BRIEF ANALYTICAL REPORT

Topic: molecular, genetic and cellular mechanisms for the implementation of abnormal processes within a human body in the presence of blood disorders*(final report).*

Factor VIII and IX gene mutations were detected in 191 unrelated patients with severe hemophilia A and 61 patients with hemophilia B respectively. New 37 and 20 mutations were discovered in FVIII and FIX genes respectively. It has been established that major intron 22 inversion (Inv22) in severe hemophilia A occurs mainly in spermatogenesis. Some gene FVIII oligo(A) tracts are shown to be hot-spots of mutagenesis with their microdeletions and microincertions being of polyphyletic origin. Inv22 predominates among mutations associated with inhibitory hemophilia A. In the course of the project, a specter of studied diseases was expanded and a molecular genetic testing of rare coagulopathies (various forms of fibrinogenemia and factor VII deficiency) and Hageman factor deficiency associated with factor XII decreased activity was initiated. Novel mutations in the genes of alpha, beta and gamma subunits of fibrinogen and in FVII gene were discovered. Mutations in factor VII and XII genes, which are major for the Russian population, were shown.

Research involving 48.5% of patients with autoimmune hemolytic anemia made it possible to discover T cell homogeneity due to the clonal expansion of CD8+ T cells. The immune clones are preserved during remission. They are not correlated with the disease severity, disease duration, and Hb level. It means there is no direct association between detection of these clones and autoimmune process. In healthy people, the detection rate of homogeneity outcomes was significantly lower as compared to patients with autoimmune hemolytic anemia. The detection rate of homogeneity with the TCRG and TCRB genes as targets was 11.3% and 3.2% of cases respectively. Based on the analysis of isolated T-cell subpopulations it has been found out that CD8+CD57+ cells contain clonal products. It is not a pathology. In the majority of examined healthy people (21 of 25), CD8-CD57 cells are still polyclonal. Thus, it has been found out that T-cell immune status undergoes significant changes in patients with autoimmune pathologies. Basic repertoire shifts such as the clonal and oligoclonal cellular expansion occurs within CD8+CD57+ population of T-cells.

A standardized method of growing thrombi under an intense flow was developed to examine their dissolution under the influence of various effects. Calibration experiments aimed to destruct thrombus formation under flow influenced by various factors (hydrodynamic, fibrinolytic, etc) were conducted. The protocol of an experimental study of PLT hydrodynamic activation was developed and an experimental setting was created and well-adjusted. Calibration experiments were conducted to determine platelet activating critical conditions of a hydrodynamic flow. An effect of some (accessible) forms of thiol isomerase inhibitors on blood coagulation under flow was examined.

Topic: studying immune modulating properties of multipotent mesenchymal stromal cells (MMSC) to improve therapeutic effectiveness in patients after allogeneic stem cell transplantation *(interim report).*

It has been found out that culturing MMSCs with 500 Units/ml of interferon (IFN) for 4 hours with a subsequent environmental change avoids additional efforts to expand a large number of MMSCs. Long-term culturing of MMSCs with 500 Units/ml of IFN did not result in significant changes in relative expression of any studied immunomodulatory genes. When MMSCs are cultured using a similar concentration of IFN for 4 hours with a subsequent environmental change, expression of ID01 was 360 times higher. After IFN addition, this gene expression varies significantly. It means that MMSCs exhibit individual differences. Expression of IL6 and CSF1 was 2.4-2.5 and 5.5-7 times higher in an accurate and dose-dependent manner ($p<0.05$).

Short-term incubation of MMSCs and IFN does not affect the proliferative potential and HLA molecules don't have enough time to be expressed at the cell surface. However, as long as MMSCs stay within a body, they will be expressing HLA-DR, and cells will become immune-competent. In co-culturing of MMSCs with lymphocytes, the cells produce an effect on one another. Not only subpopulations of lymphocytes, but also basic properties of MMSCs and IFN-MMSCs are changed. In co-culturing of MMSCs with lymphocytes, changes in MMSCs are more pronounced as compared to when the cells are activated with IFN. Co-culturing with MMSCs or IFN-MSCs prevents transition of naive T-cells into an effector condition. MMSCs and IFN-MMSCs affect the transition of activated T-cells from one state to another state. MMSCs also affect the markers of human lymphocytes stimulation by phytohemagglutinin (PHA). In 5 cases of 7, intraosseous administration of MSC was followed by restored hematopoiesis in a donor.

Topic: study of molecular, cytogenetic, morphological basis of blood system diseases to detect molecular and biological markers, improve diagnostics, adequate selection of differential therapy and disease monitoring *(final report).*

Typical changes in the absolute, and relative number of hematopoietic progenitor cells were found among patients with hemoblastosis following the performed splenectomy. While analyzing the relative change in the number of leukocytes in the peripheral blood it was detected that 24 hours after the surgery they sufficiently increased and subsequently decreased.

A PNG-clone found in patients with aplastic anemia prior to immunosuppressive therapy can be taken as a favorable prognostic factor displaying effectiveness of immunosuppressive therapy, and as a factor of an earlier and more complete response to the conducted therapy.

Detecting expression of JAK2, MAL, PDL1, PDL2, TRAF1 genes enables differential diagnostics of primary mediastinal B-cell lymphoma and diffuse large B-cell lymphoma with an isolated damage to mediastinal lymph nodes. Minimal disseminated disease during diagnosis verification is not a predictor of unsuccessful therapy.

T-cell clonality detected by rearrangement of T-cell receptor gamma chain in the bone marrow and/or peripheral blood among patients with mycosis fungoides/Sezary's syndrome is an unfavorable prognostic factor.

Genotyping of 19 hemoculture-derived extended spectrum beta-lactamases allowed to find genetic differences between two isolates of *E. Coli* only, obtained from one patient at two-month intervals. In paired genotyping of hemoculture- and rectal mucous-derived extended spectrum beta-lactamase producers, 26% pairs of isolates were genetically related. This is how the endogenic infecting path was supported. Probable intestinal colonization with extended spectrum beta-lactamase producers amounted to 91% in patients with lymphomas and 84% in patients with acute myeloblastic leukemia (AML). The probability of preserved colonization with beta-lactamase producers amounted to 30.3%. Newly occurring colonization of ESBLP with positive enterobacetria was found in 13 (39%) of 33 patients with a median of 37 days. Probably returned colonization with beta-lactamase producers amounted to 49.4%. Parenteral nutrition ($p=0.05$) and continuous stay at hospital ($p=0.002$) were significant risk factors of colonization of intestinal mucous membrane with ESBLP-positive bacteria.

Del17p13/TP53, t(8q24)/cMYC and amplq21 are factors of unfavourable prognosis among patients with multiple myeloma in the presence of more than 1 additional copy of lq21 locus. Increased expression of c-MYC gene can be an unfavourable prognostic factor only if MAPK signalling path is activated due to RAS mutant gene. In patients with MM and extramedullary plasmocytoma, the histologic pattern of the brain differs from that in patients with and without bone plasmocytoma. A negative effect of plasmocytoma-based CD56+ and

c-MYC on the course of the disease and tumor sensitivity to medicinal drugs was recorded. It makes further molecular and biological studies of a tumor substrate in MM necessary.

A highly sensitive and highly specific molecular diagnostic method of JAK2, MPL, CALR, SRSF2 and SF3B1 gene mutations was developed. It was established that detection of JAK2V617F mutation and further histologic testing of the bone marrow make it possible to confirm the presence of myeloproliferative disorders (MPD) if Ph-negative MPDs are hidden and have venous portal thromboses as clinical signs.

A system that tests the RHOA-gene based on allele-specific PCR was developed. Nucleotide sequences of IgVH genes were studied in 45 patients with splenic marginal zone B-cell lymphomas (SMZBCL). Thus, 23 patients with non-mutated VH genes (51%) and 22 patients with mutated VH genes (49%) were detected. Within the studied group, VH genes of 23 patients (51%) belonged to VH1 family. VH3, VH3-7 and VH4 family genes were detected in 12 cases (26.7%), 3 and 8 (18%) cases respectively. VH6 and VH7 were found once each (2%). Distribution by VH1 and VH3 incidence significantly differed among B-cell chronic lymphocytic leukemia (CLL) and healthy B-cells. In case of SMZBCL, all cases related to VH1 family (except for one) expressed a IGHV1-2 gene (49% of the sample). In patients with CLL, the gene was found in 5% of cases. Moreover, in patients with CLL the gene was often non-mutated (84%), whereas patients with SMZBCL had a similar number of mutated and non-mutated cases.

During the study of a karyotype complex of patients with myelodysplastic syndrome (MDS) and AML (n=36), 6 abnormalities per karyotype (2-16) were detected using molecular and cytogenetic methods. Chromosomal disturbances mainly included translocations (97%). Deletions were found in 94% of patients. Additional abnormalities and/or additional chromosomal breakpoints were recorded in 36.1% of patients. The greatest number of abnormalities were detected on chromosomes 5 (80.6%), 7(63.9%), 11 (36.1%), and 17 (38.9%). True monosomy for chromosome 7 was found in one patient; no true monosomy existed for chromosomes 5 and 17. Molecular and cytogenetic research methods were used to detect fragments of chromosomes 5, 7 and 17 within simple reciprocal and complex translocations. Except for one case, they were accompanied by deletions of 5q31, 7q31 and 17p13 regions. A regular cytogenetic study of G-differentiated stained chromosomes is not enough to analyze a karyotype complex. Additional molecular and cytogenetic research methods are required. Patients are stratified into risk groups based on the results of a regular cytogenetic study. However, it can't be excluded that the detailed elaboration of complex karyotypes using modern molecular and cytogenetic research methods can influence selection of a therapeutic method.

It has been found out that DSP30 and IL-2 culturing results in an increased frequency of aberrant karyotype in patients with CLL as compared to mytoses obtained during LPS+TPA culturing.

In myeloid/lymphoid neoplasms, clinical and laboratory signs with PDGFRA gene rearrangement correspond to myeloproliferative diseases; in case of lymphadenopathy, in 30% of cases there is a high probability of a biphenotypic disease. It should be accompanied by determination of PDGFRA gene expression within lymphoid cells of a lymph node. In PDGFRA-positive myelo/lymphoproliferative neoplasms, clonality should be supported only when molecular and genetic research methods such as a regular cytogenetic research fail to detect chromosomal aberrations.

Topic: determination of clinically relevant minor histocompatibility antigens in HLA-similar transplantation of hematopoietic stem cells *(interim report).*

Minor histocompatibility antigens (MHA) specific cells can be detected in culturing of peripheral blood cells after transplantation with synthetic antigenic peptides. A collection of cell lines (lymphoblastoid cells) taken from patients and donors who underwent allogeneic

stem cell transplantation was created. MHA-specific T-cells express gamma-interferon during stimulation with cells presenting unmatched MHA and can mediate cytotoxicity of target cells. At the same time, autologous cells (peptide with one amino acid replacement within own Major Histocompatibility Complexes) are protected from cytotoxicity. T-cell clones were co-cultured with a patient's and a donor's lymphoblastoid cells. Cytotoxicity of T-cells in relation to target cells was estimated using flow cytometry. Reverse transcription, cloning of the obtained cDNA and Sanger sequencing were used to decipher the TCR beta chain sequences of HA-1- and HA-2- specific cytotoxic T-cell clones.

Topic: evolution of bone marrow stromal microenvironment under the effect of cytotoxic drugs and tumor cells while treating patients with hemoblastoses *(interim report).*

It has been shown that when a marrow disease is diagnosed in patients with AML and acute lymphocytic leukemia (ALL), the level of fibroblast colony-forming units is reduced in a statistically significant manner, whereas time to form a confluent monolayer after primary marrow transplantation is increased for every studied nosology (AML, ALL, CML). Aggregate cellular products do not differ significantly from those found in MMSC cultures of healthy donors. A significantly changed expression of various growth factors, differentiation markers and other regulatory molecules where found during analysis of the relative level of gene expression in MMSCs of patients before treatment. A set of obtained data shows that marrow stromal microenvironment is changed in any studied type of leucosis. These changes are far from being similar and depend on nosology. Treatment restores separate characteristics of stromal precursor cells. Other changes are preserved for a long time as soon as remission has been obtained. Further studies on the change in the stromal microenvironment will make it possible to specify the pathogenesis of various forms of hemoblastosis, reveal therapy-related remote consequences and ultimately optimize treatment.