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ANTIGENS OF THE BRONCHOPULMONARY SYSTEM AND THEIR ROLE IN THE DIAGNOSIS OF AUTOIMMUNE PROCESS IN CHILDREN WITH BRONCHIAL ASTHMA

Chernusky V. G., Popov N. N., Govalenkova O. L., Letyago A. V., Kashina-Yarmak V. L., Evdokimova T. V.

V. N. Karazin Kharkiv National University

Bronchial asthma (BA) in children is considered to be a chronic inflammatory disease of the respiratory tract causing the damage of bronchial epithelium, which leads to the development of autoimmunity and bronchi remodulation. At present research is intensively being conducted to develop diagnosticums derived from different cell and tissue structures of the respiratory system to use them in children with asthma.

The paper provides the data to identify the autoimmune process in the bronchopulmonary system in children with asthma, aged 5 to 14 in the period of exacerbation. The control group consisted of 25 healthy children aged from 7 to 14 years old.

It has been shown that lipopolysaccharide antigens from bronchopulmonary interstitial connective tissue structures derived from children, who died accidentally, have higher specificity compared to protein antigens and allow to identify morphological changes in the bronchopulmonary system, the degree of severity, and to monitor the effectiveness of therapy.

Key words: bronchial asthma, children, protein antigens, lipopolysaccharide antigens, autoimmune process.

According to modern concepts bronchial asthma (BA) is considered to be a chronic allergic inflammation of the respiratory system, the fundamental importance in its development is given to immune pathological reactions in cell and tissue structures of the tracheobronchial tree [2-4].

In this regard, autoimmune reactions in cell and tissue structures of the bronchopulmonary system are considered to play a role in the pathogenesis of asthma in children. Therefore, the creation of tissue antigens from the structures of the respiratory system and their use in diagnostic assays to detect irregularities in the structure of the trachea, bronchi and lung tissue during the development of the disease has recently been recognized to be one of the promising areas in the improvement of BA immunodiagnostics [1, 8].

The literature provides information on bronchopulmonary antigens used in the experiment and clinic for BA diagnosis. The comparative analysis has shown that the diagnostic value of antigens derived from the bronchopulmonary structures is proportional to the purity of antigenic preparation [1, 3, 9].

The purpose of the research was to study the role of antigens of the respiratory system in the diagnosis of autoimmune process in children with bronchial asthma.

Materials and methods

The serum autoantibodies were studied in 97 children with asthma who had been hospitalized to the pulmonology department of Children's Hospital of Kharkiv Railway Clinic in the period of exacerbation. Methodology and methods of the study were based on consensus provisions of the medical bioethics and principles of evidence based medicine.

The age of children who were examined in the period of exacerbation was between 5 and 14 years old. The control group consisted of 25 healthy children aged from 7 to 14 years old. 35 children had non-allergic form of asthma (NABA), 32 children had atopic asthma (ATBA), 30 children – mixed asthma (MBA). The diagnosis was made according to the classification adopted by the Congress of Pediatricians and approved by the Order of the Ministry of Health of Ukraine of 14.12.2009 № 04.01.12-8-1178 and IDC-10. The treatment groups were representative and randomized by age, gender, forms and degrees of severity of the disease.

Protein water-salt antigens from the epithelial cells of the bronchial mucosa were obtained by the method of E. F. Chernushenko et al. [11]. Antigenic material used in the studies was sectioned samples of the bronchial tubes, taken from children with I (0) blood type, who had died accidentally, 2-4 hours after the death. Supernatants, microsomes, nuclei, mitochondria derived from epithelial cells of the bronchial mucosa were used as tissue antigens. Antigenic activity of tissue antigens was established according to quantification of protein by the method of E. F. Chernushenko et al. [11]. Lipopolysaccharide antigens from homologous cell and tissue structures of the trachea, bronchi and lungs were obtained by the method of V. D. Yakovenko et al. [10]. The level of autoantibodies in the blood serum to water-salt protein antigens was determined in the reaction of passive hemagglutination (PHA) by the method of E. F. Chernushenko, L. S. Kogosova [11]. Quantitative determination of autoantibodies to protein and lipopolysaccharide antigens was performed using nephelometric reaction of Hoigné in modification of V. V. Kvirikadze et al. [6]. Mathematical processing of the research findings was carried out by conventional statistical methods using the Student's t-test [7].

Results and discussion

Despite the multi-faceted use of diagnostic tissue antigens when evaluating clinical forms and degrees of asthma severity, we proceeded from the fact that they were able, first of all, to reflect the autoimmune reactions determining the pathogenesis of the disease.

Thus, we applied one of the immunological techniques – the reaction of passive hemagglutination (PHA) – in order to identify the autoimmune process in the bronchopulmonary system in 97 children with asthma, aged 5 to 14 years old during the exacerbation.

The results of the study (in which the blood serum of children with BA was used) showed that the obtained diagnosticums were characterized by mild precipitinogenic properties, so they could not be used in PHA reaction for diagnostic purposes (see Table 1). This was primarily reflected in the low percentage of positively reacting sera of children with BA (23.7% – 58.1%). It was found that the frequency of positive results when using serum of the

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control group of healthy children was significantly higher than in children with BA for two of the four tested diagnosticums (antigens of nuclear substance and mitochondria) of the bronchial mucosa cells (see Table 1).

A comparison of the data obtained in clinical and laboratory examination of children suffering from asthma (CBC, total protein and protein fractions of the blood serum, immunograms), with antibody titers and the studied antigens of the bronchial mucosa cells showed that they were not able to detect autoimmune disorders in serological tests in this group of children. It should also be noted that except the diagnosticum of supernatants and mitochondria of the bronchial mucosa cells, all the other antigens had extremely low antigenic activity in PHA reaction, providing a positive result (1: 0,87-1: 1,38) that fully excludes their diagnostic significance. It was found that this group of diagnosticums chemically presented by protein components on the antigenic activity did not significantly differ among themselves in their ability to react with the blood serum of children with asthma (see Table1). It also negatively characterizes their prospect of target use. It was shown that the antigens of supernatant and mitochondria of bronchial mucosa cells in PHA with sera of children with asthma produced the relevant specific autoantibodies in the titers: supernatants – 1:75,4-1:108,2; mitochondria – 1: 58,9-1: 82,4. Therefore, they cannot be considered diagnostically promising, because the differences in the groups of patients with asthma were insignificant and, in addition, they were significantly reduced as compared to the data from control group of healthy children (see Table 1). The conclusion from the results of the study is that the protein antigens of the bronchial mucous cells used as diagnosticums are not able to stimulate the production of bronchopulmonary autoantibodies involved in the manifestation of autoimmune process in children with asthma.

The presence of positive results in clinically healthy children in PHA reaction with the tissue antigens obviously needs to be explained. The autoantibodies detected with the tissue antigens may seem to refer to a group of so-called normal (physiological) autoantibodies regulating the main morphofunctional manifestations of the bronchial tree in the conditions of physiological normalcy. Then the reduction of titers of these autoantibodies in children with asthma can seemingly be assessed as an indication of the overall reduction of the general immunological reactivity of the body and development of the secondary immunodeficiency. Low diagnostic ability of the tested antigens of the bronchial mucosa cells in PHA reaction is obviously connected with the fact that asthma in children is a chronic non-specific inflammatory process, which is based on the initial changes in the interstitial stroma of the bronchopulmonary system. It is known that cellular elements of the connective tissue are responsible for the development of the inflammatory process, which is a nonspecific response of the body directed to localization and elimination of the damaging factor. The nature of the immunological changes essentially depends on the adequacy of the reactive response of the elements of interstitial connective tissue and its loose structures (microphages, macrophages, eosinophils, lymphocytes), i.e., they can generate immunopathological processes, based on the regulating effect of the immune system on the excessive proliferation of cultured cells of the connective tissue elements of the bronchopulmonary system.

It means that the characteristics of asthma development in children can be identified through the use of tissue antigens, derived from the interstitial connective tissue stroma of the bronchopulmonary system in the relevant immunological tests. Anticipating the natural question of the specificity of these antigens, we can say that, in accordance with the compelling data of the literature, the elements of human interstitial stroma possess a strict antigenic and chemical specificity. In order to study this, we have tested the antigens of the trachea, bronchi and lung tissue obtained from the interstitial connective tissue of the structures of the bronchopulmonary system taken from children with I (0) blood type who had died accidentally. As a result, the tested antigens had a chemical composition, generally presented by lipopolysaccharides. Proteins were absent in the composition of antigenic preparations; the peptides were identified only as traces. Quantitative determination of antibodies in the serum of children suffering from asthma was performed using Hoigné reaction in modification of V. V. Kvirikadze et al. [6].

The immunological study of 97 children with asthma aged 5 to 14 years old in the period of exacerbation of the disease and the control group of 25 healthy children, using the method of photometric determination of immune antibodies in the blood serum, showed that lipopolysaccharide antigens of the trachea, bronchi and lung tissue, as compared to protein antigens of the bronchial mucosa cell structures, had a high antigenic activity and ability to detect differences in differential diagnosis of clinical forms of asthma in children (see Table 2).

Thus, the studies have shown that all the clinical forms of BA in children are characterized as an immunopathological process based on the initial inflammatory changes in the bronchopulmonary system, inductively predefined by different factors, including the infectious ones.

Pathogenetic characteristics of BA are determined by the productive type of recurrent inflammatory reaction manifested by activation and uncontrolled proliferation of interstitial cells of the connective stroma of bronchopulmonary structures. Therefore, the main range of immunological and immunopathological reactions in children with asthma has a clear antigenic dependence on the interstitial stroma of the bronchopulmonary system activated by inflammation. Changes in the cellular structures of the bronchial mucosa have no antigenic specificity. Therefore, bronchopulmonary antigens of protein nature, derived from different structures of the bronchial mucosa cells, usually cannot objectively diagnose the level of inherent immunopathological disorders in children with asthma.

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Table 1. Specific activity of protein antigens derived from epithelial cells in mucosal bronchial asthma in children, exacerbation (M ± m)

Clinical diagnosis	The titer of TPHA							
	The supernatants		Microsomes		Nuclei		Mitochondrions	
	the number of positive responders, %	(M ± m)	the number of positive responders, %	(M ± m)	the number of positive responders, %	(M ± m)	the number of positive responders, %	(M ± m)
Control group, n=25	0	0	23,7	1:0,96±0,89 E_x=0,018	63	1:3,74±1,98 E_x=0,022	100	1:122,3±63,2 E_x=0,35
NABA, n=25	54,6	1:108,2±17,5* <0,05 E_x=0,38	32,5	1:1,24±0,19 <0,05 E_x=0,012	45,2	1:1,68±0,41 <0,05 E_x=0,016	57,4	1:68,7±20,0 <0,05 E_x=0,27
MixBA, n=30	50,2	1:87,2±20,3* <0,05 E_x=-0,32	37,9	1:1,08±0,21 <0,05 E_x=-0,014	43,7	1:1,94±0,52 <0,05 E_x=-0,016	56,2	1:82,4±19,6 <0,05 E_x=-0,26
ATBA, n=32	56,8	1:75,4±19,7* <0,05 E_x=0,29	35,6	1:0,96±0,18 <0,05 E_x=0,15	46,3	1:1,57±0,43 <0,05 E_x=0,17	58,1	1:69,5±17,2 <0,05 E_x=0,20

Notes:
*1. * – valid difference of indicators determined in the control group of healthy children (P<0,05).*
2. Ex – indicator of sample distribution normalcy (Ex=0).

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Table 2. Comparison of protein activity and lipopolysaccharide antigens from the mucosal epithelial cells of the bronchi in reactions quantifying autoantibodies in the blood serum of children suffering from asthma exacerbation in (M ± m), cond. u

View broncho-pulmonary antigens	Clinical forms			
	Control group, n=25	NABA, n=35	MixBA, n=30	ATBA, n=32
	n = 25	n = 35	n = 30	n = 32
Protein antigens from the mucosal epithelial cells of the bronchi				
The supernatants	0,032±0,006	0,067±0,012 <0,05 E_x=0,015	0,099±0,021* <0,05 E_x=0,017	0,069±0,015 <0,05 E_x=0,020
Microsomes	0,025±0,004	0,032±0,007 <0,05 E_x=0,011	0,028±0,008 <0,05 E_x=0,09	0,032±0,007 <0,05 E_x=0,010
Nuclei	0,027±0,002	0,033±0,010 <0,05 E_x=0,06	0,030±0,005 <0,05 E_x=0,09	0,031±0,009 <0,05 E_x=0,014
Mitochondrions	0,048±0,009	0,059±0,014 <0,05 E_x=0,021	0,061±0,010 <0,05 E_x=0,018	0,067±0,018 <0,05 E_x=0,029
Lipopolysaccharide antigens of bronchopulmonary structures				
Antigen trachea	0,030±0,007	0,109±0,021* <0,05 E_x=0,23	0,171±0,018$^{@}$ <0,05 E_x=0,28	0,096±0,012* <0,05 E_x=0,06
Antigen bronchus	0,028±0,003	0,238±0,038$^{\#}$ <0,05 E_x=-0,18	0,252±0,047 <0,05 E_x=-0,20	0,188±0,016$^{@}$ <0,05 E_x=-0,19
Antigen lung tissue	0,043±0,006	0,292±0,042* <0,05 E_x=0,25	0,265±0,039* <0,05 E_x=0,19	0,221±0,043* <0,05 E_x=0,31

*Notes: 1. Q_φ – indicator of immune antibodies in conventional units. Q_φ= 0,0004 – 0,1236 – backlash; Q_φ= 0,1634 – 0,6411 – positive reaction; Q_φ= 0,1237 – 0,1633 – weak positive reaction; Q_φ= 0,6412 – 1,4248 – sharply positive reaction. 2. * – Significant differences of indicators from a group of healthy children (P<0,05); $^{@}$ – Significant differences from the indicators MixBA from a ATBA (P<0,05). $^{\#}$ – Significant differences from the indicators NABA from a ATBA (p<0,05). 3. E_x – indicator normal sample distribution (E_x=0).*

Clinical and immunological examination found that lipopolysaccharide antigens derived from connective tissue structures of the trachea, bronchus and lung tissue can accurately detect the presence and severity of autoimmune process, inflammatory and proliferative changes in the bronchopulmonary system.

This determines their useful application in clinical practice for immune diagnostics of clinical forms of asthma.

Conclusions

1. Antigens of protein nature derived from sectioned material of the bronchopulmonary system cannot be obtained industrially, as they are not standardized by protein and not sterile, which greatly limits their use and reduces the diagnostic value.

2. Multi-antigenic composition of protein homogenates derived from cell and tissue structures of the bronchopulmonary system does not allow to clearly identify the nature of morphological changes in children with BA.

3. Lipopolysaccharide antigens from bronchopulmonary structures do not contain protein in their composition, so they can be prepared industrially under sterile conditions and stored in a lyophilized state for more than two years, which allows to use them widely in immunodiagnostics of the clinical forms and degrees of asthma severity in children.

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ANTIGENS BRONCHOPULMONARY SYSTEM AND THEIR ROLE IN THE DIAGNOSIS OF AUTOIMMUNE PROCESSES IN BRONCHIAL ASTHMA IN CHILDREN

Chernusky V.G., Popov N.N., Govalenkova O.L., Letyago A.V., Kashina-Yarmak V.L., Evdokimova T.V.

Introduction. One of the promising directions of improvement of immunodiagnostic BA recently recognized the creation of tissue antigens of the respiratory system structures and their use in diagnostic assays to detect subtle disturbances in the structure of the respiratory system in the development of this disease. Each of the body tissue has an extremely wide range of proteins that characterize its species and organ specificity of the individual. It follows that satisfactory diagnostic results can be obtained only if the isolation of individual proteins characteristic lesion of certain components of the trachea, bronchus and lung tissue. **Materials&methods.** The results of studies using the blood serum of children with asthma during exacerbations, showed that the resulting diagnostic tools are characterized by mild pretsipitogennymi properties, in consequence of which they can not be used in the passive hemagglutination (PHA) response for diagnostic purposes, since there is a low the percentage of positive reacting sera from children with asthma (23.7% - 58.1%). Established that were significantly higher than in children patients with asthma for two of the four test diagnostics (nuclear antigen substance and mitochondria) cells of the bronchial mucosa frequency of positive results with serum of healthy children of the control group (63.0 - 100%). It is emphasized that except diagnosticum constituting supernatants mitochondria and bronchial mucosa cells, all other antigens differed in TPHA antigenic activity is extremely low, providing a positive result (1: 0,87 - 1: 1,38), which eliminates them diagnostic significance. It is found that the diagnostic tools derived from the cells of the bronchial mucosa, chemically presented protein components did not significantly differ among themselves antigenic activity in children with asthma, which also negatively affects the possibility of their intended use. On the supernatant antigens and mitochondria of cells in bronchial mucosa RPGA with sera of children with asthma autoantibodies determined in the following credits: supernatants - 1:75,4-1:108,2; mitochondria - 1: 58,9-1: 82,4. These results indicate that the test protein as diagnostics antigens bronchial mucosa cells are not able to stimulate development of bronchopulmonary autoantibodies involved in autoimmune manifestations component in children with asthma. Low diagnostic ability of the tested antigens of bronchial mucosa cells in PHA, obviously, is connected with the fact that asthma in children is a chronic non-specific inflammatory process, which is based on the initial changes in the interstitial stroma bronchopulmonary system. Features of asthma development in children can be identified by the use of immunologically relevant tests in tissue antigens, derived from the interstitial connective tissue stroma of bronchopulmonary system. We have tested antigens trachea, bronchi, lung tissue derived from connective tissue interstitial pulmonary system data structures. Antigens were presented lipopolysaccharide. Proteins are composed of antigenic preparations were absent, the peptides were determined only in trace amounts. This diagnostic kit used in the reaction aggregates quantitation immune serum of children with asthma. Immunological examination showed that lipopolysaccharide antigens of the trachea, bronchus and lung tissue, as opposed to protein antigens of cell structures of the bronchial mucosa, possessed high antigenic activity and the ability to detect differential diagnostic differences of clinical forms and degrees of severity of the disease. Thus, the main range of immunological and immunopathological reactions in asthma in children has a clear dependence on the antigen-activated interstitial stroma inflammatory bronchopulmonary system. **Conclusions.** 1. Antigens derived sectional proteinaceous material bronchopulmonary systems can not be obtained industrially, as they are not standardized to protein have not sterile, which greatly limits their use, and reduces the diagnostic value. 2. Multi-antigenic composition of protein homogenates derived from cell-tissue structures of the respiratory system, does not allow to clearly identify the nature of morphological changes in bronchial asthma in children. 3. Lipopolysaccharide antigens from bronchopulmonary structures contain protein in their composition may be prepared industrially under sterile conditions and stored in a lyophilized state for more than two years, which allows to use them widely in the forms and immunodiagnostics clinical severity of asthma in children.

References

1. Ado AD. General Allergy //M.: Medicine.1978.468 p.
2. Baranov AA. Pediatric Allergy // M.: GEOTAR Media. 2009. 687 p.
3. Bogdanov AV. Differential diagnosis of bronchial asthma in children // Pediatrics. 1998. № 1. P. 66-68.
4. Drannik GN. Clinical Immunology and Allergology // M.: Medical Information Agency. 2003. 603 p.
5. Karaulov AV. Clinical immunology // M.: Medicine. 2002. 651 p.
6. Kvirikadze VV. Quantitation of antibody in the blood: Method. Recommended. USSR Ministry of Health, GISCO them. L. V. Tarasevich // M.. 1984. P. 1-9.
7. Lakin GF. Biometrics // M.: Higher School.1990. 352 p.
8. Mayansky DN. Chronic inflammation //M .: Medicine. 1991. 271 p.
9. Resnick IB. Features of airway inflammation in asthma in children // Pediatrics. 1997. N 2. P. 9-14.
10. A process for preparing tissue for diagnosis of allergen autoallergichesky processes in chronic tonsillitis: AS 1084025 USSR MC and 5 and 61 K 39/00 / VD. Yakovenko, AD. Bazavluk, GP. Cherkass, stated. 10/11/82; publ. 09/12/84 // Bull. open. invented. 1984. N 13. P.19.
11. Chernushenko EF. Immunological studies in the clinic // Kyiv: Health Protection. 1978. 159 p.

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