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FUNCTIONAL STATE OF CELLS OF BRONCHOALVEOLAR LAVAGE FLUID OF PATIENTS WITH BRONCHIAL ASTHMA OF VARYING SEVERITY

The cellular composition of the oxidation-state of metabolic function and cytokine-producing activity of cells of bronchoalveolar (BAL) fluid of patients to clarify the mechanisms that determine the severity of the clinical course of bronchial asthma (BA) was studied.

The increased number of neutrophils in BAL fluid of patients with asthma as the worsening of the disease was marked. Also it was found that with the worsening of the severity of BAL fluid cells of asthmatic patients secrete more Th2 cytokines profile. The authors substantiated the fact that these changes in the functional state of pulmonary cells are the basis of the mechanisms that determine the severity of the clinical course of asthma.

Keywords: bronchial asthma, bronchoalveolar lavage, neutrophil, macrophage, active oxygen metabolites, cytokines.

Introduction.

Respiratory diseases are the leading place in the overall morbidity of the population of the Russian Federation, which determines not only their medical significance, but also the social burden on the economy of any country in the world.

As you know, the most important factors in the onset and progression-trivial bronchial asthma (BA) are the changes in immune regulation, among which the leading role belongs to the IgE-mediated allergic reactions [1], which, of course, depend on the functional state of the effector cells of inflammation and allergy [7].

Currently in clinical practice for diagnosis and treatment of patients with asthma are widely used methods of bronchoscopy, with the result that it became possible to carry out morphological study of bronchial biopsies to obtain pulmonary cells and study their structural and functional status [5]. In our view, in contrast to the evaluation of cytologic features of sputum, bronchoscopic method and further cytological and morphological study of the biological material is obtained in the course of its holding, allows us to understand the true picture of pathological processes occurring in the airways and lung tissue. At the same time it is worth noting that the sputum is often used in

pediatric patients because it is noninvasive, has no side effects and contraindications [11, 13, 16].

Thus, the present study examined the cellular composition, oxidative-metabolic function and cytokine-producing activity of cells of bronchoalveolar lavage fluid of patients to clarify the mechanisms that determine the severity of the clinical course of asthma.

Material and methods.

We examined 18 patients with mixed form of bronchial asthma (allergic and infectious-dependent) who were hospitalized in pulmonology department of Amur Region Hospital (Blagoveshchensk). Age of patients ranged from 18 to 68 years (mean age 43±2,8 years). Among the examined patients, 10 were diagnosed as moderately of BA (I group), and 8 - severe severity (II group).

This paper has been used to study the bronchoalveolar lavage (BAL) fluid cells of patients with asthma received in the course of therapeutic and diagnostic bronchoscopy performed by standard methods [12]. BAL fluid was centrifuged at 1000 rev/min for 10 min to obtain a suspension of lung cells. For differential cell counting BAL fluid smear cell sediment was fixed in formalin vapor and stained by the Romanovsky-Giemsa.

BAL fluid cells were cultured in the number of 106/ml in RPMI-1640 added 10% fetal calf serum, gentamicin 80 ug / ml, 2 mM L-glutamine, 5x10-5 mM mercaptoethanol. To stimulate the cells of the BAL fluid are parallel wells were added LPS E. coli in a concentration of 0.5 mg / ml. The content of immunoregulatory cytokines IL-1β, IL-4 and TNF-α in the conditioned culture medium of cells BAL fluid was assessed after 24 hours of incubation with a commercial ELISA test systems ("Protein contour", St. Petersburg), according to the protocol productivity shipment.

To assess the oxidative-metabolic function (OMF) BAL fluid cells of patients with BA was assessed by luminol-dependent chemiluminescence (CL) [4]. In this case, BAL fluid cells are used immediately after their selection. Measuring the intensity of the CL response BAL fluid cells was performed using biochemiluminometer "SKIF-0306M" (SKTB "Nauka", Krasnoyarsk, Russia). As the luminophor was used purified preparation of luminol (5-amino-2, 3 - digidroftalazindion-1, 4) («Serva», USA). To assess the reactivity of the BAL fluid cells using a yeast polysaccharide zymosan (Zymosan A, «Sigma», USA) at a concentration of 5 mg / ml. Registration of the intensity of CL emission BAL fluid cells was performed after 3 min for 30 min. Results are expressed as CL studies combined spontaneous (sp-CL) and zymosan-induced (Z-CL) CL response of cells BAL fluid: sp-Isum and Z-CL-Isum = imp/103 BAL cells /30 min, where n - number of pulses emitted by BAL fluid cells within 30 minutes of study. To assess the reactivity of the BAL fluid cells was calculated stimulation index (IS) using the formula: IS = sp-CL / Z-CL, conv. units.

Statistical analysis was carried out of the material licensed software package Excel 7,0 and Statistica 5,0, using the arithmetic mean, the average error, t-test. Results were considered significant at $p < 0,05$.

Results and discussion.

The results of calculation of the relative numbers of BAL fluid cells have shown that patients with severe asthma severity (II group), the number of pulmonary macrophages (PMf) was smaller, and neutrophils (Nph) - more than in patients with moderate disease severity (I group). The relative number of eosinophils and lymphocytes in BAL fluid of patients in both comparison groups was approximately equal. At the same time, patients of group II the number of other cellular elements (bronchial epithelial cells, basophil and mast cells, etc.) was greater than in patients in group I (Table 1).

In patients with severe asthma severity (II group), spontaneous CL response of cells BAL fluid was higher than in patients with moderate disease severity (I group) ($p < 0,05$). However, the additional stimulation of cells BAL fluid zymosan A their Z-CL response was weaker than that of patients in group I (Table 2).

Thus, in patients with severe asthma severity found increased BAL fluid cells of OMF. However, the reactivity of the BAL fluid cells of patients in this group was significantly lower than patients in group I (Table 2).

According to D.N. Mayansky [7], the key effector cell of acute inflammation is the Nph. Hence, judging by the increase in the relative abundance of Hph in the BAL fluid in patients with severe asthma severity has an intense inflammatory process caused by the increase in the functional state of Nph and PMf. Our results showed that the BAL fluid cells of patients with severe asthma the severity actively generate reactive oxygen metabolites (ROM), as evidenced by the data of sp-CL response. As you know, in the etiopathogenesis of bronchopulmonary system, including allergic nature, play an important role on the one hand, microcirculatory disorders, on the other - the activation of Nph and macrophages, generating ROM. ROM-generated Nph and macrophages have a direct toxic effect on cells of the microenvironment [14]. Probably, the increase in desquamated epithelial cells of the BAL fluid of patients with severe asthma severity (II group) due to the effect of the damaging effect of ROM, produced by Nph and hard PMf. In addition, ROM may activate mast cells that are actively begin to secrete biologically active substances, such as biogenic amines - histamine, serotonin, which have a pronounced constrictor action that aggravates the clinical course of BA [3].

The content of pro-inflammatory cytokine IL-1β in the conditioned cell culture medium

BAL fluid of patients with comparison groups were about equal. At the same time, the conditioned cell culture medium BAL fluid of patients with asthma, severe gravity of the content of TNF-α was significantly lower than in patients with moderate disease severity (I group) ($p<0,05$). However, in the conditioned cell culture medium BAL fluid of patients with asthma, severe gravity of the contents of an anti-inflammatory cytokine IL-4, by contrast, was significantly higher than in patients with moderate disease severity (I group) ($p<0,01$) (Table 3).

Stimulation of BAL fluid cells of asthmatic patients of both groups of LPS E. coli resulted in the increase of cytokine-producing activity. In the conditioned culture medium BAL fluid of patients with asthma, stimulated by LPS E. coli increased levels of both pro- (IL-1β and TNF-α), and anti-inflammatory (IL-4) cytokines. Thus, upon stimulation of cells BAL fluid LPS E. coli in patients with moderate severity of asthma (I group) in the conditioned cell culture medium concentrations of proinflammatory cytokines IL-1β and TNF-α increased, respectively, 2.99 and 3.4 times ($p <0,001$). At the same time in patients with severe asthma severity of the content of proinflammatory cytokines IL-1β and TNF-α in the conditioned cell culture medium BAL fluid increased at 1,6 ($p <0,01$) and 2,2 ($p <0,001$) times respectively (Table 3).

For cell stimulation BAL fluid LPS E. coli in patients with moderate severity of asthma (I group) in the conditioned cell culture medium concentrations of anti-inflammatory cytokine IL-4 increased 1.9-fold ($p <0,01$), whereas in patients with severe gravity - in 3.1 times ($p <0,001$).

Thus, BAL fluid cells of patients with moderate severity of asthma actively produced the proinflammatory cytokines, including TNF-α, and in patients with severe gravity - IL-4. In addition, it was found that the BAL fluid cells of patients with severe asthma severity, according to the results of LPS-induced cytokine-producing activity, preconditioned to enhance Th2-cytokine profile (IL-4).

The key to determining the development of asthma is a violation of the ratio of Th1-and Th2-cytokine profile associated with the lack of Th1-response due to decreased production of IL-12 by macrophages with a decrease in the level of IFN-γ and increase activity Th2-cells in the form of increasing production of IL -4, IL-10, IL-13 [15].

During an asthmatic inflammatory cells that control the production of antibodies, produce regulatory factors that lead to the production of antibodies mainly class IgE (IL-4, IL-13), which attract eosinophils to the site of inflammation and promote their subsequent activation of IL-5, GM-CSF, G-CSF . These cells are called Th2 lymphocytes and their secreted biologically active regulatory proteins (IL-4, IL-13, IL-5) - Th2 cytokine profile [2, 8]. Drawn into the inflammatory mast cells and eosinophils also secrete cytokines, Th2 profile, inducing Th2 lymphocytes. This creates a vicious cycle that supports the characteristic inflammation of the airway wall.

Inflammatory changes associated with bronchial hyperreactivity - a typical sign of the functional asthma [6, 10].

In summary, the results of the study suggest that the allergic inflammation of the respiratory system, including asthma, is one of the variants of chronic inflammation [7, 9], which is based on mononuclear infiltration, and major effector cells are phagocytic cells - monocytes / macrophages and lymphocytes, that implement its functionality enhancement products ROM, pro- and anti-inflammatory mediators, etc. [7] However, the increase in BAL fluid Nph in patients with asthma as the worsening of disease severity may reflect the activation of inflammation in the airways and lung interstitium. At the same time set the activation of BAL fluid cells OMF, which is also indicative of inflammatory activity with increasing destructive processes. In addition, it was found that as the worsening of the severity of BAL fluid cells of asthmatic patients secrete more Th2 cytokines profile. All these changes are likely to underlie the mechanisms that determine the severity of the clinical course of asthma.

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Table 1.

The cellular composition of BAL fluid of patients with asthma of varying severity

Cellular composition, %	Group of patients with asthma	
	I	II
PMf	74,3±4,7	63,2±3,1*
Nph	11,2±3,3	23,3±3,3*
Eosinophils	1,5±0,5	1,7±0,4
Lymphocytes	11,8±0,3	10,6±0,06
Other cellular elements	1,1±0,5	2,1±0,1*

Note: * - significant difference from those of patients in group I. In other cellular elements including single bronchial epithelial cells, basophils and mast cells, etc.

Table 2.

The total CL response of cells BAL fluid and their reactivity (IS) in patients with asthma of varying severity

Groups	CL response, imp/10^3 cells BAL/30 min		IS, conv. units
	Spontaneous	Z-induced	
I	6,7±1,2	$16,4±2,7^X$	2,4±0,1
II	9,8±1,6*	11,3±1,9	1,13±0,05*

Note: * - significant difference from those of patients in group I and ^{X} – compared with those of the spontaneous CL response of cells in the corresponding group.

Table 3.

Cytokine-producing activity of cells in BAL fluid of patients with asthma of varying severity

Cytokines, pg/ml	Groups	Cytokine-producing activity of cells	
		Spontaneous	LPS- induced
IL-1β	I	75,3±2,4	$225,7±15,2^X$
	II	67,5±4,3	$111,2±9,3*^X$
TNF-α	I	99,5±5,8	$337,4±23,5^X$
	II	65,3±7,4*	$145,8±11,4*^X$
IL-4	I	63,8±5,8	124,4±9,8
	II	109,2±9,7*	$342,4±33,3*^X$

Note: * - significant difference from those of patients in group I and ^{X} – compared with those of the spontaneous production of cytokines in the corresponding group.

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