Morphogenesis of Chronic Lung Lesions under the Influence of Proteolytic Enzymes

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Acute and chronic pathological processes induced in murine lungs by influenza virus or intranasal administration of proteolytic enzymes chymotrypsin and proteinase K were compared. Histologic and electron microscopic analysis showed that a number of characteristics of these two processes were similar. These characteristics included serosanguineous lung edema on days 1-5 postinfection, infiltration of lung tissue with cell elements, metaplasia of bronchial epithelium, and presence of mycoplasma in chronic pneumonia foci. These parameters persisted during 150 days of the experiment without regression. The results suggest the key role of primary cell destruction and proteolytic activity enhancement in the development of chronic influenza-induced pneumonia.

Key Words: *influenza, chronic pneumonia, proteolytic enzymes*

Chronic pneumonia, one of the most serious diseases of respiratory tract, can be caused by various agents, in particular, viruses, bacteria, mycoplasma, and fungi flora and requires complex treatment including specific and nonspecific therapy.

Apart from specific etiological features pneumonia is characterized by some parameters resulting from the body reaction to damage independent on its origin, in particular a disbalance between tissue content of proteolytic enzymes (PE) and their inhibitors resulting in increase in proteolytic activity [2].

Chronic influenza infection are characterized by the appearance of adenomatous structures consisting of epithelium with signs of metaplasia in the inflammation foci and dense lymphoid infiltration of the damaged tissue [1,6,7,11]. These chronic features persist for a long time after infection and lead to respiration impairment in the whole areas or even lobes of the lungs.

The increased proteolytic activity in the lungs involved in influenza pneumonia [3,5] and beneficial therapeutic effect of protease inhibitors in respiratory viral infections [12] confirm the important role of proteases in the genesis of chronic influenza infection.

The aim of the present study was to elucidate and compare morphological peculiarities of pathological processes caused by intranasal administration of PE to animals with chronic influenza infection and controls.

MATERIALS AND METHODS

Influenza virus A/PR/8/34 (H1N1) passaged in chick embryos to hemagglutination of 1:256-1:512 was used in the experiments. The infection was performed with 0.1 ml allantoic fluid (10^{-4}, 1 LD_{50}).

Albino outbred mice weighing 20 g were infected intranasally under ether anesthesia. At the end of experiment the animals were killed, the lungs were removed, fixed in 10% formaldehyde, and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and examined under a light microscope.

Proteinase K (0.2 mg/ml) and chymotrypsin (0.5 mg/ml) in 0.1 M phosphate buffer (pH 7.4) were administered intranasally to mice under ether anesthesia. The animals were sacrificed on days 1, 3, 5, 10 and 150 after treatment and their lungs were examined under a light microscope.

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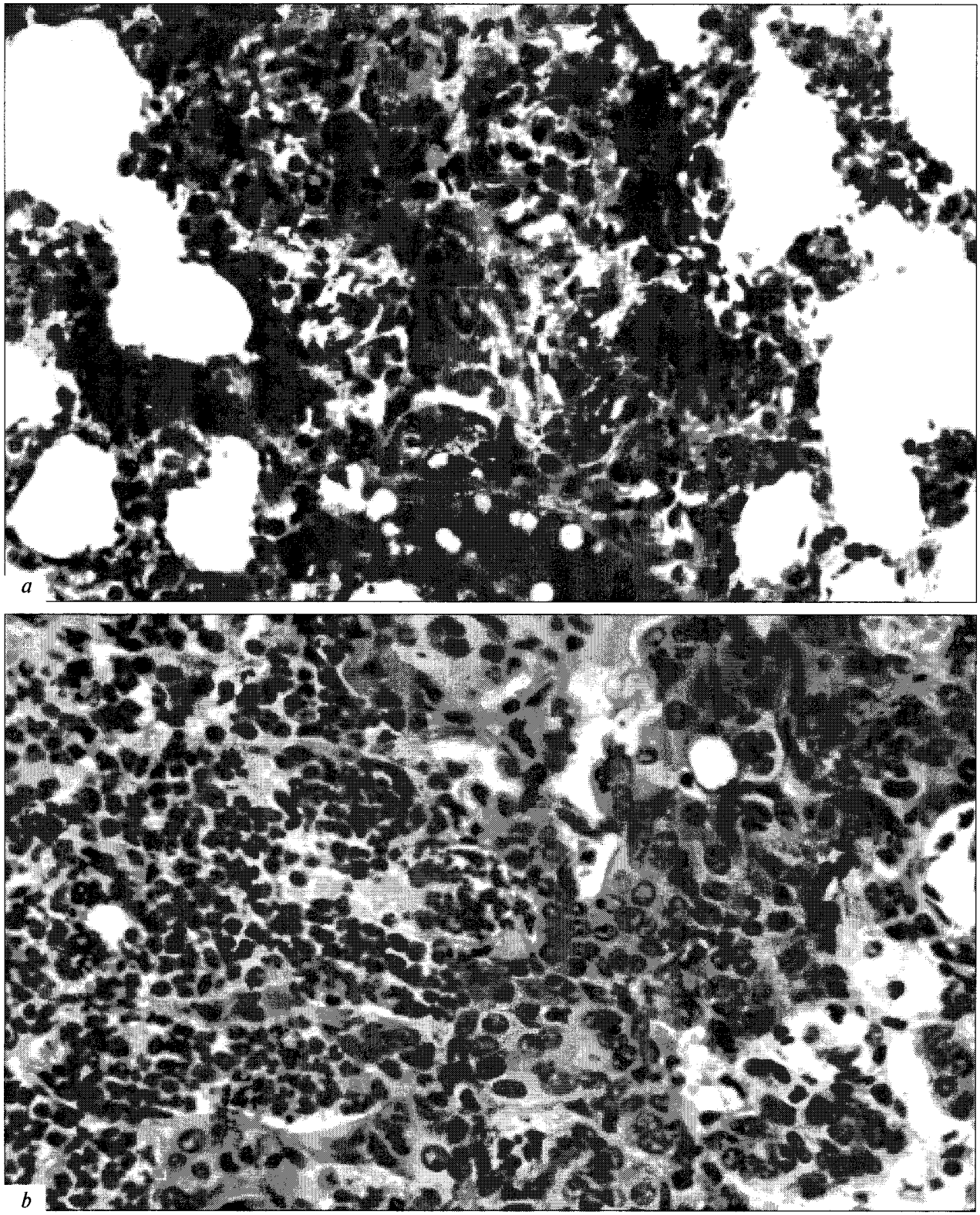

Fig. 1. Metaplasia of broncial epitheliocytes in the focus of chronic pneumonia. Ten days after intranasal chymotrypsin administration (*a*) or infection with A/PR/8/34 influenza virus (*b*). Hematoxylin and eosin staining, ×400.

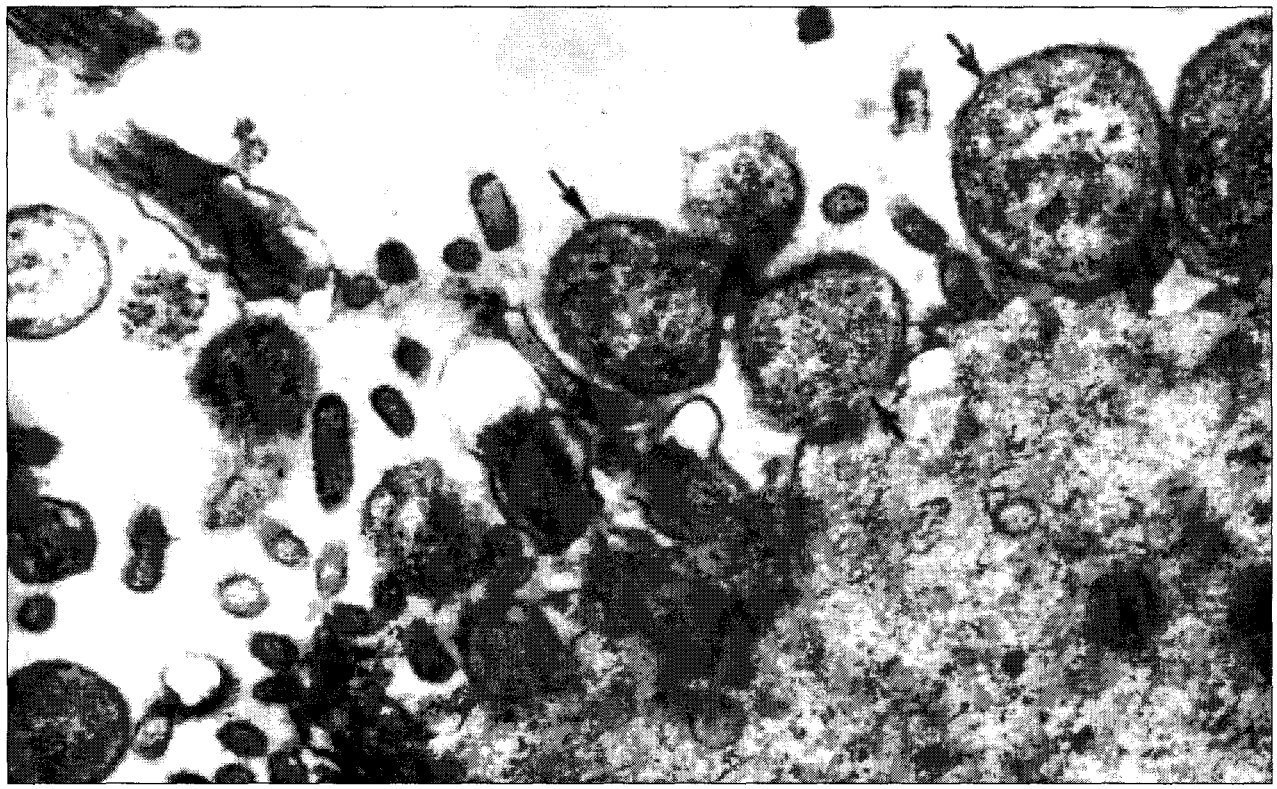

Fig. 2. Micoplasma (arrows) on surface of proliferating bronchial epitheliocytes in chronic pneumonia focus in mouse lung 150 days after intranasal proteinase K administration.

For electron microscopy, affected lung areas were fixed with 2.5% glutaraldehyde in cacodylate buffer, postfixed with osmium tetroxide, contrasted with uranyl acetate, dehydrated, and embedded in epon. Ultrathin sections were contrasted with lead citrate and examined under a JEM-100S electron microscope at 80 kV accelerating voltage.

RESULTS

Macroscopic examination of the lungs of infected and PE-treated animals revealed similar pathologic processes caused by these two interventions. Thus, influenza caused hemorrhagic pulmonary edema on days 1-3 of the experiment. PE induced pulmonary edema as soon as 1 day after treatment, and its intensity strongly depended on enzyme concentration. The doses exceeding 0.2 or 0.5 mg/ml of proteinase K or chymotrypsin respectively, caused death of all animals within 1 day.

Hemorrhagic edema caused by both treatments was then replaced by infiltration process in the lesion focus (days 5-10 of the experiment).

Histological examination showed that pathological changes in the lungs induced by different proteases were similar, but differed from those caused by influenza infection. Thus, influenza induced the formation of basophilic inclusions in the cytoplasm of respiratory epitheliocytes, while PE induced destruction of alveolar epithelium, desquamation and degeneration of alveolocytes. No basophilic inclusions were found in the cytoplasm of these cells. Alveoli and small and middle bronchi were filled with serosanguineous discharge.

On day 3 of after influenza infection, typical destruction of bronchial and alveolar cells accompanied by the formation of specific cytoplasmic inclusions was observed. Alveoli were filled with hemorrhagic discharge. During the same period proteases caused hemorrhagic edema due to fibrin condensation and neutrophil infiltration. Neutrophils were also found in the bronchi close to damaged epithelium.

From day 5 of the experiment, multilayer epithelial structures were formed in the lungs and the interstitial space was filled with free round cells indicative of intense regeneration. These processes resulted in the formation of adenomatous structures separated from the normal tissue by inflammatory swelling (Fig. 1). Epithelium, alveoli, and bronchi were infiltrated mainly with neutrophils, whereas peribronchial space contained round cells (lymphocytes and macrophages). Cell differentiation in distal bronchial regions was disturbed, cilia were absent on these cells. These changes persisted to the 150th day of the experiment.

Electron microscopy showed that hemorrhagic edema pulmonary during the acute period (days 1-3)

resulted from destruction of the alveolar epithelium and exposure of naked basal membrane. The protease-induced destruction was accompanied by impairment of cell-cell contacts and desquamation of alveolocytes. Influenza infection was characterized by virus-induced destruction of these cells and high permeability of the blood-air barrier. Starting from day 10 of the experiment, numerous mycoplasma bodies were revealed on the surface of regenerating cells in the bronchi and alveoli, which persisted during 5 months (Fig. 2).

Thus, our experiments allowed to elaborate a model of PE-induced chronic pneumonia in mice. We elucidate the mechanisms of primary damage to the blood-air barrier, e. g. destruction of cell-cell contacts in the bronchial epithelium, edema of the basal membrane, and accumulation of serosanguineous discharge in the alveoli, which accompany pneumonia induced by both influenza and intranasal protease administration. Chronic stage of both pathologic processes was characterized by stable metaplasia and hyperplasia of the bronchial epithelium and massive infiltration of affected zones with neutrophils and round cells. Influenza caused extensive cell destruction, stimulated migration of neutrophils and macrophages releasing endogenous proteases, and destroyed cells producing protease inhibitors, thus causing a disbalance between proteases and their inhibitors [2] and enhancing proteolytic activity in affected tissue.

Influenza stimulated synthesis of M2 viral protein functioning as a ion channel [10,13], which can destroy lysosome and plasma membanes and mediate the release of endogenous proteases into the extracellular space and their damaging effect on the blood-air barrier.

Further chronicity can be promoted by concurrent bacterial infection [14] and long-term functioning of epitelial cells under conditions of oxygen deficiency.

Some authors reported potent effect of influenza infection on cytokine production by cells [8,9]. It is possible that individual reaction to enhanced proteolytic activity, which depends on persisting influenza virus, determines quantitative features of influenza pneumonia (more pronounced metaplasia of the bronchial epithelium compared to protease-induced chronic pneumonia).

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