IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF THE BRONCHIAL MUCOSA DURING CHRONIC INFLAMMATION

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The leading mechanisms of development of a pathological process in mucous membranes are disturbances of regeneration and differentiation of the epithelial lining which, to a certain degree, are determined by the state of the stroma, the microcirculatory bed, and the system of local immunity [1, 4, 5, 7]. In particular, chronic inflammation in the bronchi leads to the formation of secondary immunologic insufficiency [14] and causes marked sensitization of the body with hetero- an dautoantigens [11], i.e., it acquires the features of immune inflammation [8, 9].

The aim of this investigation was to study relations between structural reorganization of the epithelium on the one hand, and the state of local immunity and changes in the microcirculatory bed of the human bronchial mucosa, on the other hand, during chronic nonspecific inflammation.

EXPERIMENTAL METHOD

Biopsy material taken from the walls of the main, lobar, and segmental bronchi at bronchoscopy on 31 patients with chronic nonspecific bronchitis, aged from 19 to 57 years, was studied by light and electron microscopy and immunohistochemistry. The biopsy material was fixed in a 4% solution of paraform, and after fragments had been cut off for electron microscopy, all the remaining material from the bronchial mucosa was treated by a method specially developed for bronchial biopsy specimens [3]. Fragments of tissue for electron microscopy were postfixed in 1% OsO_4 solution and embedded in a mixture of Epon and Araldite; semithin sections were stained with azure II and by the PAS reaction. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope. Immunohistochemical investigations were carried out on paraffin sections, and immunoglobulins of the A. G. and M classes were determined by the indirect Coons' method and immune complexes by the method of Goldwasser and Shepard. Paraffin sections were stained with hematoxylin and eosin, by Van Gieson's method with counterstaining of elastic structures with Weigert's fuchselin, and by the PAS reaction.

EXPERIMENTAL RESULTS

Depending on the state of the epithelial lining, all bronchial biopsy specimens were divided into four groups in accordance with the classification in [4]. Changes in the epithelium correlated distinctly with the state of local immunity and the character of the immunopathological reactions.

Group 1 included biopsy specimens in which the normal histoarchitectonics, ultrastructure, and local immunity of the mucosa were mainly preserved. The epithelium was stratified cyclindrical in appearance with a well-preserved ciliated border and with moderate secretory activity of the goblet cells. In the apical regions of the ciliated cells and on the surfact of the cilia IgA was detected. Mononuclear infiltration of the subepithelial layer was represented mainly by lymphocytes and plasma cells producing IgA; IgM — and IgG-containing cells were less frequently seen. This type of structure of the mucosa was observed infre-

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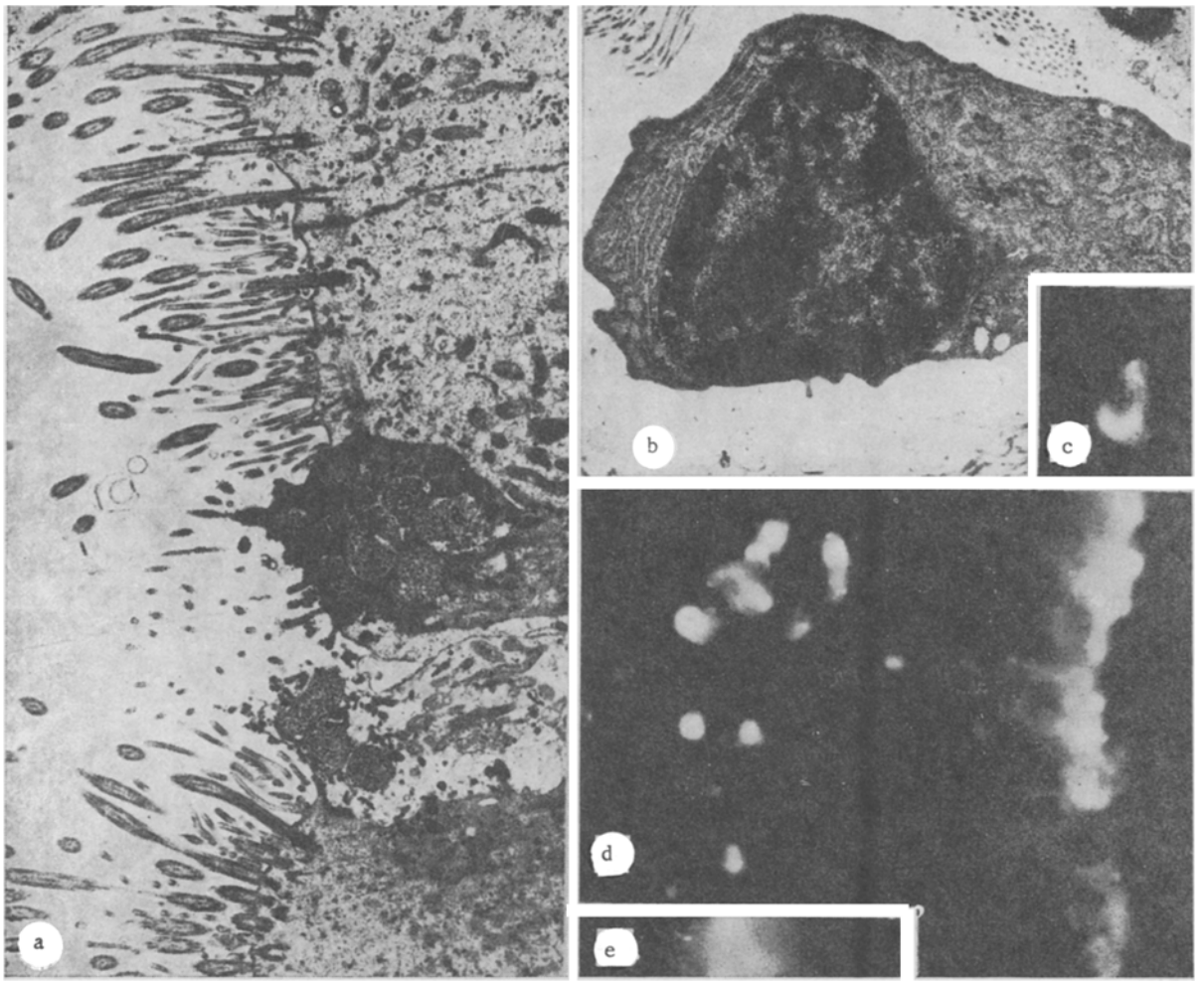

Fig. 1. Local immunity and ultrastructure of bronchial mucosa in chronic bronchitis: a) mucosa of segmental bronchus in chronic catarrhal inflammation (epithelium of group 2). 8000 ×; b) Mature plasma cell. 20,000 ×; c) IgA-producing cell in bronchial mucosa. 400 ×; d) IgA in cells of lamina propria of bronchial mucosa and apical zones of ciliated cells in chronic catarrhal inflammation. 200 ×; e) Ciliated cell (fragments of Fig. 1d). 600 ×; c-e) Indirect Coons' method.

quently and corresponded endoscopically to the normal bronchologic picture. Local immunity was preserved or even a little activated.

Changes in the epithelial lining in the specimens of group 2 were characterized by a combination of signs of alteration of the ciliated cells, hyperplasia and hyperproduction of the goblet cells, and changes in local immunity (Fig. 1a). The basal cells were unchanged, but the intercellular spaces were widened, and interepithelial lymphocytes were frequently seen. In the subepithelial zone and stroma of the glands, infiltration became polymorphocellular: plasma cells and lymphocytes were a constant component, and polynuclear neutrophils, eosinophils, mast cells, macrophages, and fibroblasts were observed. The number of globulin-producing cells in these biopsy specimens was considerably increased; hyperplasia of the clone of IgA-producing plasma cells predominated (Fig. 1b, c), and they were diffusely distributed in the lamina propria of the mucosa, with concentration in the subepithelial zone and in the stroma of the bronchial gland. Specific fluorescence of IgA was observed in the apical regions of the ciliated cells and in areas where the ciliated border was preserved (Fig. 1d, e). IgG-containing cells were numerous, IgM-containing cells fewer in number. These changes in the epithelial lining corresponded to chronic catarrhal bronchitis, and the state of local immunity resembled the immune response arising after aerosol vaccination [15].

Changes in the epithelium of the group 3 material were characteristic of chronic catarrhal-sclerosing inflammation. The ciliated cover was almost completely destroyed. The stage of hypersecretion in the goblet cells was replaced by dystrophic changes: secretory granules

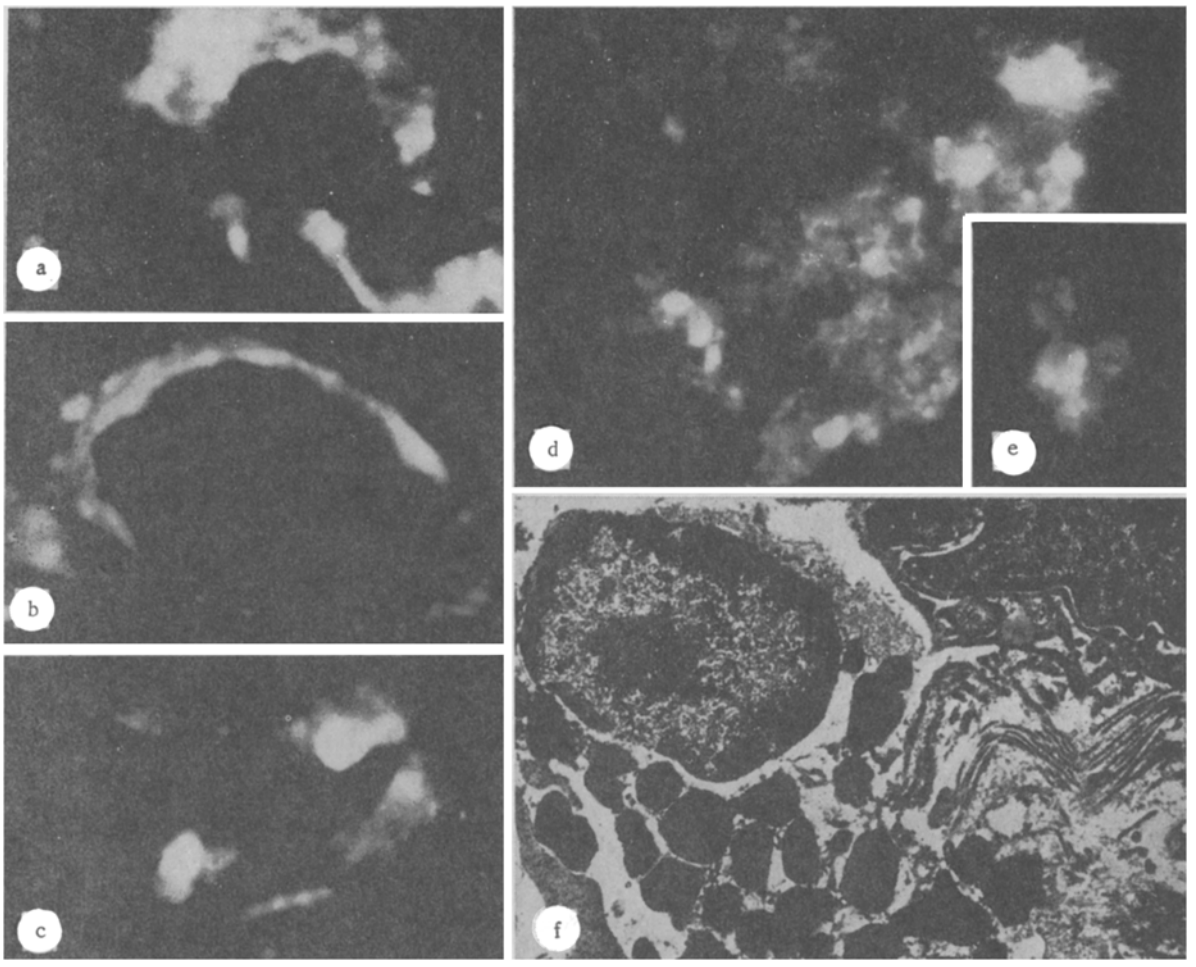

Fig. 2. Immunopathological reactions in bronchial mucosa in chronic bronchitis: a) granular fluorescence of fixed immune complexes in vascular wall of bronchial mucosa; b) IgM in wall of a small vein; c) deposits of IgG in vascular wall; d) immune complexes in zone of polymorphocellular infiltration of lamina propria of bronchial mucosa; e) granular fluorescence of phagocytosed immune complexes; f) degranulating mast cell. a, d, e) Goldwasser—Shepard method; b, c) indirect Coons' method. Magnification: a) 300; b, c, e) 400; d)200; f) 20,000 ×.

were few in number, and lysosomes and autophagosomes were frequently seen near them. A characteristic feature of changes in the epithelium of this group was the formation of cells of a special type, polygonal in shape with long, narrow processes, which insinuated deeply between the surface cells. The polygonal cells had many desmosomes and their cytoplasm contained bundles of thin fibers and many small osmiophilic granules. Infiltration of the lamina propria of the mucosa corresponded to the character of inflammation: either mononuclear cells or polynuclear leukocytes were predominant in it. The state of local immunity was characterized by a unique reorganization of immunoglobulin synthesis by the plasma cells. A relative IgA deficiency was formed, the number of IgM-producing cells was virtually unchanged, and the number of cells producing IgG was sharply increased.

Considerable changes took place in the microcirulatory bed under these circumstances. The epithelium of the capillaries and postcapillary venules was hypertrophied, PAS-positive substances accumulated in the walls, and immune complexes and IgG and IgM were fixed (Fig. 2, a-c). Infiltrating polymorphonuclear cells were concentrated near these vessels and immune complexes were detected in them, both extracellular and phagocytosed by polynuclear cells, giving a characteristic granular type of fluorescence of the cytoplasm (Fig. 2d, e). Mast cells with signs of degranulation were often found in these same zones (Fig. 2f).

Accumulation of immune complexes in the tissue, activation of mast cells and basophils by them, and their phagocytosis by polynuclear leukocytes were accompanied by release of histamine, serotonin, and hydrolytic and proteolytic enzymes, causing a marked disturbance of

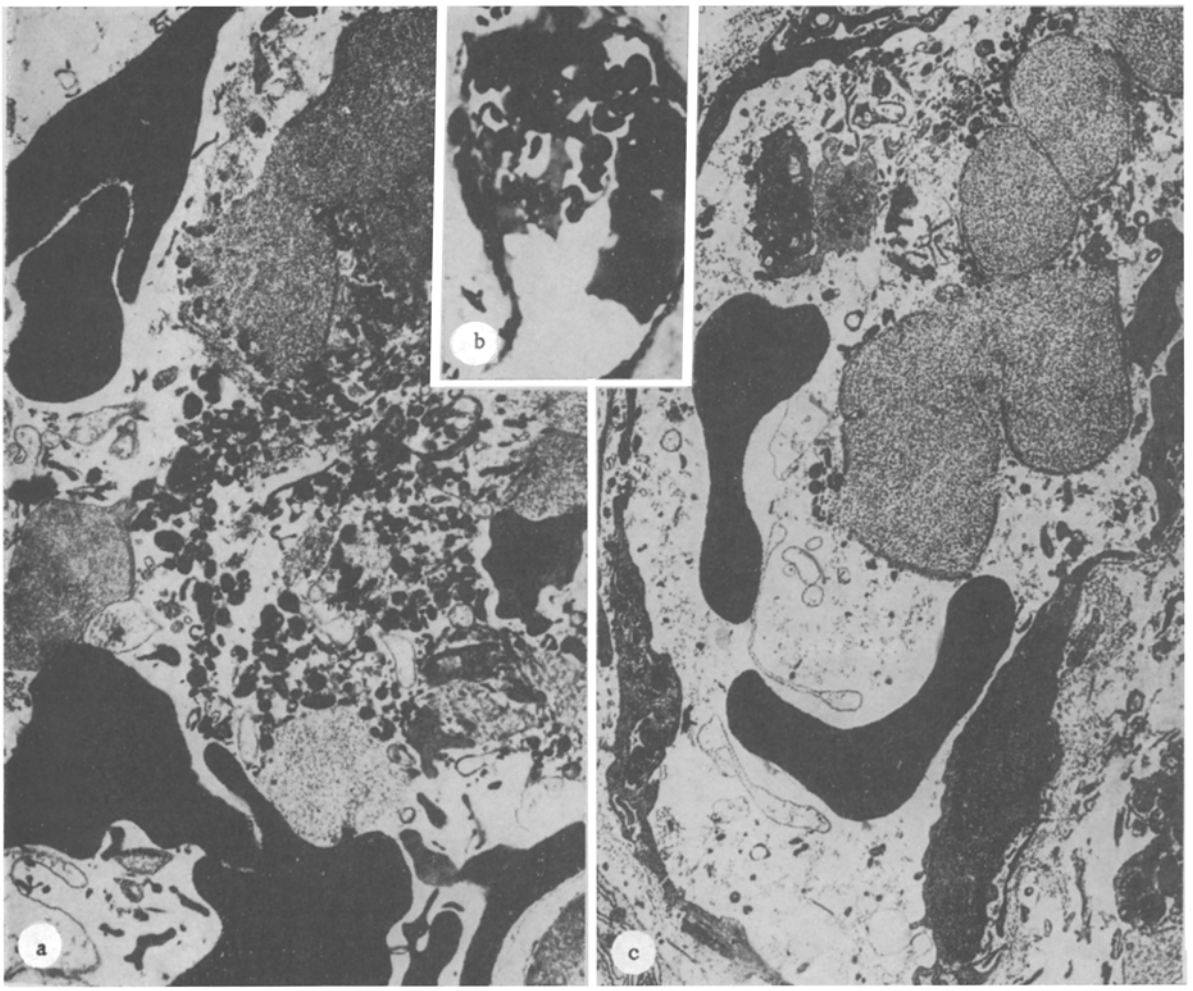

Fig. 3. Disturbance of microcirculation of bronchial mucosa in chronic bronchitis: a) response of release of platelets, in center — granules escaping from platelet, aggregated platelet, and hemolyzed erythrocytes; at periphery — sludging of erythrocytes. 15,000 ×; b) Beginning of aggregation of erythrocytes and protein coagulates appearing in lumen of a small vein. Semithin section, azure II. 400 ×; c) Formation of globules from platelets and hemolyzed erythrocytes. 10,000 ×.

vascular permeability [9, 10, 16]. One result of this was activation of the clotting system of the blood and, in particular, of thrombus formation [9, 10]. Pictures of platelet adhesion were frequently observed, followed by escape of granules from the platelets through the system of externally opening microtubules (Fig. 3a), after which numerous microfilaments appeared in the cytoplasm and the changes ended with the formation of large protein coagulates, or "globules" [6]. Besides platelets, these could also contain disintegrating erythrocytes (Fig. 3b, c). Circulation of the globules led to focal thrombosis of the microcirculatory bed and disturbance of metabolism of all tissue elements in this part of the mucosa, resulting in reorganization of differentiation and regeneration of the epithelial line.

The epithelium of the group 4 specimens corresponded to squamous-cell metaplasia. The epithelial layer consisted of flat cells with numerous desmosomes, and with fibrils diffusely arranged in the cytoplasm, forming regular bundles in some places. Infiltration of the lamina propria of the mucosa was sparse and consisted mainly of fibroblasts and small lymphocytes; solitary plasma cells producted IgG. The lamina propria of the mucosa was sclerosed and perivascular sclerosis was well marked. No deposits of immune complexes could be found in these biopsy specimens.

The character of the immunopathological reactions and changes in the microcirculatory bed in the bronchi in chronic inflammation recalls the picture of a chronic immunocomplex disease, culminating in pervascular sclerosis but without any marked proliferative changes or leukocytic infiltration [8, 12, 16].

In their general form reactions of local immunity in chronic bronchitis can be represented as follows: prolonged persistence of the antigen, initially bacterial in nature, followed by the formation of autoantigens, arising as a result of prolonged alteration of the bronchial mucosa [11], leads to secondary deficiency of secretory IgA, due to disturbance of synthesis of the secretory components in the epithelial cells or to the switch to synthesis predominantly of IgG antibodies, of a type similar to the dysfunction of isotype switching of B-lymphocytes [13]. As a result of the local IgA deficiency neutralization of heteroantigens on the surface of the mucosa does not take place, and they enter the lamina propria of the bronchial mucosa by familiar pathways [2].

In chronic bronchitis many antibodies circulate in the blood stream, including autoantibodies [11]. The basic condition is thereby created for realization of the immunocomplex reaction: one of its components must be in the blood stream, the other introduced locally [8, 9, 16]. It must be assumed that the main mass of immune complexes is formed locally and eliminated locally, which is responsible for such characteristic features as the focal character of the process, the absence of any marked alteration to the endotheliocytes, and participation of platelets, whereas in the classical Arthüs reaction they do not participate [10]. Subsequent reorganization of the microcirculatory bed is the basis for disturbance of epithelial-stromal interaction [5], leading to a change in direction of differentiation of the epithelial lining. Its metaplasia into stratified squamous epithelium is accompanied by loss of ability to form secretory IgA, and this in turn reduces the protective properties of the mucosa. A vicious circle is thereby created, and an essential role in its development is played by the state of local immunity and by immunopathological reactions.

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