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THE IMMUNOGLOBULIN`S LEVEL OF SICKLY CHILDREN OF GENE POLYMORPHISM (ASP299GLY) TOLL-4 AND (SER249PRO) TOLL-6 RECEPTOR WITH ACUTE RESPIRATORY VIRAL INFECTIONS

ABSTRACT

In the article the role of polymorphisms of genes Toll-4 and Toll-6 receptor in the development of low antiviral defense of sickly children is examined. It is shown that in the blood of children the content of IgM, IgG and its subclasses increases, and the concentration of IgA is reduced. At the genetic mutations in (Asp 299 Gly) Toll-4 and (Ser 249 Pro) Toll-6 receptors synthesis of explored immunoglobulins is reduced, that is regarded as one of the reasons for the low antiviral defense of children with genetic defects in the immune system signaling receptors.

Keywords: immunoglobulins, SARS, Toll-4 and Toll-6 receptors.

INTRODUCTION

It is now well known, that low anti-infective protection of sickly children with acute respiratory viral infection is associated with failure of the innate immaturity of the adaptive immunity [1,2,3,7]. These children have a selective deficiency in T and B-lymphocytes, which is reflected in the level of immunoglobulins. The concentration of IgG, IgM, IgA, sIgA is reduced in the blood of sick children [6,8]. It is considered, that the immunological disorders can be either transitory in nature or genetically determined [9]. The search for the genetic bases of the high susceptibility to viral infections and it is in its infancy and mainly affects the polymorphism of genes regulatory cytokines. So, the group of authors [5] have established a link between the gene polymorphism of Il-1β, TNF-α, and increased risk of acute pathology of the upper respiratory tract. Some patients showed a genetically determined increased production of Pro-inflammatory mediators IL-1α and IL-1β. This leads to more pronounced symptoms of inflammation and prolonged disease course [9,10]. In earlier studies [2,3] we have found that 55.6% have a genetic defect in (Asp299Gly) Toll-4 receptor (genotype Asp/Gly and Gly/Gly) and 75% of genetic mutations in a marker (Ser249Pro) Toll-6 receptor (genotype Ser/Pro and Pro/Pro) among sickly children. The ligand for Toll-4 becomes a receptor of DNA viruses, and Toll-6 – lipopolysaccharide (LPS) of gram-negative bacteria. When the structure of these receptors is disturbed, then there is poor contact between the pathogen and receptors that, in turn, disrupts intracellular signaling and production of cytokines, which are regulators of adaptive immunity (4,10).

The object: to Determine the content of immunoglobulins IgA, s.IgA, IgM, IgG and its subclasses in the blood of frequently ill children with gene polymorphism (Asp299Gly) Toll-4 and (Ser249Pro) Toll-6 receptor, during viral respiratory infections.

MATERIALS AND METHODS

In the beginning of our research, we determined the content of immunoglobulins in the blood of 60 frequently ill children with URTI without considering the polymorphism (Asp299Gly) Toll-4 and (Ser249Pro) Toll-6 receptor. For this series the control was the blood of 35 healthy children. The age of the patients was from 1 year to 3 years (both in the experimental and control groups).

Next, we conducted a population-based research on 90 sick children with gene polymorphism (Asp299Gly) Toll-4, and 100 sick owners of polymorphism (Ser249Pro) Toll-6 receptor.

The etiological causes of the disease were: influenza 49%, parainfluenza in 26%, adenovirus infection 5% and 4% for respiratory syncytial virus. The criteria for inclusion in the study were: history of at least 6 episodes of URTI per year, ranging in age from 1 to 3 years, the first 3 days of illness.

The study did not include children with chronic bronchopulmonary diseases (asthma, recurrent bronchitis, malformations of the respiratory system, allergic diseases). The research was performed in the research Institute of medical ecology. The studied material was venous blood.

We used a sample of 76 healthy children (30 boys and 46 girls) aged from 1 year to 10 years as population control. DNA extraction was carried out using sets of "DNA-Express-blood" (NPF "LitEks", Moscow, Russia). The synthesis used in the work oligonucleotide primers are made by SPC "LitEks", Moscow. Detection of mutations was performed by PCR. The concentration of IgA, s.IgA, IgM, IgG with subclasses identified by solid-phase ELISA using reagents JSC "Vector-best", Novosibirsk.

The research was conducted on 90 sick children with URTI with gene polymorphisms of Toll-4 (Asp299Gly) and 100 sick owners with polymorphism of Toll-6 (Ser249Pro) receptors. Patients were divided into 7 groups: 1st group – healthy children (control); 2nd group – patients with URTI children with the genotype Asp/Asp gene polymorphism (Asp299Gly) Toll-4 receptor; 3d group – children with the genotype of Asp/Gly gene polymorphism (Asp299Gly) Toll-4 receptor; 4th group – owners of genotype Gly/Gly gene polymorphism (Asp299Gly) Toll-4 receptor; 5th group – owners of genotype Ser/Ser polymorphism of the gene (Ser249Pro) Toll-6 receptor; 6th group - owners of genotype Ser/Pro polymorphism (Ser249Pro) Toll-6 receptor and the 7th group were owners of genotype Pro/Pro polymorphism of the gene (Ser249Pro) Toll-6 receptor.

Statistical analyses were performed by the method of variation statistics using the software packages Microsoft Excel 2007, STATISTICA 6,0. Before the analysis, the variation series were tested for normality using the Shapiro-Wilk`s W. test. The criterion of student (t-test) was used in a normal distribution. The measurements were taken in the form of mean values with standard deviation (M±SD). The Mann-Whitney's test (U-test) was used in the abnormal distribution of the trait. The results are presented in a form of median (ME [25th; 75th percentiles]).

RESULTS AND DISCUSSION

In our studies we have found a blood, that was taken in the first days of admission of children to the hospital regardless of genotypes contains a high concentration of IgM, IgG and its subclasses IgG1, IgG3, IgG4 (table 1).

Such a rapid response of b-lymphocytes cannot be associated with the formation of the antigen-specific clone. Probably, as a result of frequent infections of children, memory cells remain and when the next contact with the antigen (AG) existing antigen specific cells of adaptive immunity, turning in the activated state, produce appropriate immunoglobulins. Perhaps, part of the immunoglobulin remains in the period of remission, and on this background begins the next respiratory infection.

However, the concentration of IgA of pediatric patients with URTI in the very beginning is low; the synthesis of sIgA is not increased, so the barrier antiviral protection of such children is weakened (table.1).

Table 1

The content of immunoglobulins in the blood of children with URTI (M±SD) (mg/ml)

The immunoglobulins	Healthy children, n=35	Patients with URTI, n=60
IgA	3,2±0,6	1,51±0,2*
sIgA	1,41±0,1	1,51±0,2
IgM	1,3±0,11	2,3±0,3*
IgG	4,3±1,32	13,6±1,3*
IgG_1	3,0±0,9	10,8±2,3*
IgG_2	1,2±0,3	1,8±0,3
IgG_3	0,3±0,7	1,3±0,2*
IgG_4	0,3±0,6	1,16±0,2*

Note: *– significance of differences between indicators of healthy and sick children.

Further, there were selected patients with gene polymorphisms (Asp299Gly) Toll-4 receptor and (Ser249Pro) Toll-6 receptor among the children. The concentration of immunoglobulins is determined by genotype. So, sick children with a polymorphism in the Toll-4 receptor have a low IgA level in all the analyzed genotypes (tab.2). Synthesis of sIgA is increased only in patients with the genotype Asp/Asp (gr.2).) The concentration of sIgA of the heterozygote (genotype/Gly) and homozygotes (genotype Gly/Gly), significantly less than that of the genotype Asp/Asp in the first days of the disease.

Regardless of genotype, the level of IgM of patients is higher than in controls (in controls to 1.3±0.1 mg/ml) on the first days of the disease (table.2). The content of this immunoglobulin of heterozygote Asp299Gly (gr.3) and of the mutant homozygote Gly/Gly (gr.4) are reduced, compared with the genotype Asp/Asp.

The concentration of IgG is high in all groups of patients with URTI, regardless of the presence or absence of genetic problems, however, in the group of sick children with complete replacement of alleles in the gene encoding Toll receptor-4 (Gly/Gly) synthesis of IgG is somewhat limited (to 15.2 mg/ml) compared with the genotype Asp/Asp genotype and Asp/Gly.

Table 2

The content of immunoglobulins of patients with URTI - owners of the Asp299Gly genotype in the gene Toll-4 receptor (median, 25-75 percentiles) (mg/ml).

Immunoglobulins	ealthy children (n=76) (1)	Asp/Asp (n=40) (2)	Asp/Gly (n=18) (3)	Gly/Gly (n=32) (4)
IgA	3,4 [2,7-4,7]	2,1 [1,2-2,8]	1,6*# [1,5-2,9]	1,4*# [1-2,1]
sIgA	1,4 [1,2-1,7]	3,4* [1-4,5]	1,5# [1,2-1,9]	1,6# [1,1-2]
IgM	1,3 [1,1-1,8]	2,4* [1,1-2,9]	1,7*# [1,1-2,3]	1,8*# [1,6-2,2]
IgG	4,3 [3,8-6,4]	17,3* [14,6-20,6]	16,6* [11,5-22,1]	15,2# [11-19]
IgG_1	3,0 [2,8-4,6]	15,3* [11-18,5]	14,8* [11,9-19,5]	16,0* [12,1-19]
IgG_2	1,2 [1-2,7]	2,3* [1,8-2,8]	1,9# [1,2-2,5]	1,7# [1,5-2,1]
IgG_3	0,3 [0,1-1,9]	1,3* [1-1,9]	1,6*# [0,8-1,9]	1,2* [1-1,8]
IgG_4	0,3 [0,1-1,8]	1,8* [1-2]	1,2*# [1,8-1,9]	1,1*# [1,1-1,7]

Note: U – Mann Whitney's test; * – significance of differences compared with the control. # – significance of differences compared with the group of owners of genotype Ar/Ar gene (Asp299Gly) Toll-4 receptor.

The high content of IgG was mainly due to IgG1 subclass, which level in all polymorphic variants of the gene Toll-4 to 5 times higher than the reference value (Asp/Asp – 15,3 mg/ml; Asp/Gly 14.8 and Gly/Gly -16,0 mg/ml).

A similar trend was observed for IgG3 and for IgG4. Their concentration was increased in all the analyzed groups of polymorphic alleles of the Toll-4 receptor. The content of IgG2 by owners of the mutant homozygote Gly/Gly and heterozygotes Asp/Gly was lower (1.7 mg/ml and 1.9 mg/ml) than by the owners of genotype Asp/Asp (2,3 mg/ml).

More striking defects in immunoglobulin synthesis was detected when was the genetic changes in the gene Toll-6 receptor (tab.3)

The concentration of anti-virus defender of IgA is significantly lower with patients who have the heterozygous variant of Ser/Pro in the gene Toll-6 receptors (1.4 mg/ml) and mutant homozygotes Pro/Pro (1.7 mg/ml) than of homozygotes Ser/Ser (2.1 mg/ml). This analogy is observed also for sIgA. Abnormal variants of polymorphic gene of Toll-6 have the sIgA level of 2.3 mg/ml and 2.4 mg/ml, that is significantly lower than of genotype Set/Ser (3.4 mg/ml).

Table 3

The immunoglobulin`s content of patients with URTI - holders of genotypes Ser249Pro in the gene Toll-6 receptors (median, 25-75 percentiles) (mg/ml)

The immunoglobulins	ealthy children (n=76) (1)	Ser/Ser (n=25) (5)	Ser/Pro (n=50) (6)	Pro/Pro (n=25) (7)
IgA	3,4 [2,7-4,7]	2,1 [1,1-2,8]	1,4*# [1,1-2,9]	1,7*# [1-2,2]
sIgA	1,4 [1,2-1,7]	3,4* [1-4,3]	2,3*# [1,3-3,2]	2,4*# [1-2,7]
IgM	1,3 [1,1-1,8]	2,6* [1,2-3,6]	1,8# [1-2,2]	1,9# [1,6-2,3]
IgG	4,3 [3,8-6,4]	17,3* [13,6-21]	13,6*# [12-21,2]	12,2*# [11-18,3]
IgG_1	3,0 [2,8-4,6]	15,3* [9-17,2]	12,8*# [10,2-18]	10,0*# [9-18,4]
IgG_2	1,2 [1-2,7]	4,3* [2-5,2]	1,8*# [1,2-3,1]	1,4*# [1-2,2]
IgG_3	0,3 [0,1-1,9]	1,2* [1-1,7]	1,4* [1-1,8]	1,3* [0,9-1,6]
IgG_4	0,3 [0,1-1,8]	2,8* [0,9-3,5]	1,3*# [1,1-1,8]	1,2*# [1-1,6]

Note: U – Mann Whitney's test; * – significance of differences compared owners of Ser/Ser genotype of the gene (Ser249Pro) Toll-6 receptor.

During the genetic defects in Toll-6 receptors, IgG synthesis (gr.6,7) is limited in the genotypes Pro/Pro and becomes lower than with children with the genotype Ser/Pro (13,6 mg/ml) and genotype Ser/Ser (17,3 mg/ml) (gr.5). The total background IgG is determined mainly by IgG1, which content of the mutant homozygotes (10 mg/ml) is lower than of owners of genotype Ser/Ser (15,3 mg/ml).

Limitations of the synthesis on IgG2 and on IgG4 were observed in the homozygous owners of genotype Pro/Pro in the gene Toll-6 receptor.

Thus, our studies have shown that polymorphism of genes, that's encode Toll-4 and Toll-6 receptors, affects the synthesis of all immunoglobulins (IgA, sIgA, IgM, IgG), the concentration of that varies with genetic defects in the

analyzed receptors. Perhaps genetic limit of antibody production are one of the causes of insolvency antiviral defense of sickly children.

CONCLUSIONS

The synthesis of immunoglobulins is reduced in comparison with a group of sick children from genetic defects in the receptors, during the point mutations in the genes of Toll-4 (Asp299Gly) and Toll-6 (Ser249Pro) receptors.

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