

(57°C and 55°C) and the concentration of magnesium ions in the reaction mixes (2.5 mM) were determined. The PCR conditions and primers specificity were tested using a sample of 16 previously characterized specimens. The results of genotyping by the developed methods fully corresponded to the data of sequencing.

Thus, these methods can be used for routine determination of I- and E-genotypes in rotavirus-positive samples and for identification of reassortant rotavirus strains.

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### **CXCL10 GENE PROMOTER POLYMORPHISM A-1447G MODULATES PROTEIN EXPRESSION IN SERUM AND ASSOCIATED WITH INVASIVE ASPERGILLOSIS IN FEMALE ONCOHEMATOLOGICAL PATIENTS**

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Invasive aspergillosis (IA) — is life-threatening invasive infection, especially in immunocompromised hosts, most of which are oncogematological patients. The key components of fungal infections pathogenesis are disturbances of the immune system. Chemokine CXCL10, also known as interferon gamma-induced protein 10 (IP10), is a member of CXC chemokines. CXCL10 is an inflammatory mediator, which stimulates the directional migration of Th1 cells as well as increasing T-cell adhesion to endothelium. *CXCL10* gene promoter single nucleotide polymorphisms (SNPs) affects protein expression via NF- $\kappa$ B transactivation.

The purpose of this study is to investigate of allelic variants A-1447G (rs 4508917) and G-135A (rs 56061981) effect on the amount of CXCL10 protein in serum and risk of development IA in oncogematological patients in St. Petersburg.

171 oncogematological patients on the background of cytostatic polychemotherapy with symptoms of lung injury were recruited to participate this study. 75 oncogematological patients (44.5%) either developed proven or probable IA as defined by criteria of EORTC/MSG 2008 (median age 43±14, 57% males) whereas controls (96 oncogematological patients (55.5%) without IA comparable in age and sex) did not fulfill these criteria.

SNPs was analyzed by the method of restriction fragment length polymorphism analysis. Chemokine CXCL10 amount was determined with the use of commercial ELISA kit sets (Cloud-Clone Corp, USA). Statistical analysis was performed using SPSS 21 (IBM, USA).

The heterozygous AG rs 4508917 and homozygous GG rs 56061981 genotypes prevailed in the both studied groups and there were no significant differences in genotype distribution of A-1447G and G-135A between oncogematological patients with probable IA and without IAL. However, when dividing patients by sex in a female group G allele rs 4508917 was significant associated with the occurrence of IA ( $\chi^2 = 3.853$ ,  $p < 0.50$ , OR 3.13 95% CI (1.196–8.204).

There were no differences in serum CXCL10 levels between –135 GG and GA genotypes. However, individuals with –1447G allele had significantly higher serum levels of CXCL10 than those with –1447(A/A) genotype ( $p = 0.022$ ).

Further increase in the number of patients included in the study will allow to make conclusions about the prospect of typing the studied polymorphic variant of the gene CXCL10 as a predictive marker of the risk of mycosis development with a strong significance.

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### **DEVELOPMENT OF A PANEL OF MONOCLONAL ANTIBODIES FOR STUDYING OF LOCAL PRODUCTION OF CYTOKINES IN CHRONIC RHINOSINUSITIS**

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Cytokines as key regulators of inflammation play a central role in the pathophysiology of chronic rhinosinusitis (CRS). CRSs are divided into CRS with and without polyps of the nasal mucosa, but this difference is not sufficient for a clear definition of subgroups with the same pathophysiology and production of cytokines. This area remains open for more detailed studies. The purpose of our work was the development of a panel of monoclonal antibodies for studying the characteristics of local production of cytokines in CRS. For studies, monoclonal antibodies to human cytokines were obtained using hybridoma technologies. One or more clones producing antibodies to cytokines (IL-1 $\beta$ , IL-4, IL-6, IL-8, IL-17, TNF $\alpha$ , GM-CSF, IFN $\alpha$ , IFN $\gamma$ ) were obtained. Specificity of antibodies was proved in ELISA: direct and sandwich method. To create a panel, the antibodies were tested by indirect immunohistochemistry using the avidin-biotin-alkaline phosphatase system. Isolated peripheral blood mononuclear cells from three donors were stimulated LPS 500  $\mu$ g/ml or PHA 20  $\mu$ g/ml overnight at +37°C at 5% CO<sub>2</sub>. The cell smears on the glasses were fixed with 4% PF. The antibodies studied were used as the first antibodies, dilutions were selected in preliminary experiments. Under the optical microscope, the numbers of lymphocytes or monocyte having red staining in the cytoplasm were counted; the result was expressed as a percentage. The reaction intensity was expressed in points (1-moderate, 2-medium, 3-intensive reaction). As a result, clones were selected that produce antibodies that best detect cytokines in human cells. After induction LPS, IL-1 $\beta$  was detected in 41.0±19.3% of lymphocytes (intensity 2 points) and 90.7±1.3% of monocytes (3 points); IL-6 in 4.0±1.53% of lymphocytes (2 points) and 78.7±8.4% of monocytes (2–3 points), IL-8 in 10.0±4.6% of lymphocytes 1–2 points) and 48.0±10.6% monocytes (1–2 points); TNF $\alpha$  was rarely detected in lymphocytes, in 46.7±18.9% of monocytes (1–3 points); IFN $\alpha$  was detected mainly in monocytes (77.7±10.0%, 2–3 points); The weak but distinct GM-CSF production was determined in 56.7±18.6% monocytes (1–2 points). After PHA induction IL-4 was detected in 6.0±2.5% of lymphocytes (1–2 points), 47.3±2.0 monocytes (1–2 points); IL-17 — was determined in 38.67±15.4% monocytes (1–2 points); IFN $\gamma$  — in 16.7±11.6% of lymphocytes (1 point) and 32.3±6.38% of monocytes (1–2 points). Thus, it was shown that the obtained antibodies reliably detect the corresponding cytokines in human cells. This panel of antibodies will be used by us to assess the specific features of local production of cytokines in CRS, as well as a number of other inflammatory processes.