CHARACTERISTICS OF SURFACTANT FORMATION IN RESPONSE TO INJECTION OF EXOGENOUS PARTICLES INTO THE LUNGS

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Changes in the physicochemical properties or mass of surfactant contribute to the development of histopathological changes in the lungs [1, 2]. The principal producers of surfactant are type 2 alveolocytes, which secrete osmiophilic material and subsequently transport it to the apical surface of the cells by means of exocytosis. The most demonstrative results so far as detection of surfactant is concerned are those obtained by the use of a radioactive label [3, 4, 6] and specific antiserum [5]. The functional role of surfactant is particularly enhanced during exposure of the lungs to dust, arising in the course of human work activity, when the protective and gas-exchange functions of the lungs are primarily disturbed.

The aim of this investigation was to study secretion of the surfactant complex and its distribution on the alveolar surface after intratracheal insufficient of cytotoxic minerals (zeolites), which have found widespread application in industry, agriculture, etc., into the lungs of animals.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing initially 120-150 g, into whose lungs natural zeolite-clinoptilolites from Bulgarian (group 1), Georgian (group 2), and Siberian (group 3) deposits, were insufflated in a dose of 50 mg/ml. The 4th group comprised control animals. Each group contained at least 10 animals. The main method of studying secretion of surfactant, excretion into the lumen of the alveolus, and its distribution on the alveolar lining was electron microscopy. The animals were killed 3 and 18 days after insufflation of the dust. Pieces of lung tissue for electron microscopy were fixed in glutaraldehyde and OsO_4, dehydrated, and embedded in Araldite. Ultrathin sections, after staining, were examined in a "Hitachi-HS-9" electron microscope. Semithin sections from the same tissue specimens, stained with toluidine blue also were examined to determine the ratio between the numbers of type 1 and type 2 alveolocytes in larger areas of lung tissue than is possible with ultrathin sections.

EXPERIMENTAL RESULTS

The study of the lung tissue by light-optical and elcctron microscopy 3 and 18 days after insufflation of the mineral particles showed that the initial stage of pneumoconiosis had developed in it, in the form of an intensive cellular and vascular reaction. An essential structural feature of the architectonics of the respiratory regions of the lung was preservation of numerous dust particles of varied morphology, including fibrous or needle-shaped forms, in the alveolar lumen. The presence of free dust particles in the alveolar lumen evoked an intensive phagocytic reaction of the alveolar macrophages and this continued for a long time. Besides phagosomes, many lysosomes of varied maturity also were present in the cytoplasm of the macrophages.

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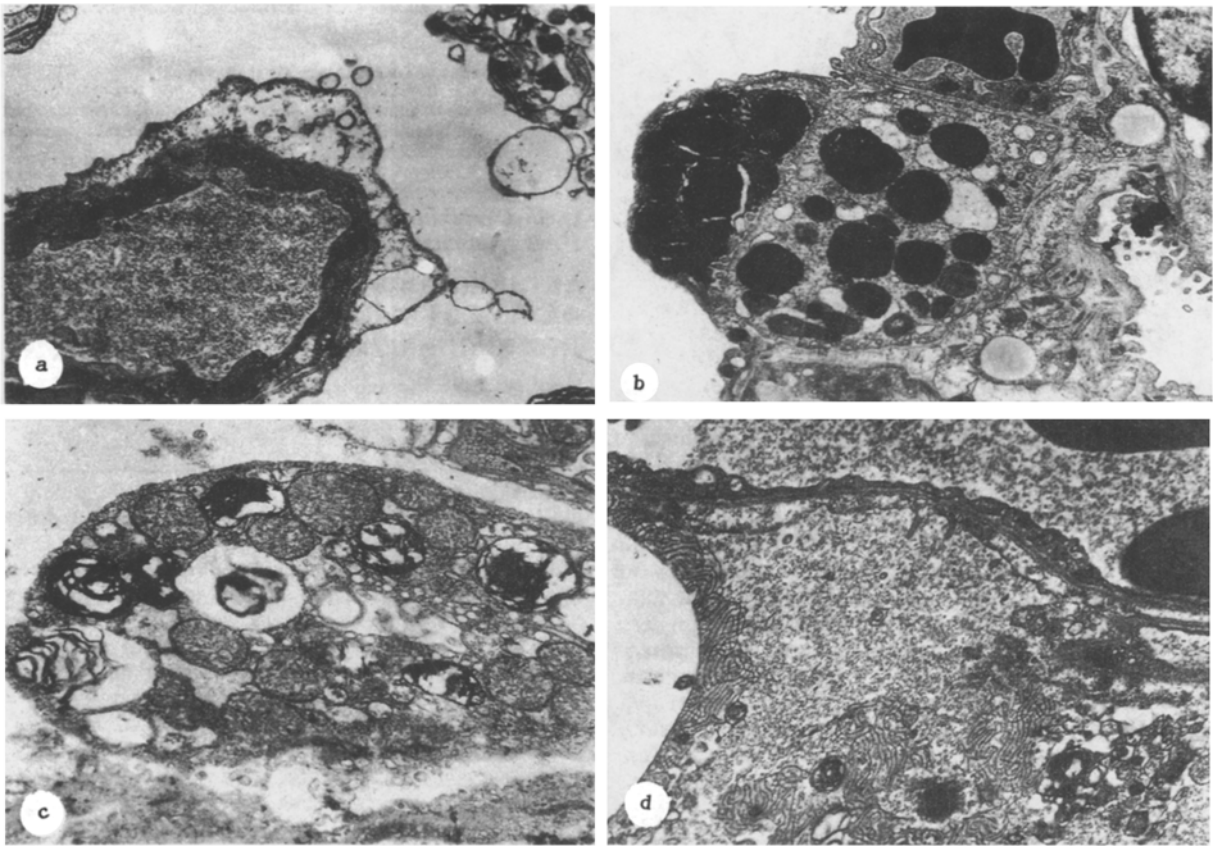

Fig. 1. Formation and structure of surfactant on alveolar surface. a) Edema and plasmatosis in type 1 alveolocyte (10,500×, here and in Fig. 1b); b) accumulation of mature osmiophilic bodies in apical surface of type 2 alveolocyte; c) exocytosis of osmiophilic body in type 2 alveolocyte (15,000×, here and in Fig. 1d); d) structure of surfactant on alveolar surface.

Abundant deposition of dust particles in the alveolar spaces and ducts also evoked an intensive reaction in structures of the air–blood barrier. Marked concentration of the plasma and signs of stasis of erythrocytes were frequently observed in the alveolar capillaries. An abundance of pinocytotic vesicles of low electron density, which moved toward the basement membrane and epithelial surface, appeared in the endothelial cells. The basement membrane in areas of intensive pinocytosis was greatly loosened in structure and edematous. Widening of the intercellular spaces could be seen between the peripheral regions of endothelial cells. The structural features observed indicated an enhanced transport function of the endothelium during dust loading. Reactive changes also were observed in the alveolar epithelium. Thickening of the cytoplasm of extranuclear zones, accompanied by edema and by the formation of numerous vesicular structures, took place in the type 1 alveolocytes; as a result of plasmatosis, these structures became separated from the surface of the alveolocytes and entered the alveolar cavity (Fig. 1a). Intensive formation of osmiophilic lamellar bodies took place in the type 2 alveolocytes, followed by concentration on the apical surface and secretion of merocrine type on the surface of the alveolar lining (Fig. 1b). Numerous enlarged mitochondria with signs of swelling and edema were found in the hypertrophied type 2 cells. Secretion of the newly formed bodies took place in the apical part of the cell through the formation of a cavity in the plasmalemma, through which the maturing lamellar body could leave it (Fig. 1c). Type 2 cells, filled with mature lamellar bodies, grouped in the apical part and secreting them, often no microvilli could be seen. The structural changes observed in the alveolocytes and pulmonary capillaries reflected intensive secretion of the components of the pulmonary surfactant in the experimental animals. The newly formed surfactant covered a large surface area in the alveolar cavities, in which both the hypophase (basal layer) and the membranous structures (osmiophilic lamellar bodies and reticular formations) were clearly visible (Fig. 1).

Besides phagocytosed dust particles, elements of surfactant also were seen in the alveolar macrophages: reticular structures surrounded by a phagosome membrane. These observations indicate a role for macrophages in the formation of elements of the surfactant.

Under the influence of the mineral particles injected into the lungs, intensive synthesis and subsequent exocytosis of elements of the surfactant thus took place in the alveolar spaces. The main suppliers of surfactant are type 2 cells, in which marked secretion of osmiophilic bodies and their secretion onto the surface of the alveolar lining, by a merocrine mechanism, were observed. This conclusion is confirmed not only by the structural and functional activity of the type 2 cells, but also by the predominance of membranous structures in the general mass of the surfactant complex of the respiratory regions of the lung. One of the main factors responsible for this process is evidently the presence of a considerable proportion of free dust particles in the alveolar cavities, where their special physical properties (shape and size) prevent both phagocytosis and insinuation into the interstitial tissue of the lung. Deposition of exogenous indigestible dust particles in the alveolar spaces is a powerful stimulating factor enhancing the secretory function of cells of the alveolar lining. Under normal physiological conditions secretion of the surfactant complex is much less intensive, and for that reason detection of surfactant in normal lung tissue may often give rise to technical difficulties, and special methods of fixation are necessary in order to preserve it completely [7]. Desquamation and destruction of alveolar epithelial cells and also of macrophages promotes surfactant formation. Increased synthesis and accumulation of a large mass of surfactant in the alveolar spaces under these experimental conditions, compared with normal lung tissue, can be regarded as a manifestation of the protective function of the alveolar cells. However, the positive role of the protective function of surfactant when present in excess (during developing pneumoconiosis) may be transformed into a negative quality, causing disturbance of the gas exchange. At the structural level this is manifested as congestion of the vessels, concentration of the plasma in them, cellular edema, and an increase in the number and swelling of the mitochondria.

Hypersecretion of surfactant and also, possibly, changes in its physicochemical properties (for example, an abundance of membranous structures) aggravate the pathogenesis of pneumoconiosis, developing through the action of dust particles of natural zeolites.

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