Head: Associate Professor Wojciech Gorczyca, Ph.D.
Function and physicochemical properties of proteins involved in Ca^{2+} -dependent signal transmission by cAMP and cGMP in cells of the immune system

Cyclic 3',5' guanosine monophosphate (cGMP) is an important second messenger in almost all types of eukaryotic cells. Its intracellular level depends on the activity of opposite enzymes: guanylyl cyclases (GCs) and phosphodiesterases (PDEs). Numerous reports indicate that cGMP is involved in the regulation of immune processes. However, the mechanisms responsible for the nucleotide synthesis and its signalling pathways in immune cells are still not well recognized. Therefore, the aim of our studies was to elucidate which factors are responsible for cGMP metabolism in cells of mouse and rat immune systems.

Cells isolated from murine thymus, lymph nodes, and spleen were treated with GC activators. Since two distinct forms of GCs, particulate (pGC) and soluble (sGC), are recognized in vertebrates, we used known activators of both forms: peptides (ANP, BNP, and STa), which activate different isoforms of pGCs, and donors of NO (SNP or SNAP), which activate soluble GCs. SNP elevated intracellular cGMP twofold in thymocytes and lymph node cells and about tenfold in spleen cells. ANP caused a modest but statistically significant increase of cGMP in cells of all the organs. Additionally, spleen cells elevated their cGMP content about twofold in response to CNP. To see whether the analyzed organs express isoforms of protein kinase G (PKG1), known as a possible target for synthesized cGMP, cellular homogenates of these organs were subjected to Western-blot analysis. In all the homogenates the antibodies specific to PKG1 recognized a band corresponding to the molecular mass of the PKG1. The results obtained indicate that in all the investigated organs cGMP may be synthesized mainly by soluble guanylyl cyclases in response to nitric oxide. The modest increase of cGMP, upon stimulation by ANP, suggests that in all these organs either there exists only a small subpopulation of cells that express particulate cyclase GC-A

or GC-A is expressed at very low levels. In spleen cells, however, cyclase GC-B appears to be the more active enzyme. Elevated cGMP concentration may, in turn activate PKG1.

It was also found that both forms of GCs contribute differently to cGMP synthesis in rat cells isolated from peripheral blood and cells provoked to the peritoneal cavity. Moreover, it was determined that the metabolism of cGMP in rat cells is modulated by other intracellular signaling pathways mediated by Ca^{2+} and cAMP and that at least three isoforms of PDEs are possibly involved in cGMP hydrolysis in rat macrophages.

DEPARTMENT OF MEDICAL IMMUNOLOGY
Head: Professor Andrzej Górski, M.D.

Laboratory of Bacteriophages
Head: Professor Andrzej Górski, M.D.
The immunobiology of bacteriophages and their application in the therapy of bacterial infections

Effect of phage therapy on the turnover and function of peripheral neutrophils

The levels of circulated neutrophils and their precursors were determined in 37 patients with suppurative bacterial infections as well as the ability of neutrophils to phagocytize *Staphylococcus aureus in vitro*. The results showed significant changes in neutrophil precursor count and the ability of neutrophils to phagocytize bacteria. The results showed that successful phage therapy accelerates the turnover of neutrophils accompanied by a decrease in their ability to phagocytize bacteria.

The sensitivity of the uropathogenic *Escherichia coli* strains to antibiotics, bacteriophages and bactericidal serum activity

Escherichia coli strains isolated from children with urinary tract infections (UTI) were investigated for their sensitivity to antimicrobial drugs, bacteriophages and the bactericidal activity of human serum. It has been proved that the resistance to the bactericidal effect of serum is not the dominant feature of uropathogenic *Escherichia coli* strains. A significant percentage of the strains appeared to be sensitive to the most popular drugs ordered during UTI treatment in children. No simple relationship between the sensitivity of the strains to the drug and to human serum was found. Three of 44 bacteriophages specific to *Escherichia coli* showed the lytic effect towards 50-60% of the strains under investigations.

Mutations in the bacteriophage T4 genome

Bacteriophage T4 is one of the best-known phages and its genome is completely sequenced. As a model of living systems, bacteriophage T4 has many technical advantages. It can be very easily grown in the large quantities, manipulated by classical genetics, and reengineered by directed mutagenesis. Many substances were first tested for mutagenicity in T-even phages. The results are very often applicable to experiments carried out in higher organisms, because the mechanisms of mutagenesis induced by a specific mutagen are similar. T4 is also important as an appliqué bacteriophage in phage therapy, which has presented an alternative treatment since bacterial resistance to antibiotics became a serious medical problem. Directed mutagenesis is a method that enables one to introduce mutations which can influence a bacteriophage's affinity to bacteria and it can be a practical technique for enriching phages collection and widening their specificity to new bacterial strains now insensitive to phage therapy.

Nitric oxide and its role in immunological response and immunity

Some evidence was introduced about the role of nitric oxide (NO) in different diseases, especially syphilis. It was found that nitric oxide takes part, in a similar manner to that of cytokines of Th1 lymphocytes, immunity to syphilis. Its special role is in Lues latens tarda, when Th1 lymphocytes weakly or incompletely secrete cytokines. A high level of NO at this stage of disease indicates that cells other than Th1 lymphocytes have taken over immune functions. The distinct ability of cells to secrete NO in Lues latens tarda may also be an indicator that, after a long time of latency, treponemes bacteria are starting to multiply against and that the tertiary stages of syphilis is beginning.

Laboratory of Cellular Interactions
Head: Associate Professor Danuta Duś, Ph.D.
Phenotypic characteristics of cells which determine the organ specificity of metastatic secondary growth

During blood-borne metastatis, extravasation of tumor cells is a prerequisite for distant tissue colonization and further metastasis development. A necessary steps in the extravasation process is the adhesive interactions of endothelial cell adhesion molecules with their ligands, presented by partner cell surface molecules. The overall aim of the study is to search for new molecular markers engaged in the process of the mutual interactions of tumor cells with endothelial cells at the site of tumor cell extravasation. The studies in 2002 concerned the

phenotypic characteristics of partner cells which determine the organ specificity of metastatic secondary growth.

In our previous study we demonstrated that the acquisition of a tissue specific, highly metastatic phenotype by several selected variants of LS-180 human colon carcinoma cells, was accompanied by increased expressions of glycosylated tumor antigens, which correlated with their differential adhesion patterns to endothelial cells. These highly metastatic variants of colon carcinoma cells presented also higher invasiveness and cell motility. In collaboration with prof. M. Malicka-Błaszkiewicz of the Department of Biochemistry, University of Wrocław we studied the relationship between the invasivenes and metastatic abilities of colon carcinoma cells and their cytoskeleton features. We observed that the invasive cell variants presented higher levels of actin G and actin polymerization than parental cells (Nowak et al., Acta Biochim Polonica, 2002).

The vascular endothelium is a site where in response to specific signals, adhesion molecules are induced to recruit leukocytes and metastasize tumor cells. The subject of this study was panel of eight human endothelial cell lines from distinct tissue origin which we immortalized and characterized (Patent No 99 16169, 21/12/99, CNRS, France). Their organ specificity was confirmed by their differential display of endogenous lectins and cytokine receptors, as well as tissue specific addressins and other adhesion molecules expressions (Kieda et al., Endothelium, 2002). We revealed the presence of the IL-7 functional receptor on human endothelial cells (Duś et al., Immunology Letters, in press). However, the endothelial cells do not produce endogenous IL-7 cytokine. To evaluate cell adhesion efficiency and find evidence of its specificity, an original cytofluorimetric method was developed (Paprocka et al., manuscript submitted). We also revealed that the endothelium responds selectively to inflammation stress and that this is reflected by its adhesion capacity to recruit lymphocyte populations according to endothelial cell tissue origin. The study is being performed in collaboration with Dr. C. Kieda of CBM CNRS, Orleans, France.

The studies performed in cooperation with Dr. A. Lewandowicz-Uszyńska from the Department of Pediatric Propedeutics, Medical University of Wrocław, revealed the role of the tissue specific adhesion pattern of lymphocytes from children with asthma compared with the recurrent inflammations of the respiratory tracts and those of healthy volunteers (Uszynska et al., manuscript submitted).

The studies sponsored by the Committee for Scientific Research grant No PBZ-KBN-004/po4/1998/5/f entitled “New techniques of molecular biology with fundamental significance for gene transfer therapy” – “Genetically modified dendritic, hematopoietic and

tumor cells for combined chemo- and immunotherapy of cancer", were continued, according to plan. The mouse dendritic cell line JAWS II, transduced with retroviral vectors carrying IL-2 and IL-12 genes was applied as a vaccine in the therapy of mouse colon carcinoma MC38. The results indicate a slowing of tumor growth in vaccine treated mice compared with untreated controls. The study is in progress.

New progression markers in laryngeal cancer

The treatment strategy of laryngeal cancer depends mainly on the stage of the disease. The variable outcome in patients with regard to observed clinical parameters, creates a need for searching for new, reliable prognostic indicators of nodal status, tumor recurrence, and survival.

The aim of the study was to determine the prognostic value of the nm23 potentially antimetastatic gene and p53 gene expression in laryngeal cancer. Nm23 and p53 proteins in tumor specimens from 47 patients with carcinoma of the larynx, were evaluated immunochistochemically. In a multivariate analysis, only N status and p53 score ($>70^{th}$ percentile) correlated positively with survival of the patients. However, in a univariate analysis, a relationship was found between nm23 expression and the neck nodal metastasis. The nm23-positive group had a lower incidence of metastasis ($p=0.045$). The results suggest, that nm23 gene expression may be of some value in predicting nodal metastases in laryngeal cancer. (Kręcicki et al., Onkol. Pol., 2002, 5, 69)

Angiogenesis is another of the critical determinants of tumor progression and metastasis. The microvessel count within a primary tumor has been reported to predict the clinical outcome of patients with a variety of cancers. The aim of the work was the evaluation of angiogenesis in laryngeal cancer and determination of the relationship between angiogenesis and the clinical as well as histological features of the tumor. Tumor samples from 43 patients with G1-G3 carcinoma of the larynx were examined. Two different methods of estimation of the number of microvessels in the series of laryngeal cancers were applied: the classical metod of counting the group of endothelial cells (MVD - mean vascular density) and digital image measurement of the vessel density (VD – vessel density), using original computer-aided digital image analysis system which we developed. Thanks to the method of segmentation we elaborated, we received a high repeatability of measurements independent of the degree of precision in defining the region of interest (ROI). The prognostic factors analyzed for their influence on survival were age, sex, site and size of tumor, lymph node metastases, histological grading, mean vascular density (MVD), and vessel density (VD).

Statistical analysis revealed that both MVD and VD correlated significantly with histological grading of the tumor. The correlation between VD and nodal metastases was on the statistical borderline (p=0.06). This study will be re-evaluated in a larger number of samples (Kręcicki et al., Auris Nasus Larynx 2002, 29, 271).

FasL as an early marker of progression in breast carcinoma

The functional expression of Fas (receptor CD95) and its ligand (FasL) on tumor cells reported in several neoplasms, has been hypothesized as an mechanism of tumor escape from immunological surveillance. The Fas/FasL interaction has been considered a central T cell homeostatic pathway. However, their role in natural killer and T cell cytotoxicity towards tumor cells is not yet fully understood. It has been proposed that tumor cells express FasL, presumably to avoid immune detection. There are a few reports on the role of Fas/FasL in breast carcinoma progression. The prospective study we have undertaken in collaboration with Dr. M. Bębenek, of the Lower Silesian Oncology Center in Wrocław aims to evaluate the level of Fas/FasL expression in tumor tissue of breast carcinoma patients. Tumor samples from 130 women with stage II breast carcinoma and a control group of 20 women with benign tumors, were examined immunohistochemically. The results are currently being analyzed for their correlation with lymph node metastases, recurrence and survival (manuscript in preparation).

Laboratory of Reproductive Immunology
Acting head: Assistant Professor Małgorzata Jerzak, M.D.
Immunological aspects of reproduction failures

Our data emphasized the important role of ECM proteins in the regulation of T cell function during pregnancy. Excessive T cell adhesion to ECM proteins in pregnant women with histories of recurrent spontaneous abortions (RSA) with failed subsequent pregnancy outcome was previously demonstrated. Increased T cell adhesion to ECM characterized infertile women with endometriosis. Apoptotic T cell in human decidua were also demonstrated. The highest values of T cells apoptosis were observed after peripheral T cells were cultured with fibronectin or collagen IV in samples of women with histories of RSA. In contrast, T cells isolated from the peritoneal fluid of women with endometriosis were resistant to apoptosis.

The external lamina consists of collagen-IV, fibronectin and laminin and acts as a barrier between the trophoblast and maternal cells during pregnancy. These proteins may protect the trophoblast against attack of the maternal immunological cells. The aim of our

study was to investigate the influence of two main ECM components, collagen IV (C-IV) and fibronectin (Fn), on T cells apoptosis and proliferation in women with endometriosis or uterine leiomyoma. β_1 integrin expression responsible for interactions with ECM proteins, was also studied. We also determined the level of the T cells activation marker CD69 after stimulation with anti-CD3 and costimulation with collagen IV or fibronectin. A significant increase in the S phase of the cell cycle of T cells exposed to OKT3 and collagen IV in women with both uterine leiomyoma and endometriosis compared with healthy donors was shown. An increased proliferation of T cells exposed to anti - CD3 mAb and C - IV in women with uterine leiomyoma compared with patients with endometriosis and healthy donors was observed in MTT assay. Statistically significant higher β_1 integrin expression on peripheral blood T cells in women with endometriosis or uterine leiomyoma compared with healthy donors was shown. We noticed a statistically significant decrease in the percentage of double positive $CD3^+CD69^+$ cells after exposure to OKT3 or OKT3 and fibronectin in infertile women with endometriosis compared with fertile women.

Cellular effects of novel indoloquinoline derivatives.

Previous molecular and computational studies indicated that novel indolo[2,3-b]quinoline derivatives are DNA intercalators and inhibitors of topoisomerase II. We have extended our studies on their modes-of-action to the cellular level. For the present studies we selected leukemic cell lines. Jurkat acute T cell, CCRF-CEM T lymphoblastoid, THP-1 acute monocytic, and HL-60 acute promyelocytic leukemias and well as the HL-60/MX2 subline with reduced expression of topoisomerase II were used. We evaluated the cytotoxicity and cell cycle effects of the indolo[2,3-b]quinoline compounds. We also tested if these compounds are able to induce apoptosis in the cells. Our studies revealed that novel indolo[2,3-b]quinoline derivatives are more cytotoxic to all cell lines than etoposide (used as a reference topoisomerase II inhibitor), and that their cytotoxicity depends on the substituents introduced to the indolo[2,3-b]quinoline core. Surprisingly, our studies have shown that the HL-60/MX2 cell line as well as the THP-1 cell line, resistant to etoposide, are susceptible to methyl- and methoxy- substituted indolo[2,3-b]quinoline derivatives. Treatment of HL-60 cells with etoposide results in a massive accumulation of the cells in the G2/M phase of the cell cycle leading to apoptosis. In the case of the novel indolo[2,3-b]quinoline derivatives, the cell cycle progression of HL-60 cells is different. Moreover, the cell cycle of HL-60/MX2 cells is not influenced by all the compounds studied. In contrast to etoposide, indolo[2,3-b]quinoline derivatives do not induce rapid apoptosis in HL-60 cells.

Laboratory of Virology
Head: Prof. dr hab. Zofia Błach-Olszewska
Study on nonspecific immunity in viral infection

The natural antiviral immunity in human whole blood cultures, isolated leukocytes and lymphocytes was studied. The test for detection of natural, innate antiviral immunity was elaborated. It is based on the infection of peripheral blood leukocytes with vesicular stomatitis virus (VSV) and an analysis of the kinetics of virus replication. It was found that in the leukocytes of healthy blood donors VSV does not replicate, while in the leukocytes of persons with recurrent infections of the upper respiratory tract, VSV may replicate to high titers. The differences were statistically significant. The results of the experiments showed that the immunity was non specific and was also dependent on endogenous IFNs and TNFα. During *in vitro* culture of the leukocytes, they lost the immunity before VSV infecion. Innate immunity as well as the kinetics of reduction of the immunity *in vitro,* was individually differentiated.

Bronchoalveolar lavage (BAL) and induced sputum (IS) from patients with bronchial asthma were compared as sources of pulmonary leukocytes. Depending on the asthma severity either BAL, containing mostly macrophages, or IS with a predominant neutrophil population, may be chosen for studies of the airway inflammatory response. The interrelationship between cell composition and the ability of the cells to release nitric oxide (NO), in BAL and in IS, indicates that NO may be produced by neutrophils, apart from macrophages.

On the basis of the specific assessment of which inflammatory cytokines may be involved in defining of the clinical status of patients, the predominant, unfavorable role of IFN-α is suggested in the cases of bronchial asthma complicated by coexisting fungal and myxovirus infections within the respiratory tract.

Laboratory of Tissue Immunology
Acting head: Assistant Professor Beata Nowakowska, Ph.D.
HLA profiles in the South-West Polish population

HLA class I typing: to use serology DNA, or both?

For more than 30 years serological tissue typing has been the primary technique used for HLA class I analysis. This is a fast, relatively inexpensive technique. During the last years, DNA-based methods have successively replaced the serological technique. Polymerase

chain reaction (PCR)-based methods do not depend on viable cells, antisera availability, or adequate cell-surface antigen expressions, but they are more expensive and most null alleles, which do not express a protein product, are not distinguished from express alleles in low to medium resolution typing. The question is when serology or/ and molecular techniques should be used.

The aim of our study was to compare HLA class I typing in groups: hematological disorder patients, kidney waiting-list patients, and healthy persons.

We found that the samples from healthy individuals had a lower frequency of problems in serology typing (5% samples) than those from patients group (31% of samples from hematological patients and 12% from dialysis patients).

In these cases, HLA class I typing by the microcytotoxity test was unsatisfactory for 53% samples for HLA –A and 63% for HLA-B antigens.

In the group of hematological disorder patients no serological conclusion was drawn in 60% of the sample due to the high mortality of the cell suspension. We suggest that these patients should be typed only using DNA-based techniques. In others we may use serology first and, in problem cases, DNA typing.

We observed a discrepancy rate of 18% between the results of molecular and serological typing in the group of healthy persons, in which homozygosity, in locus A or B, were typed by serology.

Genetic predispositions to cancer development in familial cancer aggregations

Among the numerous cancer genes, tumor suppressor genes are of particular importance as their germline mutations confer a high risk of cancer development. Our studies concern the molecular basis of familial cancer aggregations of the complex phenotype known as Li-Fraumeni syndrome (LFS) or Li-Fraumeni-like syndrome (LFL) and, due to a significant overlap, also hereditary breast/ ovarian cancer syndrome. The spectrum of cancers in these families comprises mostly breast cancers, bone and soft tissue sarcomas, brain tumors and leukemias. Mutational analysis of the genes associated with these syndromes, i.e. p53 and BRCA1, was performed by the PCR-SSCP method and direct sequencing. In a series of 100 cancer families from Lower Silesia, germline p53 mutations were found in three LFS/LFL families. These new germline mutations of p53 were found in exon 10 and intron 9. The p53 intronic variant 13964G>C, reported as a germline mutation, was found in cancer patients and in control individuals at the frequencies of 6.9% and 8.3%, respectively. Thus, the p53 13964G>C variant seems to be a neutral polymorphism, frequency in the Polish

population is higher than that reported for other populations (8% vs. 0-4%). In the studied series of cancer families, two recurrent BRCA1 mutations were found: 5382insC and 4153delA. The mutation 5382insC appears to be the most frequent BRCA1 alteration in Lower Silesia and also in Poland.

The study of microchimerism in some autoimmune diseases

The most important source of natural microchimerism is pregnancy. The bilateral trafficking of nucleated cells between the fetus and mother was studied in connection with a noninvasive method of prenatal diagnosis. Fetal cells can be detected as early as 4 to 5 weeks of gestation. In 1996 Bianchi et al. reported that fetal cells persist in a 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 women.

The observation that fetal cells can persist in women for decades after completion of pregnancy led to the hypothesis that non-host cells might be involved in the pathogenesis of some autoimmune diseases. Women are disproportionately affected by autoimmune diseases and fetal antimaternal graft-vs-host reaction may be involved in the genesis of some of them.

First, we examined a control group composed of healthy individuals.

We separated out a $CD34^+$ $CD38^+$ 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 microchimerism was assayed by:

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

Cellular microchimerism was found in the CD34+CD38+ subpopulation of 32% and in the peripheral 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.

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

Laboratory of Medical Microbiology
Head: Associate Professor Andrzej Gamian, Ph.D.
The pathogenesis of some autoimmune diseases of bacterial etiology: the role of sialic acid, glycolipids, endotoxins and bacterial proteins

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

The general strategy for the elaboration of the protective tools against invading bacteria involves the determination of the structures of the molecules involved in infection and immune processes, their chemical and genetic manipulations, as well as their biological activities. Thus the structures of several such antigens have been established (Eur. J. Biochem. 269, 2002, 93-99, Carbohydr. Res. 337, 2002, 1541-1546, Arch. Immunol. Ther. Exp. 50, 2002, 379-391). Sialic acid is one of the key molecules on the surface of tissue cells participating in immunological functions. In some bacteria sialic acid may be a constituent of surface antigens and the occurrence of sialic acid is associated with an increased pathogenicity of the bacteria, particularly if it is involved in autoimmune processes. In one group of bacterial pathogens studied in our laboratory, the function of sialic acid in the endotoxins is not known. Sialic acid is also structurally similar to 3-deoxy-octulosonic acid, an inherent component of endotoxin. Thus interference of these bacterial components with the functions of tissue sialic acid may contribute to the mechanisms of pathogenicity. Due to the structural mimicry of sialic acid-containing structures, care should be taken when antibacterial vaccines are constructed, to avoid the induction of autoantibodies. Structural studies of glycolipids from pathogenic actinomycetal microorganisms (Biochim. Biophys. Acta, 1594, 2002, 199-205) allow the use of glycolipids for identification and as chemotaxonomic and immunodiagnostic markers useful for classification and identification of clinical isolates such as the new species *Rothia amarae* (Int. J. Syst. Evol. Microbiol., 52, 2002, 2257-2260), and for the recognition of the opportunistic actinomycetes as well as for nocardiosis-like infections.

Most of the research in the last ten years has been focused on methods of blocking cytokine responses in sepsis. The failure of this approach has encouraged a return to basic

principles, which means to enhance the resistance to infection and to characterize the inflammatory reaction of an individual patient. Our studies are thus focused on developing methods of protection against infections and monitoring sepsis and septic shock. The neutralization of endotoxin is still one of the most effective and safe ways to protect against bacterial infections. The monitoring of specific markers for sepsis and septic shock could significantly facilitate the prognosis of these diseases and their treatment. Current work concerns the determination of endotoxins as markers for monitoring different stages of septic shock and status during treatment.

DEPARTMENT OF CANCER IMMUNOLOGY
Acting head: Professor Leon Strządała, Ph.D.

Laboratory of Tumor Immunology
Head: Associate Professor Adam Opolski, Ph.D.
Studies on the mechanisms of tumor progression, metastasis, on the effects of experimental antitumor therapy

Studies on the effects of experimental antitumor immunogenotherapy were performed. MC38 murine colon adenocarcinoma cells were adapted to *in vitro* growth conditions. The cells were characterized by high expressions of Le^x and Le^y antigens, which did not change during the adaptation to *in vitro* cultures. The newly established tumor cell line has been used for transduction with retroviral vectors carrying genes of the cytokines: IL-12 or IL-2. The transduced cell lines were tested for their ability to proliferate *in vitro* and for their tumorigenicity *in vivo* compared with cells transduced with the reporter gene Neo^r and with a wild MC38 cell line. No differences in proliferation rate *in vitro* were observed among the studied cell lines. In contrast, the tumorigenicity *in vivo* of the cells transduced with the genes of both cytokines was significantly decreased compared with control cells.

A new original procedure for the conjugation of antitumor drugs with macromolecular carriers was elaborated. The procedure was tested for the synthesis of the following conjugates: BSA-tomudex, BSA-methotrexate, immunoglobulins-antitumor drugs, fibrinogen-fluorescein-antitumor drugs, and lysozyme-haptens.

We also investigated the influence of essential mineral status (Mg, Cu) on experimental tumor growth and metastasis. The results strongly indicate that tumor growth can be modulated by Mg or Cu status. In all the models applied (LLC lung cancer, B16

melanoma, 16/C mammary cancer and C38 colon cancer), we demonstrated a significant retardation of primary tumor growth in mice receiving a Mg-deficient diet.
Interestingly, in both mice fed a Cu-deficient and those fed a Cu-supplemented diet a significant inhibition of primary tumor growth (LLC) was observed (26% and 33%, respectively, compared with control mice).

Vitamin D_3 and its analogues are known for their various biological effects, especially their influence on cell proliferation, differentiation, and apoptosis. Studies on the effect of *in vitro* treatment of different human and mouse tumor cell lines (breast, ovary, urinary bladder, and leukemia) with calcitriol or its new analogue PRI-2191 on the antiproliferative activity of cytostatics were performed. The results indicated on an increased sensitivity of these cells to the antiproliferative effects of epidoxorubicin, cisplatin, carboplatin, and taxol. Moreover, a synergistic antitumor effect *in vivo* of PRI-2191 and cisplatin was observed in 16/C mammary cancer bearing mice.

Laboratory of Cellular Immunology
Head: Professor Leon Strządała, Ph.D.
Normal and pathological development and selection of lymphoid and neuronal cells

We have continued our search for novel genes that change their expression during the intrathymic development of T lymphocytes, that is the white blood cells which play a central role in the immune system of animals and man. We have established an in vitro system to study the differentiation of immature T lymphocytes (the so called CD4+8+ thymocytes) which has allowed us to identify a number of thymocyte - specific and nonspecific cDNA sequences whose expressions correlate with the process of T cell receptor (TCR) gene rearrangement. The gene identified by one such sequence and its protein product are being characterized. We have also analyzed the kinetics of transcriptome changes accompanying the differentiation of a thymoma cell line which mimics the positive selection of CD4+8+ thymocytes.

A conditionally immortalized cell line, MB-G, and a subline, MB-10, were derived from brain of embryos of transgenic mice harboring mutant SV40 T-gene (tsA58). The cells were intensely immunostained by the monoclonal antibody anti-type III beta-tubulin, the early neuronal marker, as was visualized using confocal microscopy. Use of the RT-PCR technique revealed the expression of mRNAs encoding neuron-specific proteins, such as type III beta-tubulin, microtubule associated protein 2, and neurofilaments and a lack of mRNA for glial fibrillary acidic protein (GFAP, astrocytic marker) in the MB-G and MB-10 cells.

Treatment with phorbol 12-myristate 13-acetate (PMA) induced further neuronal differentiation of MB-10 cells, as was demonstrated by an enhanced expression in treated cells of mRNAs encoding neurofilament-H and growth-associated protein Gap-43, which have been implicated in neurite outgrowth during neuronal differentiation. In conclusion, this study demonstrates that conditionally immortalized cell lines derived from mouse embryos express components of the neuronal cytoskeleton and that these lines can be used to study the expression of genes involved in neuronal differentiation induced *in vitro*.

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

Laboratory of Immunogenetics
Head: Associate Professor Piotr Kuśnierczyk, Ph.D.
The role of the HLA region in susceptibility to psoriasis

Psoriasis vulgaris is one of most common skin diseases in Caucasians. Both environmental and genetic factors are involved in its etiology. The genetic basis is complex, with the major susceptibility gene (*PSOR1*) found in the *HLA* region on chromosome 6. We examined the association of complement component alleles with psoriasis vulgaris; this work was done in collaboration with the Clinic of Venerology and Dermatology, Medical University in Wrocław. We confirmed the association of C4A*6, described earlier by others, and found a much stronger association of C4B*3 with this disease. Continuing this work, we described a very strong association of *HLA-Cw*06* allele with juvenile a psoriasis (age at onset, 0-20 yr). On this occasion, we found a distribution of *HLA-C* alleles in healthy Polish population similar to that described for Germans and Englishmen, but different from the French, Catalans and Basques. Another gene, *LILRA3* (synonyms: *ILT6, LIR4*), whose product might influence an HLA class I-associated disease such as psoriasis was recently discovered. It pressumably codes for a soluble glycoprotein that is highly homologous to other LILR (ILT/LIR) molecules; some of which (LILRB1/ILT2/LIR1 and LILRB2/ILT4/LIR2) recognize HLA class I molecules. Therefore, if LILRA3 has the same ligand specificity, it may block recognition of HLA by other LILR receptors, thus influencing the immune response and disease susceptibility. Recently, a deletion of the *LILRA3* gene in a fraction of the British population was described. We attempted to find out (i) whether the same deletion is present in our Polish population, and at what frequency, and (ii) if present, whether it is positively or negatively associated with psoriasis vulgaris. Results: (i) the

LILRA3 deletion is present in the Polish population with the same frequency as in the British; (ii) the deletion does not influence the susceptibility to psoriasis vulgaris, as the *LILRA3* deletion frequencies were very similar in patients and controls.

Laboratory of Clinical Immunology
Head: Professor Andrzej Lange, M.D.
Genetical aspects and pathomorphological verification of alloreactivity in patients receiving allogeneic hematopoietic stem cell transplantation

The Department participates in the STEMNET project, a study supported by the European Commission (Frame 5). Professor Lange is a research coordinator of the study. The following points have been already addressed and accomplished: (1) Vocabulary for A, B, DRB1 gene description at the 4 digit level was introduced enabling translation of the data obtained with the use of different serological and genetical techniques. (2) Authentication Authorization Accountability (AAA) policy for genetical data processing and storage was introduced to meet the European Union (EU) regulations. (3) Donor-recipient matching policy was presented at the website to secure transparency of the procedure. (4) Telematic system matching European Marrow Donor Information System (EMDIS) requirements was implemented.

The Department also performs the studies supported by the Polish State Committee for Scientific Research. (1) Short tandem repeats (STR) allelic specificities located in the vicinity of the Leukocyte Receptor Cluster (LRC) was worked out on the bases of radiation hybrid, genetic linkage and genetical maps techniques. Frequency of STR alleles and a pattern of chromosome 19 haplotype inheritance were calculated to establish donor-recipient matching for LRC. It became apparent that matching for LRC region associated with an increased risk of acute graft-versus-host disease (aGvHD) and shaped the recovery of natural killer (NK) cells. (2) Analysis of single nucleotide polymorphism (SNP) and STR alleles within the first intron of IFN-gamma gene was described in 162 donor-recipient pairs and association between some STR alleles and SNP was established. STR alleles and SNP pattern associated with higher IFN-gamma generation potential was found to benefit the patients as to the survival and aGvHD incidence.

Translation to the clinical practice: The group activity focus on immunological recovery post transplantation and immunopathomorphology of the tissue biopsies taken for diagnosis of HSCT complications. This activity contributed to the transplant activity of the Bone Marrow Transplantation (BMT) Unit associated with the Department, which resulted in

2002 in 54 autologous and allogeneic transplants, including 17 the most difficult transplants from matched unrelated donors.

The outcome of transplantation with a given extent of HLA compatibility largely depends on the number of CD34+ cells transplanted. It was observed that the co-expression of CXCR4 an CD34+ cells made the marrow harvest richer in hematopoietic progenitors but the content of CD34+ cells in G-SCF mobilized PBPC significantly depended on the the presence of CD34+CXCR4- cells. It was also found that patients suffered from post transplant B cells limfoproliferative syndrome (B cell PTLD) characterize with B cell expansion and lymph nodes enlargement. In patients receiving Fludarabine (Flu) and ATG (but not in those lacking Flu independently whether received ATG) containing conditioning regimen an elevated level of CD20+ cells and lymph node enlargement were observed. CD20 immunohistochemical staining was described as an important factor for assisting decision on adjuvant immunotherapy in B cell non-Hodgkin lymphoma (NHL).

Main activity of the Department is focused on:

(1) Implementation of the EU countries best practice in donor-recipient matching in Poland.

(2) Cytokine gene polymorphism study providing data on the significance of STR allelic specificities and SNP polymorphism as risk factors for survival and aGvHD.

(3) Genetical aspects and biology of KIR genes let the discovery of an association between the matching within LRC region and aGvHD post transplantation.

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