Ultrastructural Changes in the Epithelium in Asthma Associated with Gastroesophageal Reflux

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Asthma concomitant with gastroesophageal reflux is regarded in the context of systemic multifactorial primary degenerative process, based on discoordination of neurohormonal regulation and systemic metabolic and degenerative changes. Degeneration and atrophy of the structural components of the bronchial tree and gastric wall are the leading phenomenons determining the organ dysfunction.

Key Words: *asthma; gastroesophageal reflux; bronchial and gastric epithelium; electron microscopy*

High incidence of association between asthma and gastroesophageal reflux (GER), reaching 80% in different populations, is observed in recent years [3,11,13]. Gastroesophageal reflux develops as a result of reduction of the antireflux barrier because of incompetence of the inferior esophageal sphincter often combined with incompetence of the duodenogastral sphincter and retrograde passage of aggressive refluctant components into the esophagus [1,14]. The pathogenesis of association between asthma and GER includes several mechanisms. Asthma can provoke the development of GER due to anatomical characteristics, common innervation, and synchronously developing broncho- and esophagospasm. Drugs used for the treatment of asthma can induce symptoms of GER by reducing the tone of the inferior esophageal sphincter [11,12,15].

Bronchospasm is a potential trigger for temporary relaxation of the inferior esophageal sphincter. On the other hand, reflux can stimulate bronchoconstriction in asthmatics [13]. Moreover, if reflux triggers asthma and asthma causes reflux, the progress of asthma can lead to the development of a vicious circle promoting the development and persistence of GER because of increasing pressure gradient between the thorax and abdominal cavity [12].

GER alone or in combination with other conditions is one of the most frequent causes of chronic cough, caused, presumably, by stimulation of the esophago-bronchial reflex [13].

We studied ultrastructure of mucosal epithelial cells (EC) in the large bronchi and stomach in asthma associated with GER.

MATERIALS AND METHODS

Microscopy and ultrastructural analysis of bronchial and gastric EC were carried out in 25 asthmatics with GER (14 female and 11 male ones aged 32-67 years) with the disease duration of 4-20 years. Bronchoscopy with biopsy of the large bronchi and esophagogastroscopy with biopsy of the fundal and pyloric compartments of the stomach was carried out in all cases.

Paraffin sections were stained with hematoxylin and eosin in combination of Pearls reaction, after van Gieson with poststaining of elastic fibers with

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Weigert's resorcin-fuchsin, and PAS reaction was carried out. Semithin sections were prepared routinely [4] and stained with Schiff's reagent and Azur II.

The study was carried out using a Leica DM 4000B universal microscope. Microphotographs were made with a Leica DFC 320 digital photocamera and Leica OWin software. Ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

RESULTS

Paroxysmal nocturnal cough with bronchospasm predominated in the clinical picture of asthma. The bronchoscopic picture was heterogeneous and depended on the duration and severity of asthma: hyperemia and edema of the mucosa of different severity, atrophy and deformation of the bronchi. The main clinical manifestations of GER were heartburn, dysphagia, epigastric pain, and hypersalivation. Endoscopy showed erythema and erosions in the distal part of the esophagus in 61 and 8% cases, respectively, ulcerative defects in the abdominal compartment in 6% cases. Insufficiency of the cardia because of relaxation of the inferior esophageal sphincter and development of sliding hernias of the esophageal hiatus were detected in more than 50% cases. Visual evaluation of the gastric mucosa showed the absence of erosive and ulcerative defects often occurring in asthma without GER [7]. In addition to focal hyperemia of the mucosa and symptoms of the duodenogastral sphincter incompetence were detected.

In studies of bronchial biopsy specimens from patients with asthma associated with GER special attention was paid to the dynamics of structural changes in bronchial EC, which combined stereotypical reactions and some reconstructions of the epithelium and subepithelial zone, characteristic of asthma. The degree of reorganization of the epithelial and stromal compartments of the bronchial mucosa depended on the severity of asthma and reflected the dynamics of EC degeneration progress (from focal slight to diffuse significant degeneration). The progress of bronchial epithelial degeneration resulted in EC atrophy (Fig. 1, *a*) characterized primarily by decreased number of the strata of the epithelium with subsequent transformation of the mucosa epithelium into a single-layer cubical or flat, sometimes endothelium-like lining (Fig. 1, *b*) devoid of the main specific cell organelles.

In parallel, compensatory reactions of EC were induced at the expense of their proliferation, hyperplasia, and squamous-cell metaplasia (Fig. 1, *c*). Combination of degenerative reactions of the epithelium with proliferative processes created a mosaic histological picture: small biopsy specimen of the bronchial mucosa contained foci of cylindrical multi-row and multi-lamellar squamous epithelium and of atrophy and compensatory hyperplasia simultaneously; this phenotypical heterogeneity of the epithelial layer was denoted as the phenomenon of "bronchial epithelial instability" [4,6]. This indicates disorders in the regeneration and differentiation processes, manifesting by combination of different phenotypical modifications of the bronchial epithelium.

The main feature in the ultrastructural reorganization of retained ciliary EC was disordered regular location of the cilia (Fig. 1, *d*), focal destruction of basal bodies, and replacement of the lost cilia with cytoplasmic processes and microvilli (Fig. 1, *e*). Degeneration of the ciliary system was associated with destruction of the mitochondrial compartment (energetic constituent of the ciliary function): reduction of mitochondria, destruction of cristae, and clarification of the matrix. Degenerative intracellular reactions were paralleled by an increase in the number of lysosomes with subsequent formation of structurally heterogeneous autophagosomes.

Intact secretory (mucus-producing) EC of surface bronchial epithelium were in different phases of the secretory cycle. Degenerative cells with reduction of Golgi complex, condensation of secretory granules, and subsequent formation of autophagosomes predominated in the majority of biopsy specimens, particularly in severe asthma, which indicated disorders in the excretory phase of the secretory cycle.

Epitheliocytes in zones of squamous-cell metaplasia had the phenotype of polygonal process cells (Fig. 1, *f*), integrated by means of desmosomes, formed with "participation" of thick bundles of microfilaments. Elements of the protein-producing compartment, providing the production of filamentous structures, were the main elements in the ultrastructure of squamous epithelial cells.

Epitheliocytes without ultrastructural signs of differentiation into ciliary and goblet cells presenting just as cells with hyperchromatic nuclei and electron-dense cytoplasm with blurred intracellular organization were the predominant population in severe protracted asthma.

Sharp thickening of the basal membrane of surface epithelium of the bronchial mucosa is a typical, we can say, a pathognomonic sign of asthma; the basal membrane in the presence of GER is characterized by even greater thickness, electron heterogeneity, and greater number of fibrous structures.

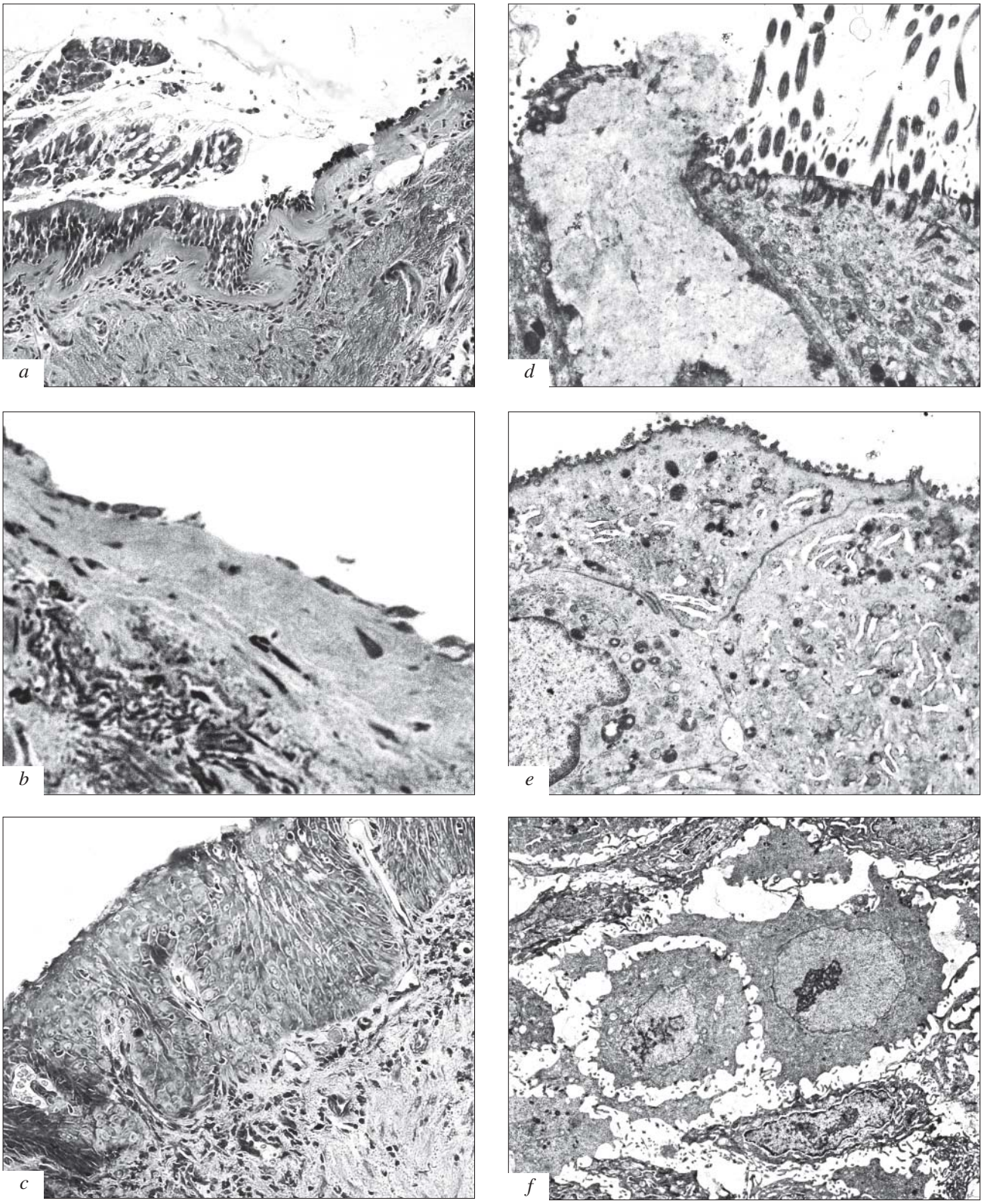

Fig. 1. Structural characteristics of lobular bronchial epithelium in asthma associated with GER. *a*) focal atrophy, ×200; *b*) atrophy with endothelium-like modification, ×600; *c*) hyperplasia and squamous-cell metaplasia, ×250; *d*) ciliary and goblet EC with the minimum destruction of organelles, ×6000; *e*) EC without cilia, ×5000; *f*) polygonal cells in zones of squamous-cell metaplasia, ×2000. *a-c*: hematoxylin and eosin staining; *d-f*: electronograms.

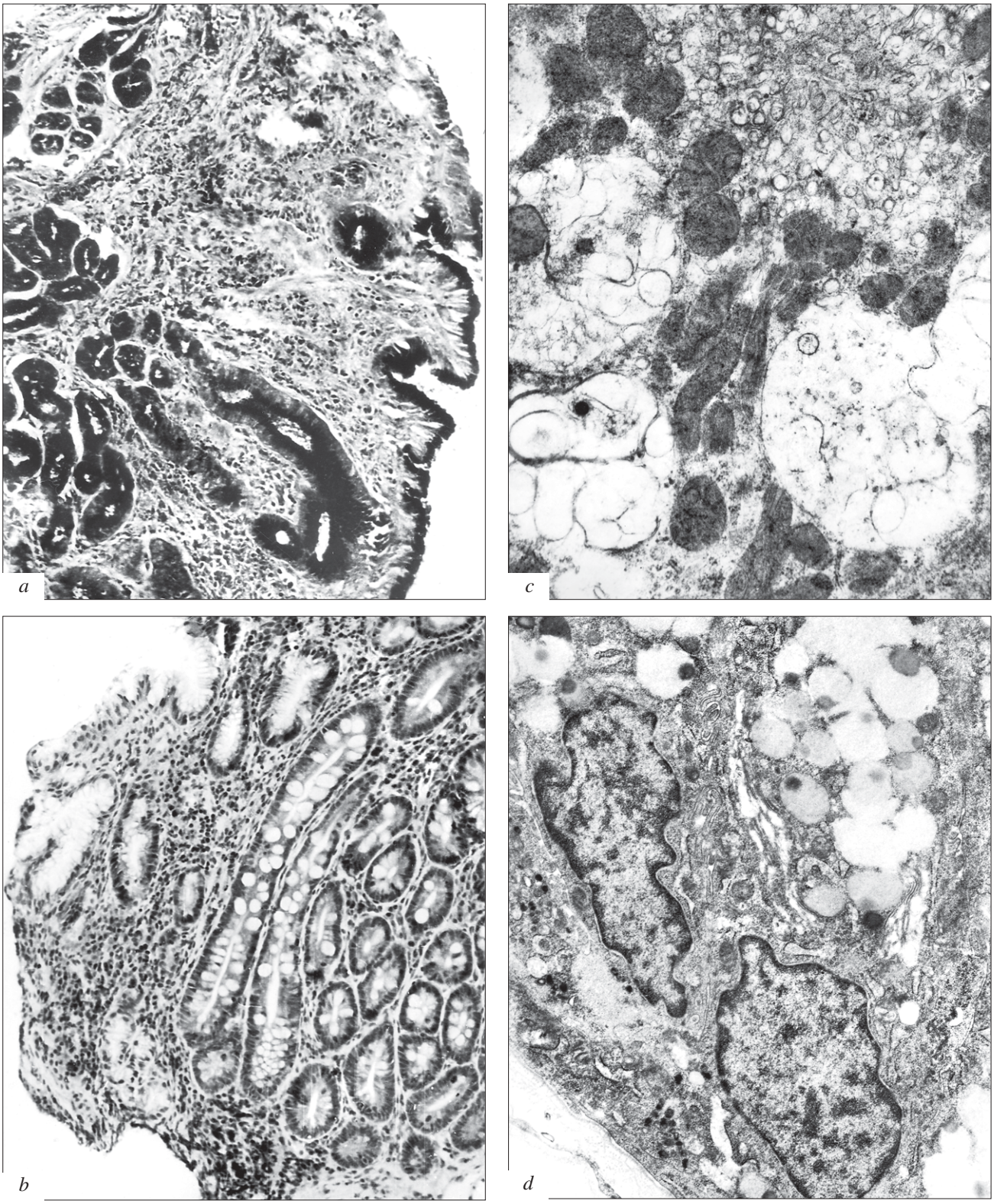

Fig. 2. Structural characteristics of the gastric mucosa in asthma associated with GER. *a*, *b*) biopsy specimens of the fundal portion; *c*, *d*) biopsy specimens of the pyloric portion. *a*) glandular atrophy. PAS reaction, ×160; *b*) small intestinal metaplasia, lymphocytic infiltration. Hematoxylin and eosin staining, ×200; *c*) parietal cells with degenerative mucoid granules, ×15,000; *d*) mucus-producing cells; fragments of endocrinocytes with small osmiophilic granules near the basal membrane, ×5000.

The basal membrane largely determines the epithelial structure and function and the epithelial-stromal interactions [4].

Changes in the bronchial epithelium in asthma with concomitant GER are characterized by more pronounced degenerative changes in ciliary EC, more incident hypoplasia and hyposecretion of goblet cells and lesser basal-cell proliferation in comparison with isolated asthma [5].

In gastric biopsy specimens from patients with asthma combined with GER, surface EC were heterogeneous, which reflected the dynamics of the pathological process (from little changed epithelium to diffuse atrophy, combined with atrophy of the glandular compartment; Fig. 2, *a*). Small-intestinal epithelial metaplasia was detected in one-third of biopsy specimens (Fig. 2, *b*). The fundal glands developed focal degeneration, partial reduction, and metaplasia; mixed cells containing along with mucin granules, elements of the tubulovesical system, intrinsic of acid-producing cells, were detected in severe asthma (Fig. 2, *c*). Erosive defects in the esophageal mucosa were associated with hyperplasia and hyperfunction of the gastric parietal cells.

Analysis of biopsy specimens from the pylorus showed hyperplasia of endocrine EC (similarly as in isolated asthma [7]), among which gastrin-producing G-cells with specific electron-clear granules playing an important role in stimulation of parietal cells [10], were most numerous. Endocrine cells of other types (Fig. 2, *d*) were often seen, particularly ECL and D (their secretion products seem to be also involved in the pathogenesis of combined disease).

Hence, analysis of the results and published data presents the scheme of morphogenesis of asthma with concomitant GER in the context of systemic multifactorial primary degenerative process, based on discoordination of neurohumoral regulation and systemic metabolic and degenerative changes [2,8,9]. Hyperplasia of endocrine EC in the pylorus is worthy of note. Degeneration and atrophy of the structural constituents of the bronchial tree and gastric wall is the leading phenomenon determining organ dysfunction. GER reflects not local defect of the inferior esophageal sphincter, but dysfunction of the entire gastrointestinal system.

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