FEATURES EXPRESSION OF THE MARKER MEDICATION OF APOPTOSIS AND ATOPY IN CHILDREN WITH DIFFERENT PHENOTYPES BRONCHIAL ASTHMA

Yu.G. Antypkin, V.F. Lapshin, T.D. Zadorozhnaya, T.R. Umanets, O. Pustovalov
«Institute of Pediatrics, Obstetrics and Gynecology, Medical Sciences of Ukraine», Kyiv

The research results of apoptosis and atopy in children with different phenotypes of asthma (BA) were adduced. The expression of proapoptotic marker Bax and its regulator Bcl-2 on cells of the bronchial epithelium, eosinophils, neutrophils and macrophages and CD4+ and IgE in induced sputum examined children was studied. Expression differences of data markers depending on the atopic status, phlogistic endotype and severity of asthma in children were fixed.
Keywords: asthma, children, phenotypes, apoptosis, atopy.

Introduction

Hitherto, asthma in children remains one of the most important medical and social problems of Pediatrics, due to its prevalence, a significant impact on the quality of patients, early disability children and significant economic losses associated with utilization of health resources [2,9,10,13].

Results of long experience suggests that asthma in children is characterized by chronic, usually allergic airway inflammation that forms their hypersensitivity and the main symptoms of the disease [2,5,8–10,12,17]. Development of inflammation in the respiratory tract of asthmatic is defined as a profile of effector cells that take part in it, and the organization of intercellular relationships.

According to modern concepts, a special role in the regulation of inflammatory processes plays an effector of apoptosis and structural cells [1,3,17]. Condition apoptosis mechanisms make a significant contribution to the immune response to external factors, the manifestations of immunoprotection and determine the issue of inflammation. The difference in the ratio of proapoptotic and antiapoptotic factors determines the future walk of cell, which is involved in the inflammatory process [3].

If the processes of induction and suppression of apoptosis of cells that are important in the pathogenesis of atopic asthma (ABA), just presented in the literature, the data for these processes with no IgE-dependent mechanisms of development, different subtypes of inflammation in children with asthma are absent.

New immunocytochemical research in the application of non-invasive methods such as induced sputum (IS), allow to study the peculiarities of intercellular interactions in causing inflammation in different phenotypes of asthma in children.

Including the heterogeneity of the population of children with asthma with different phenotypes according to the presence of atopy, different subtypes of inflammation, severity of disease, it is important to examine the features of apoptosis and expression of markers of atopy in relation to their phenotypic traits.

The purpose of the study is to explore the features of apoptosis and expression of atopy in children with different phenotypes of asthma.

Materials and methods

The study involved 48 children (study group) aged 6–12 years diagnosed with asthma of varying severity, without recrudescence, with newly diagnosed, due to excluding the impact of several factors, primarily the use of inhalated glucocorticosteroids («cocorticosteroid» children with asthma) on the studied parameters IS. It is known that inhalated glucocorticosteroids affect eosinophil apoptosis by altering the inflammatory cell profile of IS [17]. The control group consisted of 20 healthy children of the same age. For the purpose of clinical validation non atopic children with asthma phenotype (have a significant) examined 20 children with recurrent bronchitis (RB) in remission who were made group comparisons identical in age and gender.

The diagnosis of asthma is established in accordance with the approved 12th Congress of Pediatrici-

ans classification of disease (12–14 October 2010) [4]. In order to determine the objectification greater severity of asthma children surveyed used the criteria of international guidelines of the Global Initiative for asthma (GINA, 2011) and the American Thoracic Society (ATS) [9,15,18].

Clinical examination of children at the same time with life and case history, allergological amnesis, according to general examination included a set of special clinical and functional researches.

The presence or absence of atopic status, severity of asthma and inflammatory endotype of BA were the criteria for the study of asthma phenotypes observed in children. [6,8,12].

Atopic status as the main criterion is defined by means of skin testing (prick-test) with the main airborne allergen (household, pollen, epidermal, fungal) and food allergens (LLC «Immunolog», Vinnitsa). The presence of papules over 3 mm was considered a positive result.

Total and specific IgE determined by indirect enzyme immunoassay to 20 major allergens. In the presence of the surveyed children positive prick-test for more than one of the major allergens, increased levels of total and specific IgE was identifying atopic phenotype.

Respiratory function was studied in the computer Spirograph Micro Guark № 10603172 the definition of generally static (lung volume) and functional parameters of pulmonary ventilation.

IS is used to investigate the inflammatory changes of the airways. Cytomorphological IS analysis conducted by Pizzichini et al. [11]. This inflammatory subtypes according to IS in study groups of children defined by classification Simson et al. [12].

Immunohistochemistry included evaluation of the expression of pro-(Bax) and antyapoptotic (Bcl-2) antigens on the cell membranes of stratified squamous epithelium and cells of inflammatory infiltration (neutrophils, lymphocytes, eosinophils) antigens to CD4+, immunoglobulin E (IgE) in the name of the indirect streptavidin-peroxidase method using Romanovsky-Giemsa's color.

Results of immunocychemical reaction were assessed by using the methods adopted in the immunohistochemistry of determining the degree of expression (in points): 0 points — no color, 1 point — weak color, 2 points — moderate color, 3 points — a distinct color, 4 points — very expressive color. Besides, the index of apoptosis is calculated (AI), which is defined as the number of positively stained cells divided by the

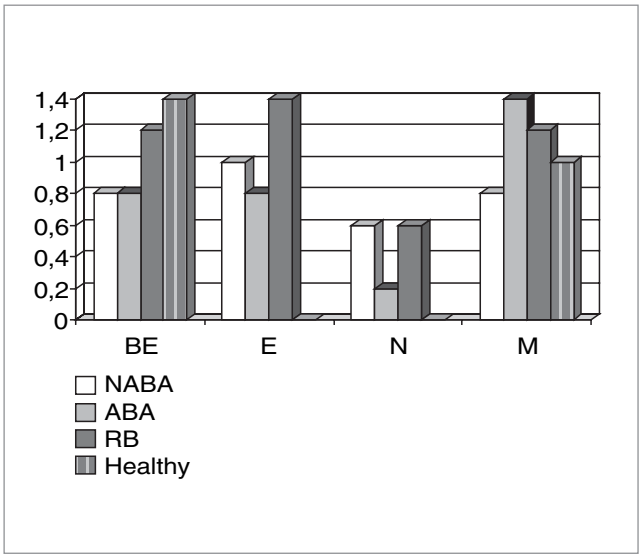

Fig. 1. Average expression of pro-apoptotic protein Bax in cells induced sputum of children studied groups: BE — bronchial epithelium, E — eosinophils, N — neutrophils, M — macrophages

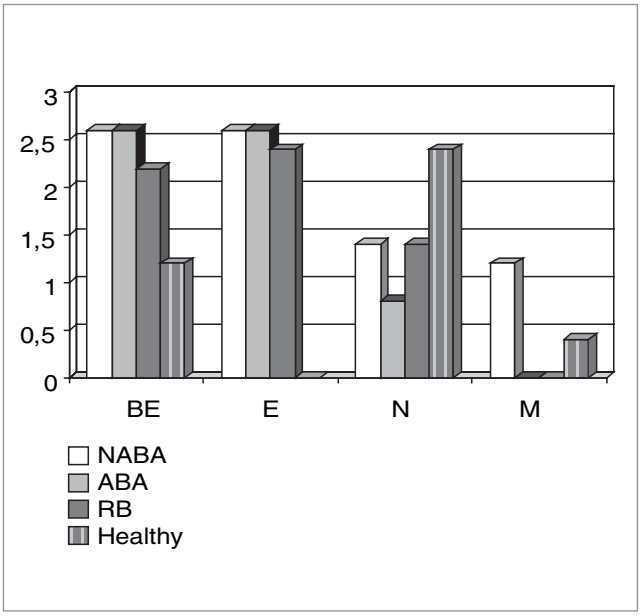

Fig. 2. Average expression antiapoptotic Bcl-2 protein in cells induced sputum children: BE — bronchial epithelium, E-eosinophils, N — neutrophils, M — macrophages

number of cells and multiplied by 100%. Study drugs in transmitted light microscope Olympus held at BH-2 (Japan).

Statistical analysis of the results carried out on a personal computer using the software SPSS 8.0 for Windows, Microsoft Office Excel 2007.

Trial Results and Their Discussion

Boys predominated by sex among the surveyed children (56.3%), by age — children of primary school (62.5%). According to the severity of asthma surve-

yed children were distributed as follows: mild course — 64.5%, moderate — 20.8%, severe course — 14.6%.

Analysis atopic status showed that 81.2% of children had ABA and only 18.8% — have a significant. The structure of sensitization in children with ABA dominated household (89.7%). Less epidermal (69.2%), pollen (41.0%) and fungal (25.6%) sensitization was recorded. Monosensitization detected in 25.4% of children, and the rest (74.4%) children had sensitization to more than one group of allergens.

Evaluation results subtype inflammation in surveyed children with asthma allowed to establish «eosinophilic» endotype in 62.5% of children and «neutrophilous» — in 37.5%. It should be noted that children with ABA (84.6%) was more typical «eosinophilic» cytogram type, and for children have a significant (77.8%) — «neutrophilic». These results are consistent with the literature data and characterize the impact of various effector cells of the inflammatory process in different phenotypes of asthma [6, 8].

Assessing the ratio of proapoptotic Bax and antiapoptotic Bcl-2 in all cells of the bronchial epithelium IS surveyed children with asthma, was found that their index is not dependent on the presence of atopic status and indicated a decrement in the intensity of the process of apoptosis in these cells by increasing the expression of its regulator Bcl-2 in all children with ABA and NABA compared with the control group children. The opposite direction in the expression of protein data was observed in children with RB. This is confirmed by calculating the index of apoptosis of epithelial cells, which was significantly reduced in the group of children with asthma compared with the control group (6.4±0,03% vs 12.8±0,04%, $p<0.05$). Children with severe asthma phenotype characterized by increased expression likely Bax in the cells of the bronchial epithelium (from 0.8 for mild asthma to 2.6 score in children with severe asthma) and a tendency to increase their apoptosis index (8.20±0.03% in children with severe asthma versus 7.00±0.03% — in children with mild asthma, $p>0.05$).

To assess the reparative activity of the bronchial epithelium is important to determine the relative number of cells with cytological features of proliferative activity: increase the number and size of nucleoli, increase core, the presence of mitotic figures. In children with asthma these cells constituted only 8.5±1,6% compared with children with RB (16.3±0,4%, $p<0.05$), indicating a compensatory ability of bronchial epithelium to repair in children with RB.

Research and proapoptotic and antiapoptotic proteins in IS eosinophils children of different clinical groups allowed to establish that in children with asthma regardless of atopic status prevailed antiapoptotic mechanisms in these cells compared with the control group ($p<0.05$) with a corresponding increase in apoptosis in neutrophils. This is consistent with their higher apoptotic index compared with the control group (11.40±0.03% vs. 0.0±0.0%, $p <0.05$;), which can explain the advantage of forming a «eosinophilic» endotype inflammation in children with asthma. In addition, children with «eosinophilic» asthma endotype also identified the likely increase of apoptotic index neutrophils compared with a group of children with «neutrophilic» type (9.0±0.03% vs. 3.40±0.01%, $p<0.05$), which practically did not differ from children with RB (9.0±0.03% against 10.0±0.03%, $p>0.05$). These data support the role of apoptotic neutrophils mechanism with simultaneous superiority antiapoptotic mechanisms of eosinophils in shaping «eosinophilic» endotype inflammation in children with asthma. It was found that eosinophil apoptosis index was independent of severity in children with asthma. However Bcl-2 expression on eosinophils significantly decreased (from 2.6 to 1.0 points) in children with severe course.

However, we found probable difference antiapoptotic mechanisms of neutrophils depending on the status of children with atopic asthma. So, children with NABA was found a significant increased expression of protein Vcl-2 on neutrophils compared with children with ABA ($p<0.05$), which explained the superiority of «neutrophilic» inflammation of the air pasage in the group. By the side of this, the apoptotic index of neutrophils significantly differed with that of children with various inflammatory endotypes. Its minimum value was found in the process of «neutrophilic» inflammatory endotype in children with asthma (3.40±0.01%). This indicated that the formation of «neutrophilic» endotype inflammation in this category of children was related to their increased viability against the background of increased apoptosis of eosinophils (10.2±0.03% in children with «neutrophilic» against 3.9±0.01% in children with «eosinophilic» inflammatory endotype). In addition, the index of neutrophil apoptosis depended on the severity of asthma and meet its maximum value (9.60±0.03% vs 3.20±0.01% in children with mild asthma, $p<0.05$) in the group of children with severe course of the disease. This may explain the instability of neutrophils in IS against severe course of asthma, namely a decrease of inflammatory changes endotype

of «neutrophilic» to «eosinophilic». Bcl-2 expression on neutrophils significantly decreased (from 0.8 to 0 points) in children with severe asthma phenotype.

In children with RB compared with children with NABA was set the maximum level of apoptosis of neutrophils (10.0±0.03 % versus 3.40±0.01%), along with «hypo granulocyte» endotype proved different intensity of «neutrophile» inflammation and pathogenesis mechanisms of their formation

As for macrophages, the probable difference in orientation apoptotic processes in these cells in children with different asthma phenotypes depending on their atopic status was found. So, children with ABA defined probable increased expression of proapoptotic protein Bax at lower expression of its regulator Bcl-2, that indicated the increasing apoptosis of macrophages in children with ABA. Conflicting relationships that characterize long-term viability of macrophages and their role in maintaining inflammation of the respiratory tract accordingly were found in children with NABA. It was found that children with severe asthma significantly decreased expression of Bcl-2 on macrophages (from 2–3 to 1 point).

It is known that CD4+ T-2 cells and IgE-dependent allergic reaction mechanisms play a key role in the regulation of chronic inflammation in the ABA [2,5,10]. The expression of markers of atopy in local IS was examined, considering the possibility of predominantly local IgE synthesis in asthma, discussed in the literature [7,14,16], and unproven role of IgE-dependent systemic mechanism in its development of the surveyed children with NABA.

Conducted immunocytochemical study of children's IS for identifying antigens CD4+ and IgE showed their highest level in children with ABA (16.60±0.05% and 18.80±0.06%, respectively) compared with the control group (5.1±0,02% and 0.0±0.00% respectively). It was found that expression of IgE in children with ABA significantly different from that of children with NABA (18.80±0.06% vs. 8.04±0.02%, $p<0.05$) and children with RB (5.04±0.02%, $p<0.05$). The level of expression of CD 4+ children with asthma did not depend on their atopic status and differed from children with RB (10.3±0.03% at have a significant, 16.6±0.05% at ALA v. 5.1±0.02 % of children with RB, $p<0.05$).

It was found that a tendency to increase their expression in surveyed children's IS was observed in severe asthma. The presence of increased local synthesis of IgE in children with ABA (at levels of total IgE in serum 398,0±12,0 KU/ml) at its insignificant levels and absence of systemic signs of atopy in children with NABA (total IgE — 98.5±3,5 KU/ml) proved the major IgE-dependent mechanisms of allergic airway inflammation in children with only ABA. Children with minor NABA local synthesis of IgE may be associated with the impact of infectious factors on the one hand, and the so-called «nonatopic» IgE-response on the other. According to published data, IgE-response can be heterogenic, and its' «non atopic» variant requires significant antigenic impetus. As to «nonatopic person» became sensitized «highly allergic individual» it is necessary more intensive, high and / or long-lasting contact with the allergen than «atopic person» [5,16]. Besides, high levels of expression in CD4+ of this category children's IS may indicate cellular mechanisms involved in the development of chronic allergic inflammatory response of the respiratory tract.

Conclusions

In such a way, on the basis of the obtained data it was determined cytological and immunocytochemical criteria differentiated changes of inflammatory infiltration of the respiratory tract, the local expression of markers of apoptosis and atopy in surveyed children. So, ABA was associated mainly with «eosinophilic» endotype inflammation, and its differential diagnostic immunocytochemical markers were distinct expression of antigens IgE, CD4+, Bax on alveolar macrophages, Bcl-2 on eosinophils in IS. That indicated that the ABA is not only systemic but also local synthesis of IgE antibodies and proved superiority IgE-dependent mechanism in the pathogenesis of this disease phenotype. Besides, the characteristic feature of the inflammatory process in ABA was part of eosinophils and lymphocytes CD4+ with a decrease in macrophage response, as evidenced by strengthening the processes of apoptosis and increased viability of eosinophils (a distinct expression of apoptotic marker Bax on alveolar macrophages and All-2 on eosinophils).

Nonatopic asthma phenotype in surveyed children characterized mainly «neutrophilic» endotype inflammation. The increase of macrophage response to local background neutrophilia in IS of children of this category accompanied by an increase in their viability (expressive Bcl-2 expression on neutrophils and macrophages) against increased apoptosis of eosinophils, indicating their role in the inflammatory process. The slight expression of antigens IgE, along with high expression of CD4+ was installed, in the IS of this category of children determines mainly the role of cellular mechanisms in the development of

chronic allergic inflammatory response of the respiratory tract. In children with RB compared with children with NABA was set the maximum level of apoptosis of neutrophils, next to «hipogranulocytic» inflammatory endotype proved different intensity «neutrophile» inflammation and pathogenic mechanisms of their formation.

In children with severe asthma phenotype probable increased expression Bax on epithelial cells and reduce Bcl-2 on eosinophils and neutrophils was found.

Different inflammation endotype («eosinophilic» and «neutrophilic») was installed in the surveyed children, next to features of expression of apoptosis and atopy markers, proving the involvement of these cells and determining the key pathogenic mechanisms (IgE-dependent or cell) development and persistence of allergic airway inflammation.

References

1. Bulgakov V.A., Bulgakov V.A. Klynycheskoe importance Study Enabling markers and apoptosis of immunocompetent cells in atopycheskoy bronhyalnoy asthma in children. Pedyatryya. 2009; 87(2): 12—18.
2. Lasitsa A.L., Lasitsa T.S., Nedelska S.M. Pediatric allergist. K.: Book Plus, 2004: 367
3. Nevzorov V.A., Suvorenko T.N., Konovalov E.N. Apoptosis and inflammation in asthma bronhyalnoy. Therapeutic Archives. 2001; 12: 92—96.
4. Antypkin Yu.G. et al. Modern classification of asthma in children. Perinatology and Pediatrics. 2011; 12: 92—96.
5. Titov N.D. The role of allergic reactions DIFFERENT typical in the pathogenesis of asthma in children bronhyalnoy. Immunopathology, allerholohyya, ynfektolohyya. 2007; 4: 47—57.
6. Lotvall J., Akdis C.A., Bacharier L.B. et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J. Allergy Clin. Immunol. 2011; 127: 355—360.
7. Groot Kormelink T., Thio M., Blokhuis B.R. et al. Atopic and non-atopic allergic disorders: current insights into the possible involvement of free immunoglobulin light chains. Clin. Exp. Allergy. 2009; 39: 33—42.
8. Yong-Chen F. Clinical types of childhood asthma and nonatopic asthma. World J. Pediatr. 2006; 2: 85—89.
9. Global strategy for asthma management and prevention. National institutes of health. National Heart, lung and Blood Institute. 2011; 102.
10. Hedlin G., Konradsen J., Bush A. An update on paediatric asthma. Eur. Respir. Rev. 2012; 21(125): 175—185.
11. Pizzichini E., Pizzichini M.M., Efthimiades A. et al. Indices of airway inflammation in induced sputum: reproducibility and viability of cell and fluid — phase measurements. Am. J. Respir. Crit. Care Med. 1996; 154: 308—317.
12. Simpson J.L., Scott R., Boyle M.J., Gibson P.G. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006; 11: 54—61.
13. Papadopoulos N.G., Arakawa H., Carlsen K.-H. et al. International consensus on (ICON) pediatric asthma. Allergy. 2012; 67: 976—997.
14. Mouthuy J., Detry B., Sohy C. et al. Presence in Sputum of Functional Dust Mite-Specific IgE Antibodies in Intrinsic Asthma. Am. J. Respir. Crit. Care Med. 2011; 184: 206—214.
15. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. Am. J. Respir. Crit. Care Med. 2000; 162: 2341—2351.
16. Rob C. Aalberse Specific IgE and IgG responses in atopic versus nonatopic subjects. Am. J. Respir. Crit. Care Med. 2000; 162 (3): 124—127.
17. Spinozzi F., de Benedictis D., de Benedictis F.M. Apoptosis, airway inflammation and anti-asthma therapy: From immunobiology to clinicalapplication. Pediatr. Allergy Immunol. 2008; 19: 287—295.
18. Liard R., Leynaert B., Zureik M. et al. Using Global Initiative for Asthma guidelines to assess asthma severity in populations. Eur. Respir. J. 2000; 16: 615—620.