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## **Ultrastructural characteristics of eosinophilic leukocytes contained in the respiratory tract in patients with bronchial asthma**

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**Abstracts:** The ultrastructural study of eosinophilic leukocytes contained in the respiratory tract. Cellular elements of the respiratory system of patients with asthma were removed by bronchoalveolar lavage. Electron microscopy revealed degranulation of eosinophils performed by fragmentation of the cytoplasm. In damaged eosinophilic leukocytes in the respiratory tract in patients with bronchial asthma is missing nucleus fragmentation, there is hypertrophy of the nuclear matrix.

**Key words:** bronchoalveolar lavage, bronchial asthma, eosinophils

Eosinophils are cytological marker bronchial asthma and other hypersensitivity (5). The role of eosinophils in allergic considered from two perspectives. The first position takes into account the participation of eosinophils in the payment of diseases, it is based on the fact that eosinophils possess nuclear receptors glyukortikoidam involved in the adaptation syndrome. In the granules of eosinophilic leukocytes contained histaminase enzyme breaks down histamine and disposing of the enzyme arylsulfatase derivatives of arachidonic acid, found in the same zinc-containing granules. A number of authors draw attention to the pronounced degranulation of specific granules of eosinophilic leukocytes in the case of asthma (4, 6, 7, 8). According to these researchers eosinophil white blood cell involved in the transport complex of cytotoxic factors in the extracellular environment, which has anti-inflammatory in nature. Therefore, in the present moment remains unexplored role of eosinophils processes of compensation and decompensation of respiratory diseases.

The aim of the study was to study the ultrastructure of eosinophilic leukocytes contained in bronholavazhnoy fluid in patients with bronchial asthma.

### **Materials and methods**

Study of bronchoalveolar lavage was carried out on the basis of the central research laboratory of the National Academy of Amur Amur regional hospital and one public hospital in the endoscopy department. We investigated bronchial lavage fluid (BAL) in 240 patients with bronchial asthma (BA). We studied bronchoalveolar lavage cells in 130 patients with endogenous and in 110 patients with combined (endogenous and exogenous), bronchial asthma, among them a severe there were 100 people, and with an average over 140 people. In its properties, it is identical to the so-called bronchoalveolar washout. The procedure begins with the injection of 10 ml of warm saline subsegmental bronchus of the middle lobe of the right lung during endoscopic study by the original

method. BALF was centrifuged for 15 minutes at 1500 rev / min by gently pipetting the supernatant was taken away, leaving a 0.3 ml tube, and the residue was mixed until a homogenous cell suspension, after which the cell suspension was precipitated by the proposed method on an agarose gel, gelatin, fibrin clot by centrifugation at 800 rpm. The gelling agar substrate used is a mixture of agarose. Agarose gel formation temperature is 40-45 ° C.

Formation of fibrin clot formation set out further. BAL cells were fixed 1 hour in a 2.5% glutaraldehyd in 0.1 M phosphate buffer pH 7.4. Then samples of material were placed in a 1% solution of osmium tetroxide - osmic acid in 0.1 M phosphate buffer pH 7.4 to 1, 5:00, dehydrated in alcohols of increasing concentration of the standard method (1).

After wiring patterns embedded in a mixture of Araldite and Epon. Then prepared semi-thin and ultra-thin sections on ultramicrotome LKB-NOVA. Ultrathin sections were contrasted with a saturated alcoholic solution of uranyl acetate and lead citrate. The study was carried out and photographing in a transmission electron microscope PEM 100, JEM-100CXII (Jeol Japan).

### **Results and discussion**

By electron microscopy the cytoplasm eosinophils detected mitochondria and free ribosomes tube endoplasmic reticulum. Specific eosinophil granule ovoid shapes fill the entire cytoplasm. Characterized by a crystalloid size of 0.6-1 microns. Electron-microscopically in the equatorial zone of eosinophil granule identified single crystalloid structure with lamellar structure.

Crystalloids eosinophilic granules contain major basic protein. Granules of eosinophils are different in structure from the lysosomes of macrophages. Attention is called to change the structure of the lysosomes of macrophages. We are in the study found that the degeneration of lysosomes leads to a change in the shape of these organelles that are characteristic of macrophages. Since lysosomes contact tanks advanced endoplasmic reticulum. As part of the alveolar macrophage lysosomes elektronnoplotty contain material that resembles crystalloids eosinophils.

In the cytoplasm of pulmonary macrophages are found free ribosomes. At the periphery of the cytoplasm are phagocytic vacuole. On the cytoplasmic membrane bounding wall of the phagosome microbubbles are found, the function of which is not clear.

In the case of asthma in cytological preparations of bronchoalveolar lavage fluid is constantly observed decay of eosinophils, which confirms the findings of other authors.

The outer membrane of the cell to lyse. Through its massive defects remains of cytoplasm communicate with the environment. The cytoplasm is divided into cell membrane structures. The cytoplasm is filled with electron-dense granules. Organelles in the cytoplasm of most destroyed. As a result, we see cell vacuolation. In the cytoplasm, remain fine fibrillar structure between the components of the cytoplasm with each other.

At the ultrastructural characteristics there is a high stability of nuclei of eosinophils to the damaging effects of the microenvironment factors cells.

The cells contained two fragments of a segmented nucleus. They are filled with electron-dense structureless homogeneous material, which are found in the areas of low electron density of irregular shape. Nuclear membrane retains its structural integrity and completely separates the nucleus from the cytoplasm.

Thus, for ultrastructural analysis of cells in the bronchoalveolar lavage fluid contained confirmed that cytoplasmic degranulation of eosinophils is one of the mechanisms of asthma in humans. In a number of cellular elements of eosinophils in BAL fluid contained intracellular structures observed fragmentation characteristic of apoptosis. However, in this case no pronounced shrinkage of the cytoplasm and nucleus fragmentation of eosinophilic leukocyte cells characteristic of apoptosis. In this case, it seems that the change in the ultrastructural core eosinophils, may be the result of hypertrophy of the nuclear matrix, the cytoskeleton of the nucleus.

In turn, it can be assumed that the histopathology core in eosinophils in the case of asthma, indicates the activation of proteins of the laminin. At the same time, the study of the fragmentation of the cytoplasm of alveolar macrophages in patients with bronchial asthma draws attention to the

presence of lipid droplets informativeness near the nucleus, which may reflect the development of the phanerozoic (2, 3). For that reason, we believe that there is a single mechanism for the fragmentation of the cytoplasm of alveolar macrophages, and eosinophils in the lungs in the case of respiratory diseases. This view is confirmed by the fact that in the study of partial weight bronchoalveolar cytogram in the case of asthma found a reliable correlation coefficient, confirming the existence of an inverse-proportional relationship between the number of alveolar macrophages, and eosinophils (2).

#### **Conclusion:**

1. In patients with bronchial asthma in the respiratory tract revealed degranulation of eosinophils, which is done by the fragmentation of the cytoplasm.
2. In the respiratory tract in patients with bronchial asthma, eosinophilic leukocytes in damaged cells no shrinkage, nuclear fragmentation, marked hypertrophy of the nuclear matrix.

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