



FEATURES OF CHANGES IN IMMUNE HOMEOSTASIS IN PATIENTS WITH BRONCHIAL ASTHMA

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ABSTRACT: 92 patients with bronchial asthma (BA) were examined. Among them, 48 patients with allergic BA (52.2%), 12 patients with non-allergic BA (NBA) (13%) and 28 patients with mixed BA (SBA) (26.8%). In 71 (77.2%) patients, the hereditary history was delayed. Determination of total IgE in blood serum was carried out by the ELISA method using a set of the company "Hema-Media", the level of IFN- γ , IL-4 in blood serum using test systems for quantitative determination by the solid-phase ELISA method (CJSC "Vector-Best", Russia). Genotyping of DNA samples was performed by PCR amplification.

The results obtained show a certain dependence of the levels of total IgE, IL-4, and IFN-g in patients with BA with a pathogenetic variant of the disease, the distinctness of which is more pronounced in patients with ABA. In patients with ABA and SBA, there is a pronounced inhibition of IFN- γ production, while in patients with BA with a predominantly allergic mechanism of the disease, a significant increase in the level of IL-4 is recorded. According to the results of our studies, there was no significant relationship between the Gln27Glu polymorphism of the β 2-Barenoreceptor gene and the activity of inductive cytokines. The Gln27Gln genotype of the β 2-Barenoreceptor gene in patients with BA and, especially, in the allergic form of the disease, is associated with IgE hyperproduction.

Keywords: bronchial asthma, Gln27Glu polymorphism of the β 2-Barenoreceptor gene, cytokines IL-4, IFN- γ , IgE.

I. Introduction

The increase in the incidence of bronchial asthma (BA), along with an increase in the number of patients with severe asthma, indicates the need to disclose the features of the body's homeostasis and its immunoreactivity in response to the influence of pathogenic factors in the formation of the pathological process in the lungs. The study of immune regulatory mediators in patients with BA, on the one hand, contributes to obtaining information about the functional activity of various types of immunocompetent cells, the severity of the inflammatory process, the ratio of Th1 and 2-type activation processes in various variants of the course of the pathological process, and on the other hand, to investigate the involvement of specific immune links in the hereditarily determined mechanisms of the pathogenesis of the disease [1,3,4,5,7,10].

A large number of different inflammatory mediators take part in the formation of inflammation, their functions are interdependent in many ways, but the influence of the inflammatory process can be judged with a certain degree of probability by some cytokines that play a key role in the development of the inflammatory response. The systemic content of IL-4 and IFN- γ in the blood serum of patients with BA, as many authors note, reflects the course of the disease, and with the exacerbation of BA, an imbalance in the content of these cytokines is observed [1,3,4,5]. Th2 helper cells, which are regulators of IgE synthesis, produce IL 3, 4, 5, 6, 9, 10 and 13, and Th1 secrete IFN- γ , and IL-2, TNF- α , and- β . The ratio of these cells is the main factor in the regulation of IgE production [11, 12].

With the development of the Human Genome program, it became possible to conduct so-called genome-wide searches. As a result of these studies, candidate sites of structural changes that contribute to the formation of BA were verified. As candidate genes for IL-4, β 2-BArenoreceptor (BARB2), TNF- β , IFN- γ , T-cell receptors, mast cell chymase, and others [13,15].

It is known that the activation of BARB2 leBAs to a rapid relaxation of the smooth muscle cells of the bronchi and an increase in the lumen of the airways, so in BA, much attention is drawn to the possible violations of BARB2, which may underlie the pathogenesis of the disease. In this regard, the search for the association of the BARB2 gene with a predisposition to BA has become the task of many studies [2,6,8,9,16].

Objective: to study the various forms of BA in relation to the features of immune homeostasis and to assess the contribution of hereditary mechanisms in the pathogenesis of the disease.

II. Material and methods of research.

92 patients with BA were examined. Patients with BA were divided into groups according to the WHO international classification and in accordance with the GINA diagnostic criteria. For a comparative analysis of clinical and pathogenetic variants of BA, 48 patients with allergic BA (37%), 12 patients with non-allergic BA (NBA) (13%) and 32 patients with mixed BA (SBA) (26.8%) were identified based on differential diagnostic criteria. The average age of the patients was 41.6 ± 1.33 years. The duration of the disease, on average, was 11.3 ± 0.68 years. The control group consisted of 25 practically healthy individuals.

The examination of all patients was carried out using generally accepted clinical and laboratory-

instrumental methods of research. Immunological methods of research were carried out in the laboratory of immunocytokines of the Institute of Immunology of the AcBAemy of Sciences of the Republic of Uzbekistan. Determination of total IgE in blood serum was carried out by the ELISA method based on the principle of a two-layer enzyme immunoassay using a set of the company "Hema-Media". The determination of the level of IFN- γ in the blood serum was carried out using the test systems of quantitative determination by the method of solid-phase ELISA "ELISA-IFN-gamma" (CJSC "Vector-Best", Russia). The level of IL-4 in the blood serum was determined by the ELISA method using the test systems "ELISA-IL-4" (CJSC "Vector-Best", Russia).

DNA extraction from whole blood was carried out with a set of Diatom™ DNA Prep 200 reagents (manufactured by IsoGen Laboratory LLC). DNA isolation was performed according to the standard DNA isolation protocol using the Diatom™ DNA Prep 200 reagent kit. The DNA supernatant was then directly genotyped by PCR amplification.

III. Results

We conducted studies of the serum levels of total IgE and cytokines - antagonists of IL-4 and IFN- γ in patients with BA. Analysis of the level of serum immunoglobulins showed that the total IgE content in the examined cohort of patients with BA was 769.7 ± 123.37 pg / ml, which is significantly higher than in the group of practically healthy individuals (221.3 ± 66.11 pg / ml; $P < 0.01$). As it turned out, the level of IgE varies significantly in the compared groups of patients with different forms of BA. This indicator was highest in patients with ABA (1201.3 ± 233.97 pg / ml), significantly different from the parameters of the healthy group (221.3 ± 66.11 pg/ml; $P < 0.001$) and patients with NBA (236.9 ± 37.49 pg/ml; $P < 0.001$). In patients with SB, the IgE level was also almost three times higher than in the control group ($P < 0.05$).

Analysis of the IL-4 content in the examined patients with BA showed that its level is 13.9 ± 1.92 pg / ml and significantly exceeds the values characteristic of practically healthy individuals (5.1 ± 1.88 pg/ml, $P < 0.001$). When comparing the studied parameters in groups of patients with different pathogenetic variants of the disease, it was revealed that the highest level of IL-4 was registered among patients with ABA. In this group, the IL-4 content is 16.4 ± 1.49 pg / ml, which is more than 3 times higher than in the control group. In contrast, IL-4 levels were relatively low in NBA and hBA intermediate values in patients with SBA.

The level of IFN-g in patients with BA was, on average, 0.14 ± 0.013 pg / ml, which is almost twice lower than the values of the control group ($P < 0.05$), while its lowest values are recorded in patients with ABA (0.08 ± 0.02 pg/ml; $P < 0.05$). It should be noted that the IFN-g parameters in patients with NBA and ABA differ almost three times (0.24 ± 0.04 pg / ml and 0.08 ± 0.02 pg / ml, respectively; $P < 0.05$).

Analysis of the frequency of alleles and genotypes of the Gln27Glu polymorphism of the BARB2 gene among patients, depending on the clinical variants of the pathological process, showed that the Gln27 allele in the group of patients with ABA is significantly more common than in the group of practically healthy individuals (86% compared to 70.2%, respectively, $\chi^2 = 4.2$; $P < 0.05$). Consideration of genotype polymorphism showed that in this subgroup of patients, the frequency of the homozygous variant of the Gln27Gln gene significantly exceeds that of the control group of healthy individuals

(73% vs. 44.6%, respectively, $\chi^2 = 4.7$; $P < 0.05$). At the same time, the frequency of Gln27Glu heterozygotes in the group of patients with ABA was significantly lower than the level typical for the healthy part of the examined population (27% compared to 51%, respectively, $\chi^2 = 3.5$). Variants of the Glut27Glu homozygous genotype were not observed among patients with ABA.

The following values for the frequency of polymorphic markers were found in patients with NBA: Gln27 allele-73.5%, Glut27-26.5%; Gln27Gln genotype – 47%, Gln27Glu-53%, patients with Glut27Glu genotype were not noted. In patients with SB, these indicators, respectively, were: 69%, 31%; 48%, 43% and 9%. Thus, the analysis of the genotype features in the groups of patients with NBA and SBA showed no significant statistically significant differences in the frequencies of alleles and genotypes of the Gln27Glu polymorphism of the BARB2 gene compared to the control group of healthy individuals.

It was important to study the features of the production of total IgE and immunoregulatory cytokines in patients depending on the genotypes of the Gln27Glu polymorphism of the BARB2 gene. It was found that in the general group of BA patients with Gln27Gln polymorphism, the average level of total IgE is 996.9 ± 186.49 pg / ml, significantly exceeding the indicators of the group with the intermediate Gln27Glu genotype (453.7 ± 125.59 pg/ml; $P < 0.05$).

In the group of ABA patients with Gln27Gln polymorphic variant of the BARB2 gene, IgE values (1664.2 ± 293.29 pg/ml) were almost four times higher than the level recorded in patients with this form of the disease with the Gln27Glu genotype (338.4 ± 121.33 pg/ml; $P < 0.01$). According to the results of our studies, there was no significant correlation between the Gln27Glu polymorphism of the BARB2 gene and the activity of inductive cytokines.

Conclusion

In general, our results show a certain dependence of the levels of total IgE, IL-4, and IFN- γ in patients with BA with a pathogenetic variant of the disease, the distinctness of which is more pronounced in patients with ABA. The serum level of mediators in patients with BA with various forms of the disease is characterized by multidirectional changes in the production of both pro-inflammatory and anti-inflammatory cytokines: in patients with ABA and SB, there is a pronounced inhibition of IFN- γ production, while in patients with BA with a predominantly allergic mechanism of the disease, a significant increase in the level of IL-4 is recorded. In the family aggregation of cases of the disease among patients with BA, there is a marked increase in the level of serum IgE.

Taking into account the role of cytokines in the development of allergic inflammation, we studied the association of alleles and genotypes of the polymorphic DNA locus of the BARB2 gene with IL-4, IFN- γ , and IgE levels in various pathogenetic variants of BA in patients with severe persistent course. When analyzing the immunological parameters of BA patients, depending on the polymorphism of the BARB2 gene, some differences in the distribution of alleles and genotypes were revealed. Thus, the association of this genotype with IgE hyperproduction was established in patients in the general group of BA, patients with ABA, who mainly have the Gln27Gln genotype.

The results of the study of serum levels of IgE and cytokines - antagonists of IL-4 and IFN- γ , the producers of which are Th2 and Th1, in the pathogenetic forms of BA, namely ABA, NBA and SBA, in relation to the polymorphism of the BARB2 gene, indicate a multivariate nature of immunological insufficiency in BA. This also reflects the relationship of immunological defects with the clinical and genetic polymorphism of the studied pathology, i.e., indicates the genetic determinism of the pathogenetic forms of BA.

Thus, the study of the cytokine profile and a differentiated approach to patients with BA, depending on the clinical and pathogenetic variant of the disease, increases the possibility of diagnosis, prognosis of the course of BA, necessary to achieve control over the disease and contributes to the optimization of therapy.

References:

1. Dugarova I. D., Anew E. H., Chuchalin A. G. On the role of cytokines in bronchial asthma // Pulmonology, 2009. - No. 4.-P. 96-102.
2. Kurenkeeva A. K., T. M. Sooronbaev About Pak. A. Hyperreactivity bronchi and its relationship with gene polymorphism β 2-BAREnergic receptors in patients with bronchial asthma Kyrgyz // Pulmonology. - 2006. - No. 1. - pp. 76-80.
3. Turdibekov Kh. I., Umarova A. A., Khaitova N. M., Aripova T. U., Petrova T. A. Study of the serum level of immunoregulatory mediators in various variants of severe bronchial asthma. - Moscow, 2008. - No. 6. - pp. 354-356.
4. Barnes P. J. Targeting of cytokines for the treatment of asthma and chronic obstructive pulmonary disease // Nat. Rev. Immunol. 2018; 18: 454-466.
5. Che X. W., Zhang Y., Wang H., Wang W. The effect of injection ligustrazine on levels of interleukin-4 and interferon-gamma in patients with bronchial asthma // Chin. J. Integr. Med. – 2008. - T. 14, №3. – p. 217-220.
6. Dewar J. C., Wheatley A. P., Venn A. β 2-BAREnoreceptors polymorphism is in disequilibrium, but is not associated with asthma in the BAult population // Clin. Exp. Allergy. - 1998. - Volume 28. - pp. 442-448.
7. Godard, M., Desvart, K., Vergoth, K., Saunders, M., de Haard, H., HammBA, H., Blanchett, S., Lambrecht, B. N. Bispecific antibody strategy for targeting multiple type 2 cytokines in asthma. Immunol. 2018; 142: 1185-1193. e 4.
8. Hakonarson H., Bjornsdottir U. S., Ostermann E. Allelic frequencies and patterns of single-nucleotide polymorphisms in candidate genes for asthma and atopy in Iceland // Am J. Respir. Crete. Care Med. - 2001. - Volume 164. - p. 2036-2044.
9. Holloway, J. W., Dunbar, P. R., and Riley, G. A. Association of β 2-BAREnoceptor polymorphisms with bronchial asthma. Exp. Allergy. - 2000. - Vol. 30, No. 8. - pp. 1097-1200.
10. Lambrecht, Bart and HammBA, Hamida and Fahi, John. Cytokines of bronchial asthma // Immunity. 2019; 50. 975-991.
11. Lambrecht B. N., HammBA H. Immunology of bronchial asthma // Nat. Immunol. 2015; 16: 45-56.

12. Mishra JK, Rashid Parvez, Usha, Girish Singh. Immunological study of bronchial asthma with special attention to interleukin-4, interferon- γ and immunoglobulin E / / Journal of Public Health Management. 2015; 02(1), 47-50.
13. Reichsauss, E., Innis, M., McIntyre, N., and Liggett, S. B. Mutations in the beta-2-BArenergic receptor gene in normal and asthmatic patients. Cellular Mole. Biol. - 1993. - Vol. 8. - p. 334-349.
14. Santillan A. A., Camargo K. A. Jr., Ramirez-Rivera A. Association between beta2-BArenergic receptor polymorphisms and asthma diagnosis in BAult Mexicans. The wedge. Immunol. - 2003. - T. 112. - R. 1095-1100.
15. Turdibekov Kh. I., Ziyadullaev Sh. Kh., Holliiev R.Kh The significance of the β 2-BArenoreceptor gene polymorphism molecular and genetic mechanisms of bronchial asthma formation// International Journal of Pharmaceutical Research//January - March 2020, Volume 1 2, Issue 1. pp. 1243-1249
16. Wang Z., Chen S., Niu T. Association of asthma with B2-BArenoreceptor gene polymorphism and cigarette smoking. Crete. Care Med. - 2001. - Vol. 163. - p. 1404-1409.

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