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Structural Modifications of the Bronchial Epithelium in Asthma

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A complex of structural changes in the parenchymatous and stromal compartments, characteristic of the primary degenerative process (bronchial epithelium degeneration and subsequent diffuse atrophy, reactive sclerosis of the stroma) develops in the large bronchi in asthma. Induction of the regeneratory reactions of the bronchial epithelium with synchronously developing reorganization of the stroma (loosening of the connective tissue and stimulation of its vascularization) is shown during correction of asthma. Association of positive structural changes with lymphoid tissue hyperplasia in the bronchial wall can be interpreted in the context of significant role of lymphocytes in induction of the regeneratory reactions.

Key Words: *asthma; bronchial epithelium; ultrastructure; morphometry*

Asthma was investigated not once in studies of bronchopulmonary diseases. Among the factors essential for the appearance and course of this disease, pollutants (sulfur and nitrogen dioxides, black smoke), ozone, aeroallergens, and genetic liability to allergic reactions are considered as the most significant [4,7-9,11,12].

Compensation for changes developing in asthma is realized at the expense of endotheliocyte and myofibroblast proliferation, which leads to collagen deposition in the lamina reticularis of the basal membrane and its characteristic thickening [10], as well as to other structural changes including hypertrophy and hyperplasia of the bronchial smooth muscle cells, increase in the count of goblet cells, and restructuring of the bronchial connective tissue. Many mediators, responsible for modification of the respiratory tract architecture, are not identified, and hence, cytokines and growth factors are now considered to play the leading role in this process [13].

We studied restructuring of the bronchial epithelium in asthma during its correction in order to evaluate the regeneratory potential of epitheliocytes.

MATERIALS AND METHODS

A retrospective analysis of bronchial biopsy specimens (n=250) from 70 asthmatics (40 females and 30 males aged 17-58 years) was carried out over the course of treatment. In group 1 patients (n=44) the tone of sympathetic regulation of the airway smooth muscles was reduced by crossing the internal branch of the superior laryngeal nerve, group 2 patients (n=26) received drug therapy for asthma (inhalation β_2-agonists and antiinflammatory drugs,

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long-acting theophyllines, inhalation corticosteroids).

Functional and X-ray studies were carried out in all patients, bronchoscopy with biopsy was carried out before and after treatment (after 1 week, 1 month, 1 and 6 years in 10 patients). Paraffin sections of bronchial biopsy specimens were stained with hematoxylin and eosin in combination with Pearles' reaction, after Van-Gieson with poststaining of elastic fibers with Weigert's resorcin-fuchsin, and using PAS reaction. Semithin sections were stained with Schiff's reagent and Azur II, ultrathin sections were contrasted with saturated uranylacetate solution in ethanol and lead citrate and examined in a JEM 1010 electron microscope at accelerating voltage of 80 kV.

Morphometrical and stereological analysis of bronchial biopsy specimens was carried out on semithin sections using an ocular multipurpose test system. Volume and surface densities of capillaries, structural density of fibers and main substance of connective tissue and cell infiltrate were calculated as the primary parameters. The thickness of the epithelial basal layer was measured with an ocular micrometer.

The data were statistically processed using Student's *t* test; the differences were considered significant at $p<0.05$.

RESULTS

Diffuse degeneration and atrophy of the surface epithelium with pronounced desquamation (Fig. 1, *a*), glandular degeneration and atrophy, pronounced stromal sclerosis, and reduction of the microcirculatory bed were present in all biopsy specimens before treatment. The epithelial basal membrane was significantly thickened; hyperelastosis of the mucousa was observed (Fig. 1, *b*).

Ultrastructural analysis showed intracellular changes characterizing the development of progressive atrophy of the bronchial epithelium. The ciliary system was the first to be destroyed in the ciliary epitheliocytes; cytoplasmic organelles were disorganized and altered (destruction of the mitochondria, reduction of the cytoplasmic reticulum, decreased number of ribosomes and polysomes). Goblet granulocytes had few secretion granules, some granules were incorporated into the lysosomal phagocytic complexes, thus forming polymorphic autophagosomes. Epitheliocytes devoid of morphological signs of differentiation into ciliary and goblet cells were the predominant population: cells with electron-dense cytoplasm and sharply modified ultrastructural organization (Fig. 2, *a*).

Before treatment, the structural changes in bronchial biopsy specimens were similar in patients of both groups, while after the operation more positive changes were observed in biopsy specimens from this group: induction of regeneratory reaction was detected as soon as 1 week postoperation. After 4 weeks this trend was more pronounced; the integrity of the surface epithelium was partially restored, with reversion of its morphological phenotype (Fig. 1, *c*) or at the expense of squamous-cell metaplasia (Fig. 1, *d*); the intensity of cell infiltration in the lamina propria decreased and its vascularization increased. During the delayed period, cell infiltration in biopsy specimens was minimum and the type of structural rearrangement in the bronchial mucosa reflected high activity of the proliferative processes in the epithelium.

In group 2 positive shifts were slower and not always easily discernible, manifesting 1.5-2 months after the start of therapy by partial recovery of the integrity of surface epithelium mainly at the expense of its squamous cell metaplasia. More pronounced and rapid positive changes were observed in cases with the formation of lymphoid aggregations in the bronchial wall; the changes were minimal in pronounced atrophic sclerotic changes in the bronchial mucosa.

A clear-cut correlation between activity of the epithelial proliferative reaction and reorganization of the underlying connective tissue (more intense vascularization, formation of vascularized connective tissue papillae, lymphoid infiltration with formation of lymphoid aggregations and lymphodiapedesis), reflecting previously described regularities of the parenchymatous-stromal relationships [5], was observed in both groups.

Stereotypical ultrastructural changes in the bronchial epithelium and capillary endotheliocytes were observed over the course of treatment in both groups. Many epitheliocytes contained numerous ribosomes, polysomes, well-developed granular cytoplasmic reticulum; cylindrical cells, containing mitochondria, basal bodies, and regenerating elements of the ciliary system in the apical zone were often seen (Fig. 2, *b*). Secretory epitheliocytes had well-developed lamellar complex and polymorphic secretory granules. Capillary endotheliocytes were large, with large nuclei, high content of pinocytic vesicles, and cytoplasmic processes on the inner surface of the capillary, this being characteristic of functionally active cells.

Morphometrical studies were carried out in both groups, the results before and after treatment were compared. The thickness of the epithelial basal membrane varied greatly (from 3 to 9 μ). The mean va-

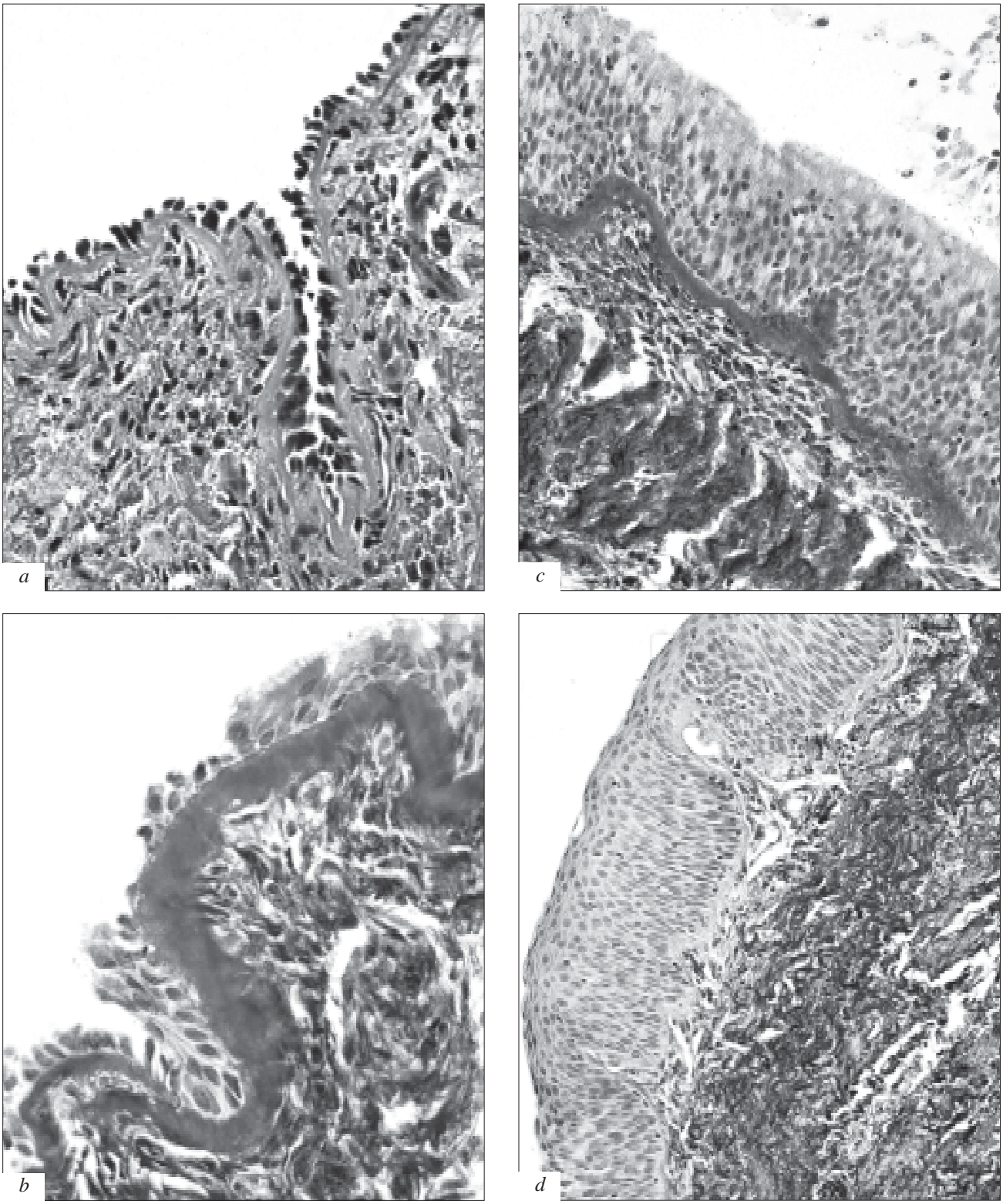

Fig. 1. Photo-optic characteristics of bronchial biopsy specimens during correction of asthma. *a*, *b*) before treatment; *c*, *d*) after treatment. *a*) degeneration, atrophy, and desquamation of bronchial epithelium, ×350; *b*) atrophy and desquamation of bronchial epithelium, sharp thickening of subepithelial basal plate, pronounced hyperelastosis, ×600; *c*) proliferation of bronchial epithelium, mononuclear cell infiltration of the stroma with lymphodiapedesis, ×200; *d*) proliferation and squamous-cell metaplasia of bronchial epithelium ×160; *a*) hematoxylin and eosin staining; *b*) Van-Gieson staining.

lue before treatment was 7.13±1.05, 1 month after treatment it decreased more significantly in group 1 (4.59±0.74 μ in group 1 *vs.* 6.45±0.80 μ in group 2). The thickness of the basal membrane varied in group 1 one month after the operation, the mean value being 4.59±0.74 μ.

The volume density of cells, fibers, and main substance of the connective tissue was evaluated in a stereological study of the large bronchial mucosa. Special attention was paid to vascularization of the subepithelial zone; structural density of capillary vessels was evaluated. Stereological study showed that the volume density of subepithelial capillaries increased more significantly in group 1 after treatment. The increase in the surface density of capillaries and decrease in the structural density of connective tissue fibers in the subepithelial zone were in line with loosening of connective tissue and its more intensive vascularization, shown by photo-optic microscopy.

Volume density of cell infiltration in the lamina propria of the bronchial mucosa in asthmatics varied greatly in both groups after treatment. Stereological parameters of cell infiltrate were characterized by considerable variability; this parameter often increased during treatment and, according to findings of photo-optic analysis, this increase was mainly at the expense of mononuclears, among which lymphocytes predominated, forming lymphoid aggregations. Hyperplasia of the lymphoid tissue reflected the immune system strain and, according to the findings of analysis of bronchial biopsy specimens, played a positive role in repair reactions of the bronchial wall during therapy.

Hence, without setting one treatment protocol for asthma against the other, we see that both are sufficiently effective, which is confirmed by positive dynamics of the clinical picture and positive restructuring of the bronchial mucosa.

On the whole, the structural changes in the bronchial biopsy specimens from asthmatics correspond in the majority of cases to the morphogenesis of a primary degenerative process (atrophic bronchopathy) with an allergic component (primary degeneration, subsequent atrophy, and synchronously developing diffuse stromal fibrosis). It is noteworthy that cell infiltration was absent or minimum in the majority of the studied biopsy specimens before treatment and, which is still more important, was presented by lymphocytes and fibro-

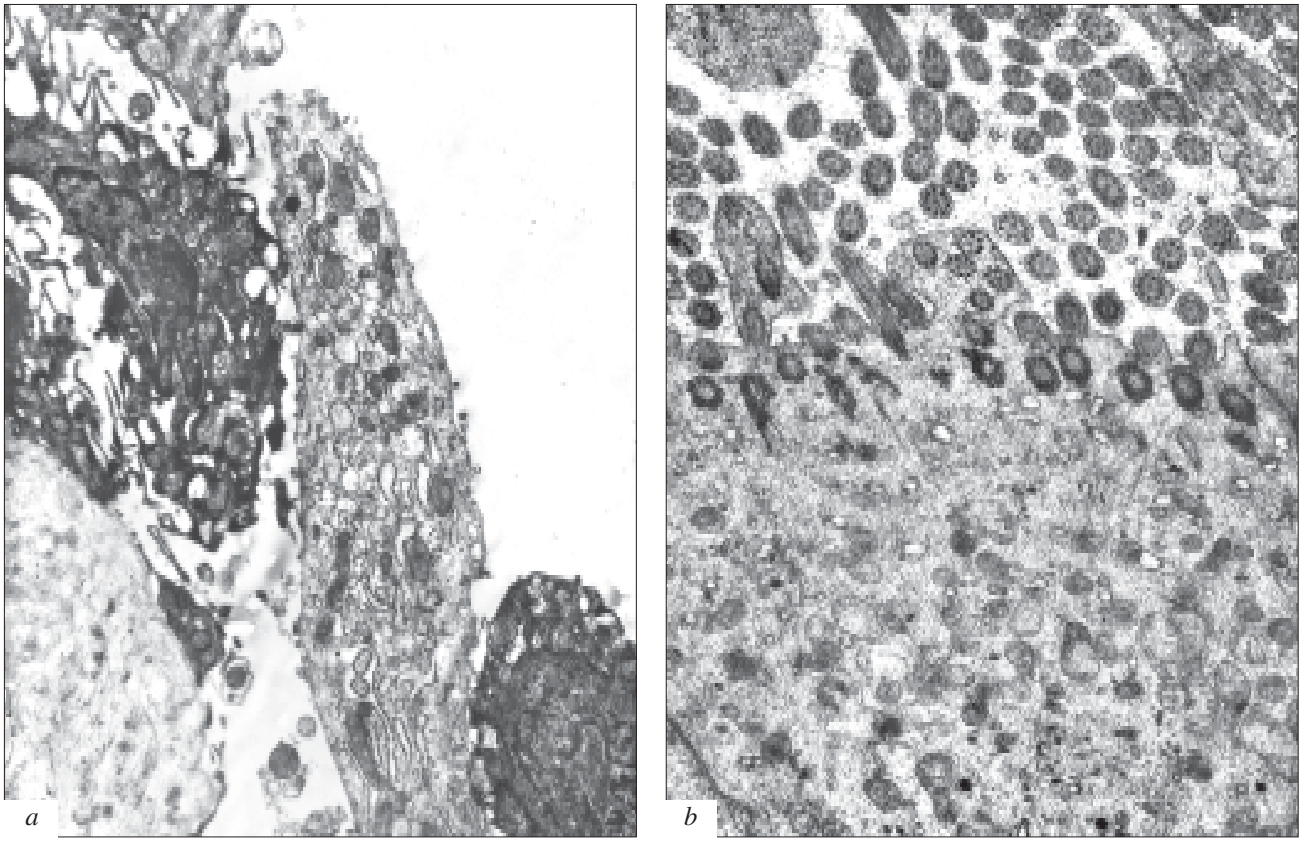

Fig. 2. Ultrastructural characteristics of bronchial epithelium in asthma. *a*) before treatment: disorders in epitheliocyte ultrastructure and differentiation, ×5000; *b*) after treatment: fragment of ciliary epitheliocyte, ciliary regeneration, ×10,000.

blasts and could not be interpreted as inflammatory infiltration. In a lesser part of biopsy specimens cell infiltration was significant and polymorphic and could be regarded as inflammatory reaction, but by the complex of structural changes it was facultative and secondary, as it developed as a result of decreased barrier function of the bronchial epithelium.

The type of cell infiltration of the bronchial wall changed in the course of treatment, particularly in group 1. Infiltration became more intense and often diffuse, but was presented by mononuclears with predominance of lymphocytes. These cells exhibited a trend to the formation of lymphoid aggregations and intraepithelial lymphodiapedesis. It is a special type of inflammation, not paralleled by suppuration and destruction, but linked with positive reorganization of the stroma and induction of the regeneratory reactions of the epithelium. It was noted that pronounced lymphoid infiltration, lymphoid follicles, multiple interepithelial lymphocytes in the biopsy specimens before treatment and virtually no eosinophils in the cell infiltrate were associated with more positive shifts in the bronchial wall after treatment. This can be interpreted in the context of significant role of lymphocytes in induction of epithelial cell regeneration and differentiation [1,6].

Other pathogenetic variants of asthma are not ruled out, as, like many other diseases, it can be polymorphic by etiology and pathogenesis. The nervous neurotrophic or corticovisceral genesis cannot be excluded [2]. Many scientists regard neurogenic degeneation as a component of virtually all pathological processes [4]. It seems that an attempt at presenting the pathogenesis of asthma in general as a chronic inflammation deserves further discussion [11].

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