THE MIMICRY ANTIGENS OF BRONCHOPULMONARY SYSTEM AS FACTORS OF AUTOIMMUNE PROCESS INITIATION IN CHILDHOOD BRONCHIAL ASTHMA

Chernuskiy V. G,. [1,2]Popov N. N., [2]Govalenkova O. L., [1]Letyago A. V., [1]Kashina-Yarmak V. L., [1]Tolmachova S. R., [1]Popova A. N.

[1]V. N. Karazin Kharkiv National University, Ukraine
Mechnikov Institute for Microbiology and Immunology of AMS Ukraine

Bronchial Asthma (BA) in children at the modern stage of the development is viewed as polyetiological disease, in pathogenesis of which the microbial factor has the leading role independent of its form.

In course of the present study, it was shown that the microorganisms isolated from the sputum of children, suffering from BA in the exacerbation period, have a varied antigenic potential and are able to include into their structure mimicry antigens of the tissue and cellular structures of the bronchopulmonary system. By including into their structure the mimicry antigens of trachea, bronchi and lung tissue, the microorganisms do not only determine the induction of the pathological processes in the bronchopulmonary system, but also shift it towards the autoimmune base, exacerbating the disease course.

Introduction

At the modern stage of development, bronchial asthma (BA) is viewed as a chronic allergic inflammation in the bronchopulmonary system, and the understanding of the role of opportunistic and pathogenic microflora in its pathogenesis is considered to be growing [1, 4, 7, 8, 15].

Many researchers note the absence of complete coincidence of the microbiological data and results of skin tests and provocation tests, which explains the different vies on the role of microorganisms in the etiological structure of BA in children. It is necessary to view the problem of mechanisms of influence of microbial antigens in BA in children from the point of view that the latter can potentially cause sensitization of any type: I, III, and IV [4, 8, 9, 11, 14, 15]. That is why depending on the prevalence of the hypersensitivity type, different pathological mechanisms that determine the severity of the BA course in children can be possible. For instance, during the performance of provocation tests the reaction can develop according to the immediate, delayed and intermediate type. The mechanism of the latter is not elucidated fully at the moment, which makes the problem of the study of the role of microbial factor in the BA development very pertinent.

Potential etiological determinants of BA in children manifest their etiological influence not through the range of the inherent patogenic or oportunistic pyogenic properties, but through the sensitization of the organism of the child accompanied by the local and systemic immune factors. Such interpretation of the etiologic participation of the microbial factor in the BA determination in children allows to explain the anamnesis and accompanying connection of BA development with tonsillitis and other atopic diseases [9,14,15,16].

One of the debatable points in evaluation of etiological participation of microorganisms in BA determination is the inability to explain the fact that the developing infectious allergy manifests towards the cellular and tissue structures of the bronchopulmonary system [6,7,8,9,16].

It is obvious that the positive answer on this question can be obtained based on the achievements of modern microbiology and infectious immunology [3, 9, 11, 17, 18]. In course of the study of the principles of microorganisms and viruses adaptation to parasitism in the organism and in a number of subsequent works [2, 4, 11, 18], it was proven that the effect of the obligate parasitism of the microorganisms and viruses significantly depends on the ability to vary their antigenic composition in terms of acquisition of mimicry (heterophilic) antigens, common with the certain organs and tissues of the host organism. As a result of this process, the microorganisms persisting in the body evade the adequate immune control and issue a sensitizing effect, taking into the account the antigenic components that are of the same type as the cellular and tissue structures of the bronchopulmonary system [3, 4, 9, 13, 14, 15].

At last, it is necessary to answer the question of substantiation of the etiological role of microorganisms in the BA determination in children: why in the infectious foci (mainly located in the rhinopharyngeal area) potential etiological agents of BA acquire the mimicry antigens of the same type as the cellular and tissue structures of the bronchopulmonary system? No systematization of the data concerning the questions of antigenic adaptation of microorganisms to the cellular and tissue structures of the organism is present at the moment. Single reports have shown that the differences determine not only the specific features of the cellular and tissue structures organization, but also differences in the chemical and antigenic characteristics of the interstitial connective tissue, specialized for each respective organ and system [11, 15, 16].

In conditions of the prolonged persistence of the infect zone that is removed from the bronchopulmonary system, but similar to the chemical and antigenic type of the interstitial connective tissue, microorganisms (viruses and bacteria) withstand the immune reaction of the organism, predominantly phagocytosis, and acquire components to their antigenic composition that are similar in structure type to the interstitial connective tissues of the particular organ or system [1,2,3,4].

Consequently, it follows that in course of the persistence process and mimicry antigens acquisition, the potential etiological agents acquire the ability to have a sensitizing effect on the organism of the child not only because of the truly microbial (viral), but also acquired mimicry antigens. Due to the similar type of chemical and antigenic organization of the interstitial connective tissue in the infection gate area and in the

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bronchopulmonary system, the cellular and tissues structures of this system fall under the immune control [3,11,15].

Therefore, allergizing effect of the prolonged persistence of the microorganisms in the infection gate area that possess mimicry antigens to the cellular and tissues structures of the bronchopulmonary system can be considered to be the one of the bases of the substantiation of the etiological participation of microorganisms in the BA development in children [14,16,17].

By denying the idea that microbial or viral etiological agent can have a direct toxic and destructive effect on the cellular and tissues structures of the bronchopulmonary system, while allowing for the ability of the microorganism to induce sensitizing of the organism to the antigenic structures of the bronchopulmonary system, it is possible to unite in a great measure infectious and non – infectious etiological factors of childhood BA that depends on the progressive autoimmune reaction of the organism [3,8,14,15,17].

At the same time, the dynamics of the formation of the allergic status of the children with BA is characterized by "truly" infectious allergy, connected with the high infectious index of the previous acute respiratory and infectious diseases, with taking into account the presence of microbial or viral mimicry antigens that have the same type of structure as the cellular and tissue structures of the bronchopulmonary system, and also true autoallergy, organotropic towards the bronchopulmonary structures [1,2,7,8,14,15].

The aim of the present study was to determine the mimicry antigens in the microorganisms isolated from the sputum and their role in the induction of autoimmune process in course of bronchial asthma in children.

Materials and methods

A microbiological study of the sputum of the 135 examined children with BA, aged 6 to 14 in the exacerbation period was carried out. The disease diagnosis was established according to the protocol and the directive of the Health Ministry of Ukraine from 08.10.2013 № 868. In course of examination, 45 children were shown to have non – atopic asthma (NABA), 46 had mixed bronchial asthma (MTBA) and 44 children had atopic form of BA (ATBA).

Microbiological analysis of the sputum was carried out with the help of widely accepted methods: plating onto the liquid and solid nutritive mediums with the subsequent isolation of strains and their microscopy, biochemical and serological identification [5, 6].

Identification of the isolated microorganisms was carried out according to the taxonomic test of the Berdgey's identification manual [11].

Etiological significance of the microorganisms in the disease was accepted in case of the microbial count value no lower than 10^6 (for isolation of pathogenic microflora). The international standard requires the isolation of the microorganisms in the sputum in quantity over 10^6 per 1 ml. Smears were prepared from the colonies, pure cultures were isolated and microorganisms were identified [5, 11].

In order to determine the possible etiological role of the microorganisms isolated from the sputum of 80 children aged 6 to 14 at the exacerbation period the presence of mimicry antigens to the cellular structures of the trachea, bronchi and lung tissue was determined.

We have carried out experiments on the 9 rabbits of the chinchilla breed. Animals aged 5, 5-6 months with average weight 2750-2800 g participated in the experiment. The work with animals was carried out according to the European convention of the use of experimental vertebral animals for scientific applications. All animals were kept in conditions that were in accordance with the international GLP standards GLP [19].

Before the experiment, in course of three days animals have undergone thermometry twice a day, the clinical condition of the animals was accessed daily, as well as manifestation of the instincts and reflexes, heart and breathing rates.

For determination of the mimicry antigens in the examined strains, we have prepared hyper immune monospecific rabbit serums to the trachea, bronchi and lung tissue antigens. The section samples of the accidentally deceased children with the I(0) blood type taken after 2-4 hours after the death served as antigenic material. The saline water extracts obtained from trachea, bronchi and lung tissue were used as tissue antigens. The antigenic activity was determined by the quantitative determination of protein according to the E. F. Chernuschenko method [13].

Hyperimmunization of the rabbits was carried out by subcutaneous inoculation of antigens according to the regimen in the following doses calculated according to the protein content: 50 μg – 100 μg – 150 μg – 200 μg – 300 μg. The interval between the injections was 2-3 days. The effect of immunization was evaluated with the help of precipitation reaction [13]. It was established that the obtained hyperimmune serums were characterized by the titer content to the trachea antigens – 1:230, bronchial antigens – 1:304, and lung tissue antigens– 1:309.

The reliability of the differences was determined according to the Student t-parameter. The differences were considered reliable at $p \leq 0,05$.

Results and discussion

Microbiological studies of the bronchopulmonary secretions of the children with BA were carried out in order to determine the presence of pathogenic and opportunistic microorganisms that could be considered as etiological and pathological factors in BA development.

The results of the studies are represented in table 1.

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Table 1. Qualitative and quantitative composition of the microorganisms isolated from the sputum of the examined children in the exacerbation period

Microorganisms	Microorganisms and their sputum associations %					
	NABA		MTBA		ATBA	
	n=45		n=46		n=44	
	abs.	%	abs	%	abs	%
S. aureus	8	17,9±3,5	7	15,2±2,2	4	9,1±1,5
S. pyogenes	5	11,1±1,8	6	13,1±2,6	3	6,8±1,3
E. coli	4	8,9±1,2	5	10,9±2,1	5	11,4±1,3
P. aeruginosa	6	13,3±2,3	7	15,2±3,1	7	16,0±4,2
P. mirabilis	4	8,9±1,2	3	6,5±1,2	6	13,6±1,9
C.albicans	2	4,4±0,6	5	10,9±2,1	8	18,2±3,4
S. aureus + S. pyogenes	5	11,1±2,8	4	8,7±1,2	2	4,5±0,7
S. aureus + E.coli	2	4,4±0,6	2	4,3±0,5	3	6,8±1,1
S. aureus + P. aeruginosa	6	13,3±2,6	4	8,7±1,2	4	9,1±1,5
S. aureus + P. mirabilis	3	6,7±1,3	3	6,5±1,2	2	4,5±0,7
Всего	45	100	46	100	44	100

It was established that the least frequent microorganisms isolated in children with ATBA were: S. pyogenes – 3 (6,8±1,3 %), S. aureus – 4 (9,1±1,5 %), and E.coli - 5 (11,4±1,3 %); among associations S. aureus + S. pyogenes – 2 (4,5±0,7 %), S. aureus + Pr. Mirabilis – 2 (4,5±0,7 %), the most frequent: C.albicans – 8 (18,2±3,4 %), Ps. aeruginosa – 7 (16,0±3,2 %), and among associations S. aureus + Ps. Aeruginosa – 4 (9,1±1,5 %), and S. aureus + E. Coli – 3 (6,8±1,1 %).

In children with NABA the least frequent isolates were: fungi of the genus C. albicans – 2 (4,4±1,4 %), as well as associations: S. aureus + E. coli – 2 (4,4±1,4 %) and S. aureus + Pr. Mirabilis – 3 (6,7±1,7 %), and the most frequent: S. aureus – 8 (17,9±3,5 %) and Ps. aeruginosa – 6 (13,3±2,6 %), among associations: S. aureus + Ps. Aeruginosa – 6 (13,3±2,6 %) and S. aureus + S. Pyogenes – 5 (11,1±2,8 %).

In children with MTBA the following microorganisms were isolated with the least frequency: Pr. Mirabilis – 3 (6,5±1,8 %) and fungi of the genus C. albicans – 5 (10,9±4,1 %), among associations S. aureus + E. coli– 2 (4,3±1,6 %); S. aureus + Pr. mirabilis– 3 (6,5±1,8%), with the most frequency: S. aureus– 7 (15,2±3,1 %), Ps. aeruginosa – 7 (15,2±3,1 %), among associations: S. aureus + S. pyogenes – 4 (8,7±1,9%) and S. aureus + Ps. Aeruginosa – 4 (8,7±1,9%).

The presence of the pathogenic and opportunistic microflora and their associations in children with different forms of BA in the exacerbation period can point to a dysbiotic processes in the bronchopulmonary system. The participation of the sputum isolated microflora in the disease pathogenesis can be determined by the determination in their structure of the mimicry antigens of the cellular and tissue structures of the trachea, bronchi and lung tissue, shifting the pathological process in the bronchopulmonary system towards the autoimmune basis.

The agglutination reaction of organ specific serums with microorganisms, isolated from the sputum of the children with the different forms of BA has revealed the following (table 2). In non-atopic form of BA mimicry antigens were determined both in pyogenic cocci and Gram-negative microorganisms. While the antigen titer values for the Gram-negative microorganisms were background or unpronounced, in pyogenic cocci they reached the values of 1:131-1:149. This confirms the theory that pyogenic cocci have the leading role no only in the etiopathogenesis of the disease, but in the exhaustion of the secretory and systemic immunity, development of the secondary immune deficiency state in the bronchial system, the consequence of which is the colonization by the gram-negative microflora. This phenomenon can be most significant in case of the mixed form of the BA, when the agglutination titer of the organ specific serums with Staphylococcus reaches the value of 1:170-1:213 At the same time, the increase in the level of mimicry antigens to the cellular and tissue structures of the trachea, bronchi and lung tissue in the Gram – negative microorganisms is observed, which is possible related to the development of destructive changes in the bronchi and lung tissue. The atopic form of BA in the studied aspect was the least demonstrative. In this form of the disease, the agglutination reaction results were in the range of 1:18-1:44, 8, which can obviously be explained by the insignificant role of the microbial factor in the etiopathogenesis of this form of BA (see table 2).

The mechanisms of participation of microorganisms in the allergic reactions of bronchopulmonary system lead to the development of bronchoobstructive syndrome through specific and non-specific mechanisms [13, 14, 15, 16].

In the first case, the microbial agents and their metabolites serve as antigens, in the second – they cause the development of autoimmune reactions to the "sequestered" antigens of the bronchopulmonary system.

The failure of colonization resistance leads to the development of "unstable metabolism" of the cellular and tissue structures of the bronchopulmonary system, which is the basis of the bronchial hyperactivity and worsens the bronchial obstruction syndrome in childhood BA.

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Therefore, the carried out experiments confirm the presence of mimicry antigens in clinical strains of the microorganisms isolated from the bronchial secretions of children with BA, determine the induction of the pathological process, shifting it towards autoimmunity, which allows to consider this disease as chronic inflammatory process based on autoimmune processes. That is why new therapeutic approaches, directed at the elimination of the microbial factor and autoimmune processes in the bronchopulmonary system, are necessary.

Conclusions

1. Independent of the BA form in children, the microbial factor has the leading role in its etiopathogenesis, and can lead to the increased severity of the disease course.
2. BA in children is characterized by complex etiological structure that combines Gram-positive, Gram-negative, Candida spp. fungi, and their associations.
3. Microorganisms isolated from the sputum of children with BA, through varying their antigenic potential, are able to include into their structure mimicry antigens of the cellular and tissue structure of the bronchopulmonary system.
4. Microorganisms, through inclusion into their structure the mimicry antigens of the trachea, bronchi and lung structure, not only determine the induction of the pathological process, but also shift it towards autoimmunity.

THE MIMICRY ANTIGENS OF BRONCHOPULMONARY SYSTEM AS FACTORS OF AUTOIMMUNE PROCESS INITIATION IN CHILDHOOD BRONCHIAL ASTHMA

Chernuskiy V. G,. Popov N. N., Govalenkova O. L., Letyago A. V., Kashina-Yarmak V. L., Tolmachova S. R., Popova A. N.

Introduction. Microorganisms, isolated from the sputum of children with bronchial asthma (BA) in the exacerbation period, are able to acquire mimicry antigens of the trachea, bronchi and lung tissue, and have sensitizing effect on the organism of the child not only through the truly microbial (viral) antigens, but through the acquitted mimicry antigens of the cellular and tissue structures of the bronchopulmonary system, thus shifting the pathological process towards autoimmunity. **Materials & methods.** A microbiological study of the sputum obtained from the 135 examined children with BA aged 6 to 14 years in the exacerbation period. The disease diagnosis was established according to the protocol and directive of the Ministry of Health of Ukraine from 08.10.2013 № 868. It was established that 45 children had non – atopic asthma, 46 – mixed type asthma (MTBA) and 44 – atopic form of BA (ATBA). Microbiological studies of the sputum were carried out with the help of the commonly accepted methods: plating onto the solid and liquid culture mediums with the subsequent strains isolation, microscopy, biochemical and serological identification. Strains identification was carried out according to the taxonomic tests of the Berge microorganism index. In order to determine the presence of mimicry antigens in the examined strains we have prepared hyperimmune rabbit serums to the trachea, bronchi, and lung tissue antigens. Section samples obtained from the accidentally deceased children with the I(0) blood type 2-4 hours after the moment of death served as a antigenic material.

Results & discussion. BA in children is characterized by complex etiological structure that combines Gram-positive, Gram-negative and Candida spp. fungi, and their associations. A comparative study of the quantitative composition of the microorganisms isolated from the sputum of the 135 examined children aged 5 to 14 years in the exacerbation period was carried out. It was established that the following microorganisms were isolated from the sputum of the children with ATBA with the lowest frequency: S. pyogenes - 3 (6,8 ± 2,1%), S. aureus - 4 (9,1 ± 2,5%), and E. coli — 5 (11,4 ± 2,3%); among associations - S. aureus + S. pyogenes - 2 (4,5 ± 1,3%), S. aureus + Pr. mirabilis - 2 (4,5 ±1,3%). The most frequent microorganisms were: C. albicans - 8 (18,2 ± 4,4%), Ps. aeruginosa - 7 (16,0 ± 4,2%), and among associations - S. aureus + Ps. aeruginosa - 4 (9,1 ± 2,5%), and S. aureus + E. coli-3(6,8 ±2,1%). In the children with NABA, the least frequent microorganisms were: C. albicans fungi - 2 (4,4 ± 1,4%), as well as associations: S. aureus + E. coli - 2 (4,4 ± 1,4%), and S. aureus + Pr. mirabilis - 3 (6,7 ± 1,7%), and the most frequent - S. aureus 7 (15,2 ± 3,1%), Ps. aeruginosa - 7 (15,2 ± 3,1%), as well as associations: S. aureus + S. pyogenes - 4 (8,7 ± 2,2 %) и S. aureus + Ps. aeruginosa - 4 (8,72 ± 2,2%). In children with MTBA the lowest frequency of isolation from the sputum was observed for: Pr. mirabilis - 3 (6,5±1,8 %) and Candida spp. fungi - 5 (10,9±4,1 %), among associations - S.aureus + E.coli- 2 (4,3±1,6%); S.aureus + Pr. mirabilis- 3 (6,5±1,8 %), the most frequent microorganisms were: S.aureus - 7 (15,2±3,1 %), Ps.aeraginosa - 7 (15,2±3,1 %), and among associations: S.aureus + S.pyogenes - 4 (8,7±2,2 %), and S.aureus + Ps. aeruginosa - 4(8,7±2,2%). The participation of the microflora isolated from the sputum in the etiopathogenesis of the disease can be proven based on the determination in their structure of the mimicry antigens of the trachea, bronchi and lung tissue. It was experimentally proven in course of the study that in NABA the titers of the agglutination of the organ specific serums with Gram-positive microorganisms (Streptococcus and Staphylococcus) were 1:131 — 1:149, which points out their decisive role in the etiopathogenesis in this form of BA, while in the Gram-negative microorganisms, the background values of the titers were observed - 1:17 - 1:85. In MTBA, the agglutination titer of organ specific serums with Gram-positive microorganisms (Streptococcus and Staphylococcus) was in the range (1:128 - 1:213), in Gram-negative microorganisms (E. coli and P. aeruginosa) – (1:64 - 1:160), which points to the participation of the pyogenic and Gram-negative microflora in the etiopathogenesis of this form of BA. In ATBA, the results of agglutination reaction of organ specific serums with Gram-positive microorganisms and Gram-negative microorganisms were in the range of

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1:18 - 1:44, the lowest range compared to the NABA and MTBA. It can be concluded that microorganisms, isolated from the children with BA, are able through inclusion into their structure the mimicry antigens of the trachea, bronchi and lung structure, not only to determine the induction of the pathological process, but also to shift it towards autoimmunity. **Conclusion**
1.Independent of the BA form in children, the microbial factor has the leading role in its etiopathogenesis, and can lead to the increased severity of the disease course.
2. BA in children is characterized by complex etiological structure that combines Gram-positive, Gram-negative, Candida spp. fungi, and their associations. 3.Microorganisms isolated from the sputum of children with BA, through varying their antigenic potential, are able to include into their structure mimicry antigens of the cellular and tissue structure of the bronchopulmonary system. 4. Microorganisms, through inclusion into their structure the mimicry antigens of the trachea, bronchi and lung structure, not only determine the induction of the pathological process, but also shift it towards autoimmunity.

Keywords: bronchial asthma, children, microorganisms, mimicry antigens, autoimmune process.

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Таблица 2. Antigenic mimicry of the clinical strains of the microorganisms isolated from the sputum of children with BA in the exacerbation period, to the bronchopulmonary system structures ()

Clinical form of BA	Strains isolated	The quantity of strains	Agglutination of the serum to the antigen					
			trachea		bronchi		lung	
			positive, %	titer (control 1:230)	positive, %	titer (control 1:230)	positive, %	titer (control 1:230)
NABA n=27	*Staphylococcus*	8	70	1:68,6±3,2	100	1:149,2±2,8	50	1:89,6±7,2
	Streptococcus	5	62,5	1:131,2±7,9	75	1:108,4±7,7	75	1:64,0±3,9
	E. coli	4	33,3	1:85,3±5,6	55,5	1:27,5±3,3*	44,4	1:19,2±2,2*
	P.aeruginosa	6	33,3	1:17,6±1,8*	41,6	1:18,8±2,2*	58,3	1:29,6±4,4*
	Proteus	4	42,8	1:21,3±2,9*	57,1	1:10,0±1,6*	42,8	1:21,3±1,5*
MTBA n=28	*Staphylococcus*	7	100	1:170,6±8,8	100	1:213,3±10,5	100	1:85,3±6,2
	Streptococcus	6	100	1:48±2,7	100	1:128±4,2	100	1:98±3,2
	E. coli	5	50	1:64±3,3	100	1:96±3,7	100	1:128±6,5
	P.aeruginosa	7	100	1:49±2,2	100	1:97±2,9	100	1:160±8,1
	Proteus	3	100	1:24±1,7*	100	1:64±2,3	100	1:48±3,2
ATBA n=25	*Staphylococcus*	4	80	1:44,8±2,9*	100	1:44,7±3,1*	100	1:44,8±2,2*
	Streptococcus	3	75	1:21,3±1,6*	50	1:16,3±2,8*	25	1:8,7±1,22*
	E. coli	5	66,6	1:10,0±1,3*	50	1:37,2±2,2	66,6	1:26,0±1,9*
	P.aeruginosa	7	71,4	1:25,6±1,9	57	1:28,4±1,7	57	1:18,0±1,5*
	Proteus	6	60	1:26,5±2,1*	60	1:21,3±1,1	80	1:27,3±1,2*

Note. *– reliable differences of the values from the titre control (P<0,05)