Purpose: To study the DNA-ase and catalase activity of IgG, isolated from the blood plasma of patients with multiple sclerosis.

Materials and Methods: Peripheral blood serum of patients with MS was used in the study. IgG was isolated by affinity chromatography on columns of Protein G-Sepharose. DNA-ase activity was determined by the degree of conversion of supercoiled form of pBluescript plasmid DNA into the ring, relaxed and linear forms. The reaction mixture volume of 15 ml contained: 20 mMtris-HCl, pH 7.5, 5 mM $MgCl_2$, 10-20 mg / ml pBluescript DNA, and 0.1 - 0.2 mg / ml of antibody. After incubation for 2 hours at 35°C the reaction mixture was added 5 ml buffer solution of 4X, containing 1% SDS, 30% glycerol, 30 mM EDTA, 0.1% bromophenol blue. Electrophoresis was performed in 1.2% agarose gel. DNA in the gel was stained with ethidium bromide solution (0.5 mg / ml). Determination of catalase activity was performed spectrophotometrically (λ = 240 nm.) On a spectrophotometer Specord. The reaction mixture consisted of 30 mM H_2O_2 solution at 50 mM phosphate buffer (pH = 7.0) of 1600 ml and 70 ml of a solution containing IgG in potassium phosphate buffer (pH 7.0).

Results and conclusions: The study of the catalytic activity of IgG in multiple sclerosis patients revealed a high percentage of hydrolysis of DNA, reaching 100% in some patients. Hydrolysis of DNA Ig G, isolated from blood of healthy individuals does not exceed 1-2%.

The ability of antibodies in multiple sclerosis patients to split hydrogen peroxide was first discovered. IgG, isolated from blood of healthy individuals did not have this ability. All studied antibodies were tested for homogeneity. The study of the catalytic properties of AT patients with multiple sclerosis will contribute to understanding the mechanism of pathogenesis of this disease.

CYTOKINE GENE POLYMORPHISMS AND ASTHMA SUSCEPTIBILITY, SEVERITY AND ASSOCIATED ALLERGIC MANIFESTATIONS IN MOLDOVAN CHILDREN

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Introduction: Asthma is a complex inflammatory disease, caused by the interaction of genetic and environmental factors, and its management requires understanding of its various pathogenesis and control mechanisms. Cytokines and other inflammatory mediators are important factors in asthma pathophysiology. The reported racial and/or ethnic differences in asthma-related loci define the importance of the candidate gene research in ethnically diverse populations. The study was aimed to investigate the association between cytokine gene polymorphisms asthma in a sample of Moldovan patients and controls.

Methods: The sample comprised 90 individuals with asthma, aged from 5 to 17 years (mean ±SEM age of 10,9±0,4 years), 51 males and 39 females, who were randomly selected from a group of asthmatic children referred to the Allergy Clinic of the Research Institute for Maternal and Child Healthcare, Chisinau, Moldova, during the years 2009-2010. The control group included 90 healthy children, matched by sex and age with patients' group (mean age 13,5±0,2), without respiratory symptoms or history of asthma and allergy. Asthma was defined according to the criteria of the Global Initiative for Asthma (GINA). A complete clinical history, physical examination, and pulmonary function test (PFT) in a standard fashion were performed for all the subjects. TNF-α G-308A, IL-4 C-590T and IL-4Rα Arg551Gln polymorphisms were evaluated by polymerase chain reaction.

Results: The genotypes frequency for TNF-α, IL-4, and IL-4Rα were equally distributed in the patient group in comparison with the controls. However, there were significant differences for IL-4 C-590T gene between the subgroups of asthmatics with different degree of the disease severity. Thus, *IL-4* CT+TT at position -590 was significantly overrepresented in children with severe asthma in comparison with those with the moderate one (53,8% in severe asthma vs 25,0% in moderate asthma; χ^2=2,7; gl=1; p=0,086). The same difference was found for the T allele (minor allele): 34,6% in severe asthma vs 12,5% in patients with moderate asthma (χ^2=5,3; gl=1; p<0,05). The study showed that the homozygous genotype *TNF-α* GG at position -308 has a protective role, being significantly more frequently identified in children with solitary form of asthma compared with those with allergic triad (86,2% vs 60,0%, respectively; χ^2=3,88; gl=1; p<0,05). Functionally compromised genotypes *TNF-α* GA+AA at position -308 were found more frequently in children with asthma associated with other allergic symptoms (40,0% in allergic triad and 55,6% in asthma cases associated with atopic dermatitis vs 13,8% solitary asthma, p<0,05).

Conclusions: The results of our study suggested an association between the IL-4 polymorphism at position -590 and asthma severity, and the association of the functionally compromised genotypes of the TNF-α polymorphism at position -308 with different clinical phenotypes of asthma in Moldovan children.

Key words: asthma, child, IL-4, IL-4Rα, TNF-α, gene, polymorphism, phenotype.

THE INFLUENCE OF BRONCHOALVEOLAR AND CIRCULATING TUMOR NECROSIS FACTOR-ALPHA ON APOPTOSIS IN DIFFERENT MODELS OF LUNG INJURY

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Cytokines are involved in a variety of lung diseases, but their pathogenetic role in programmed cell death is still controversial. This study tests whether the activation of an 'extrinsic' pathway to cell death is mediated by bronchoalveolar and circulating tumor necrosis factor (TNF)-alpha.

Nonlinear male rats weighing 200-230 g were used in all the experiments. For modeling of acid aspiration-induced acute lung injury anesthetized rats underwent tracheostomy and insertion of a fine-bore cannula into the anterior segment of the left lung. This was followed by the instillation of either 1.0 mL/kg HCI, pH 1.2 (n = 12) or 1.0 mL/kg saline in control rats (n = 12). All animals were studied at 6-hour after acid aspiration. For modeling of hepatopulmonary syndrome anesthetized rats underwent common bile duct ligation (CBDL) (n = 12). Sham rats underwent mobilization of the common bile duct without ligation (n = 12). All the animals were studied at 31-day after CBDL or sham operation. Bronchoalveolar lavage (BAL) and blood serum were analyzed for TNF- levels in pg/ml using commercially available ELISA kits. The level of apoptosis was analyzed with the help of Annexin V-FITS and propidium iodide using Beckman Coulter flow cytometer.

By 6 h after acid aspiration TNF-alpha values were significantly higher than in control group (in the blood serum: 17,06±1,91 pg/ml vs 11,54±0,59 pg/ml, p<0,001, in the BAL: 16,39±0,80 vs 1,73±0,13, p<0,001). It was also a significant increase in the number of early apoptotic cells present (in the blood serum: 0,63±0,14 vs 0,45±0,03, p<0,001, in the BAL: 2,41±0,15 vs 0,61±0,05, p<0,001). At 31-day after CBDL TNF-alpha values were also significantly higher than in control group (in the blood serum: 46,36±2,33 pg/ml vs 10,35±1,90 pg/ml, p<0,001, in the BAL: 11,50±0,77 vs 2,06±0,44, p<0,001). It was also a significant increase in the number of early apoptotic cells present (in the blood serum: 2,02±0,35 % vs 0,60±0,09 %, p<0,01, in the BAL: 2,77±0,45 % vs 0,47±0,06 %, p<0,01).