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CHANGES OF CYTOKINE PRODUCTION IN CHILDREN AFFECTED WITH ACUTE RESPIRATORY VIRAL INFECTIONS AT POLYMORPHISM OF TOLL-4 (ASP299GLY) AND TOLL-6 (SER249PRO) GENES RECEPTORS

ABSTRACT

The article analyzes the role of polymorphism of Toll-4 (Asp299Gly) and Toll-6 (Ser249Pro) receptor genes in the development of low antiviral protection in children with frequent episodes of acute respiratory infections. It is shown that the synthesis of cytokines IL-1-β, TNF-α decrease in the blood of patients with Toll-4 receptor gene polymorphism and IL-1RA content is increased in comparison with the group of diseased children without abnormal disturbances in signal receptors. Polymorphism in the Toll-6 receptor genes decreases the concentration of cytokines IL-1β, IL-8, TNF-α and IL-10. The concentration of IL-1RA is increased in Pro / Pro and Gly / Gly genotypes compared to the group of patients without polymorphic changes in the corresponding receptors. Genetic disorders in the synthesis of cytokines in the polymorphism of the Toll-4 (Asp299Gly) and Toll-6 (Ser249Pro) receptors are one of the reasons for the failure of antiviral protection in children who often have ARI.

Keywords: acute respiratory viral infection, TLR, polymorphism, cytokines.

INTRODUCTION

"Often ill children (OIC)" is a term for a group of children with a higher incidence of acute respiratory viral infection (ARVI) than among peers. The maximum incidence of acute respiratory viral infection among children is from 6 months to 6 years old and is more than 6 episodes a year [3,8]. According to modern ideas, the main reason for the high susceptibility of children to a viral infection is immaturity of the immune system [1,2,9] and a hereditary predisposition to infectious diseases [4,5]. Genetic changes in the immune system can occur at different stages of the immune response. First of all, this concerns receptors that come into contact with the pathogen. The main role in the recognition of the pathogen is played by Toll-like receptors (TLR). They are part of the cell membranes of all immunocompetent cells [6]. Polymorphism of Toll-receptor genes is associated with a number of diseases. From the literature it is known that SNP (Asp299Gly) of the Toll-4 gene is associated with septic shock [11], with the development of atherosclerosis and coronary heart disease [10], diabetes mellitus [7]. SNP Ser249Pro in the Toll-6 receptor gene was noted in patients with bronchial asthma [12]. In earlier works [4], we found that 55.6% of children with a chronic respiratory viral infection had genetic mutations in Toll-4 (Asp299Gly) and 75% in the gene (Ser249Pro) of the Toll-6 receptor.

Ligands for Toll-4 receptors are double-stranded DNA viruses, and for Toll-6 receptors - Gram-negative bacteria. The contact of pathogen-recognizing receptors with the ligand triggers the synthesis of cytokines, which regulate the degree of immune reactions. We believe that genetic disorders in Toll-receptors affect the level of intracellular signaling and the amount of cytokines produced.

The aim of the study was to study the effect of Toll-4 (Asp299Gly) and Toll-6 (Ser249Pro) receptor polymorphism on the production of cytokines in children who often suffer from acute respiratory viral infection.

MATERIALS AND METHODS

The clinical group consisted of 190 children of both sexes aged 1 to 3 years, often with acute respiratory viral infections. Of the children surveyed, 49% had influenza, 26% had parainfluenza, 5% had an adenovirus infection, and 4% had a syncytial virus. The criteria for inclusion in the studies were: in the history of at least 6 episodes of acute respiratory viral infection, the age of patients from 1 to 3 years, the first 3 days of the disease.

The study did not include children with chronic bronchopulmonary diseases (bronchial asthma, recurrent bronchitis, malformations of the respiratory system, allergic diseases). The work was carried out on the basis of the Scientific Research Institute of Medical Ecology. The studied material was venous blood.

As a population control was used a sample of 76 conditionally healthy children (30 boys and 46 girls) aged 1 to 10 years. DNA extraction was carried out with the help of «DNA Express Blood» kits (NPF «Litech», Russia, Moscow). Synthesis of oligonucleotide primers used in the work was carried out by NP «Litehs», Moscow. The detection of mutations was carried out by PCR. The concentration of cytokines was determined by the method of solid-phase ELISA using reagents LLP «Vector-Best» Novosibirsk.

Studies were carried out on 90 patients with ARVI children with Toll-4 gene polymorphism (Asp299Gly) and 100 patients with polymorphism carriers Toll-6 (Ser249Pro) receptors. All subjects were divided into 7 groups: 1 group - healthy children, control (n = 76); 2 group - patients with ARVI children with the genotype Asp / Asp (n = 40); Group 3 - sick children with the Asp / Gly genotype (n = 18); Group 4 - sick children with genotype Gly / Gly (n = 32); Group 5 - sick children with genotype Ser / Ser (n = 25); 6 group - children having genotype Ser / Pro (n = 50); Group 7 - children with genotype Pro / Pro (n = 25).

The statistical processing of the material was carried out by the method of variational statistics with the help of the Microsoft Excel 2007 software packages, STATISTICA 6.0. Before the analysis, the variational series were tested for normality using the Shapiro-Wilk test. Under normal distribution was used the Student's test (t-test). The indicators are presented as mean values with a standard deviation (M ± SD). With an abnormal distribution of the trait was

applied the Mann-Whitney test (U-test).

RESULTS AND DISCUSSION

Our research has shown that with the polymorphism of the genes (Asp299Gly) of Toll-4 receptors, the synthesis of IL-1ß is reduced compared to the group of patients with the Asp / Asp genotype who do not have polymorphic changes (Table 1).

Synthesis of the second anti-inflammatory cytokine TNF-α in polymorphism of the Toll-4 receptor gene in frequently ill children is increased in comparison with the control, but in the case of the Asp / Gly genotype to a lesser extent (9.9 pkg / ml) than in the Gly / Gly genotype 12.34 pkg / ml).

The concentration of chemokine IL-8 in ARVI is high regardless of the presence or absence of mutations in the gene.

Synthesis of anti-inflammatory cytokine IL-4 in the first days of the disease does not change and the polymorphic variants of the Toll-4 gene do not affect it.

The concentration of IL-10 increases in patients with ARVI - the carriers of all studied genotypes.

The level of IL-1RA is significantly elevated in the blood of patients with Gly / Gly genotype carriers. The concentration of IL-1RA in them is very high and amounts to (1984.3 pkg / ml).

Trying to relate the genetic anomalies of Toll-4 to the synthesis of inflammatory mediators, we were able to note a unidirectional decrease in the concentration of IL-1β in the Asp / Gly and Gly / Gly genotypes compared to the Asp / Asp genotype.

More precise results were obtained in the analysis of cytokine reactions in patients - carriers of polymorphism of the Toll-6 receptor gene.

In patients with all polymorphic variants in the Toll-6 gene, the level of the pro-inflammatory cytokine IL-1ß was significantly increased in comparison with the control group. Carriers of the normal homozygous genotype Ser / Ser exhibited the highest cytokine values, in comparison with the rest of the groups (Table 2).

The concentration of TNF-α in the group of patients with the Pro / Pro genotype increased by 11.7 pkg / ml in comparison with the control group (2.3 pkg / ml), but was lower than for carriers of the normal Ser / Ser genotype - 14.3 pkg / Ml. When assessing the level of IL-8, it was found that the concentration of chemokine in children with the Pro / Pro genotype was the lowest (16.8 pkg / ml). In Ser / Ser genotype carriers -22.6 pkg / ml, in carriers of the Ser / Pro genotype - 17.7 pkg / ml.

IL-4 had no distinct differences depending on the polymorphic variants. In children bearing the homozygous Pro / Pro genotype, the concentration of IL-4 was 1.3 pkg / ml, in children with the Ser / Ser genotype - 1.2 pkg / ml, in the control group - 1.9 pkg / ml.

The IL-10 content was highest in children with a normal homozygous genotype Ser / Ser of 3.7 pkg / ml, in heterozygotes somewhat lower - 3.2 pkg / ml. With the replacement of alleles (genotype Pro / Pro) the concentration of IL-10 decreased to 2.2 pkg / ml.

The concentration of IL-1RA is very high in all polymorphic variants of the Toll-6 gene and, especially, in the genomes Pro / Pro-1404 pkg / ml.

Conclusion. Thus, our studies showed that the functional insufficiency of antiviral protection arises at the level of synthesis of cytokines that are regulators of inflammatory reactions in children often sick with acute respiratory viral infection carriers of gene polymorphisms (Asp299Gly) Toll-4 and (Ser249Pro) Toll-6. Although the total number of pro- and anti-inflammatory cytokines in the first days of infection increases, it does not reach the values of diseased children without abnormal impairments in signaling receptors.

The defect of signaling reactions is

Table 1

The content of cytokines in patients with carriers of polymorphic alleles of Asp299Gly in the gene Toll-4 receptors (median, 25-75 percentile) (pkg / ml)

Cytokines	Здоровые дети (n=76) (1)	Asp/Asp (n=40) (2)	Asp/Gly (n=18) (3)	Gly/Gly (n=32) (4)
IL-1β	5,4 [3,6-6,5]	17,6* [12,9-22,1]	14,6* [9,9-20,5]	13,9* [11,2-17,5]
IL-8	7,9 [6,9-9,1]	21,8* [12,3-22,2]	20,3* [13,1-21,9]	27,7* [11-29,2]
ФНОα	2,3 [1,5-2,5]	11,3* [8,25-14,3]	9,9*# [8,0-12,1]	12,4* [11,4-15,4]
IL-4	1,9 [1,2-2,5]	1,5 [0,85-2,02]	1,4 [0,9-2,09]	1,7 [1,3-2,09]
IL-10	1,2 [0,7-1,8]	2,6* [1,7-3,9]	2,9* [1,5-2,8]	2,4* [1,9-4,2]
IL-1RA	342,9 [263,8-465,4]	1571* [1179-1649]	1139,2*# [1050-1578]	1984,3*# [1214-2033]

Note: U is Mann Whitney's criterion; * - the significance of differences in comparison with the control. # - significance of differences in comparison with the group of carriers of the homozygous Asp / Asp genotype.

Table 2

The content of cytokines in patients with ARVI - carriers of polymorphic alleles Ser249Pro In the Toll-6 receptor gene (median, 25-75 percentile) (pkg / ml)

Cytokines	Здоровые дети (n=76) (1)	Ser/Ser (n=25) (5)	Ser/Pro (n=50) (6)	Pro/Pro IL-1β (n=25) (7)
IL-1β	5,4 [3,6-6,5]	21,9* [20,6-23,3]	18,2*# [13,4-25,2]	15,4*# [10,3-21,9]
IL-8	7,9 [6,9-9,1]	22,6* [19,5-27,2]	17,7*# [12,9-19,8]	16,8*# [14,4-19,9]
ФНОα	2,3 [1,5-2,5]	14,3* [11,9-16,5]	12,3*# [8,8-14,5]	11,7*# [10-14,7]
IL-4	1,9 [1,2-2,5]	1,2 [0,8-1,6]	1,6 [1,4-1,9]	1,3 [0,9-1,7]
IL-10	1,2 [0,7-1,8]	3,7* [2,4-4,7]	3,2*# [2,1-4,1]	2,2*# [1,1-4]
IL-1RA	342,9 [263,8-465,4]	1391* [1207-1536]	1249,2*# [1053-1577]	1404,3*# [1193-1817]

Note: U is Mann Whitney's criterion; * - the significance of differences in comparison with the control. # - significance of differences in comparison with the group of carriers of the homozygous Ser / Ser genotype.

exacerbated by a high IL-1 receptor antagonist (IL-1RA), which inhibits the Th-1 cellular pathway of immunity. In addition, IL-10 and IL-1RA are suppressive factors. Their high concentration limits the development of protective antiviral reactions, which complicates the course of the inflammatory process.

CONCLUSIONS

1. In ARVI, the dynamics of cytokines has its own variations depending on the presence or absence of genetic defects in Toll-receptors that perceive the action of the pathogen.

2. Point polymorphism in the genes of Toll-4 and Toll-6 receptors is one of the reasons for the failure of antiviral infection in children who often have ARV.

3. The presence of polymorphism of the signal receptor genes will make it possible to single out a special group of dispensary patients for the organization of personal prophylaxis for routine recurrences of acute respiratory viral infection.

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