MORPHOLOGY OF EXPERIMENTAL PNEUMOCONIOSIS FOLLOWING INHALATION OF LUNAR SOIL

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Experimental studies to assess the action of lunar soil on living cells *in vivo* and *in vitro* have shown that it is not toxic for cells [3, 5]. At the same time, many neoplasms developed in mice receiving lunar soil by inhalation or intraperitoneal injection [1]. Electron-microscopic studies of phagocytosis of lunar soil by lung macrophages also have revealed pathological changes in the cell organelles. The intensity of the harmful action of lunar soil has been shown to depend on the dimensions and shape of the particles and, in particular, on their chemical composition [1]. The aim of the present investigation was to study the action of particles of lunar soil on the respiratory organs under experimental conditions.

EXPERIMENTAL METHOD

The development of pneumoconiosis was induced in male albino rats weighing 200 g by intratracheal injection of a suspension of finely dispersed lunar soil in physiological saline with starch in a dose of 50 mg per animal. The animals were killed 3 days and 3 and 6 months after the beginning of the experiment. Pieces of the lungs and tracheobronchial lymph nodes were fixed for light-optical and electron-microscopic investigation (transmission and scanning). Phagocytic activity of the macrophages was determined in squash preparations and also in semithin sections [4]. The lung tissue was investigated with the IEM-100S and Kwikscan-50 electron microscopes. Lunar soil (regolith), obtained by the Soviet automatic station Luna-16 from the Sea of Abundance was used in the experiments. The degree of development of pneumoconiosis after administration of lunar soil was compared with the standard model of silicosis, used simultaneously [2].

EXPERIMENTAL RESULTS

The pathological process in the lungs following intratracheal injection of lunar soil developed in the manner of an inflammatory reaction. As a result of disintegration of mast cell granules, exocytosis of histamine and serotonin took place, while the integrity of the cell membrane was preserved. Under the influence of the liberated mediators of inflammation the permeability of the exchange capillaries was increased and edema fluid accumulated in the interstitial tissue. In the air-blood barrier the basal layer was thickened. The alveolar epithelium and capillary endothelium contained many microvesicles, so that their cytoplasm had a frothy appearance. The lumen of the lung capillaries was dilated and they contained microthrombi. Fragments of ultrastructures and microvesicles of the destroyed endothelial cells were suspended in the concentrated plasma. The alveolar septa were grossly thickened.

In the early stages of the investigation (3 days) an intensive leukocytic and macrophagal response was observed. Light-optical microscopy on semithin sections showed that up to 30% of macrophages contained particles of lunar soil measuring 1-2 μ in their cytoplasm. Analysis of the squash preparations showed that destructively changed macrophages were rare.

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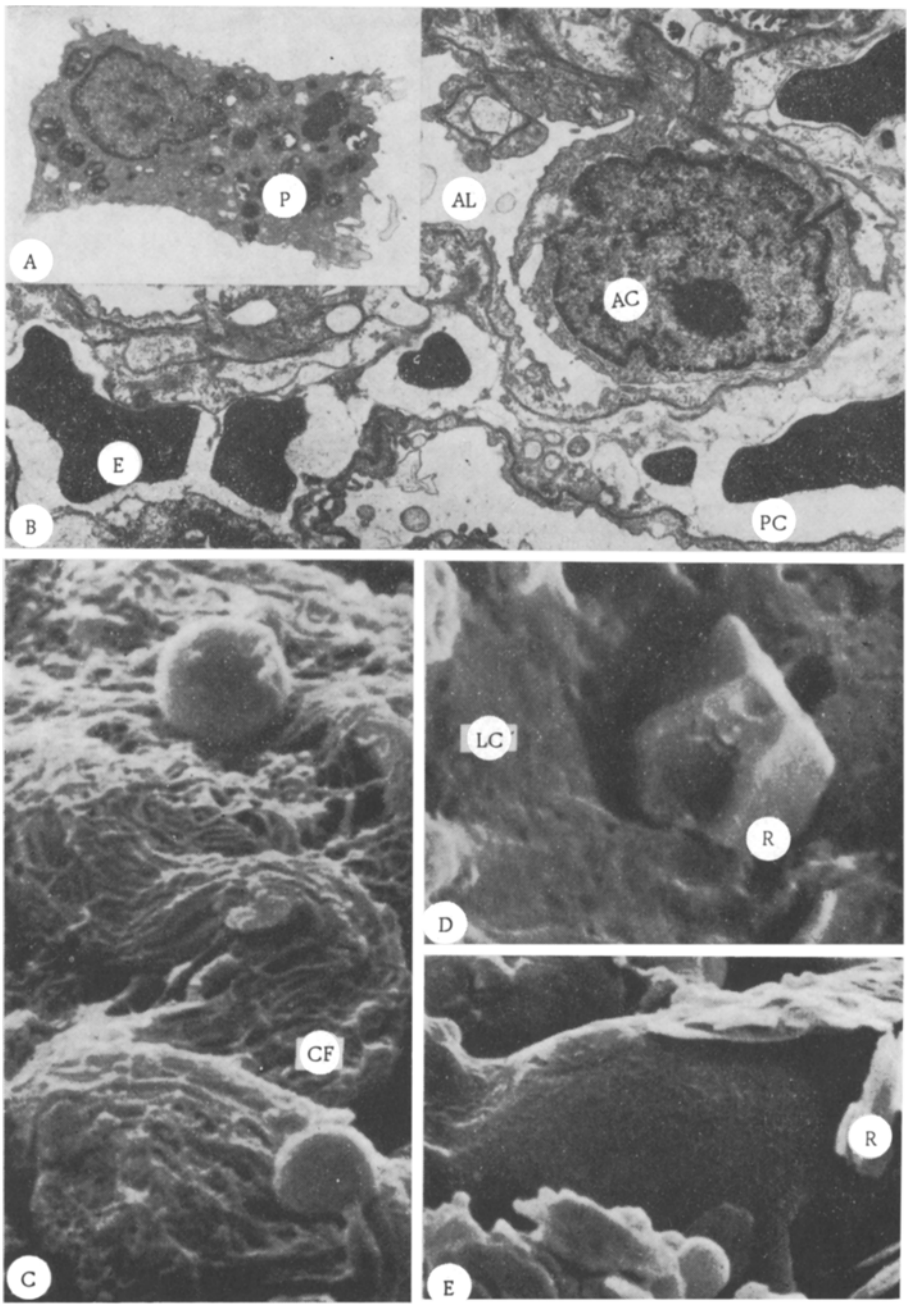

Fig. 1. Ultrastructural morphology of the lungs and paratracheal lymph node following inhalation of lunar soil. A) Alveolar macrophage, phagolysosomes contain particles of lunar soil, 4000 ×; B) edema and swelling of ultrastructures of alveolar epithelium and capillary endothelium, widening of basal layer of air-blood barrier, 6000 ×; C) collagen fibers with small spaces for circulation of tissue fluid, 20,000 ×; D) particles of lunar soil in region of filtration opening in lymphatic capillary, 20,000 ×; E) foliate particles of regolith in paratracheal lymph node, 35,000 ×. P) Phagolysosomes; R) particle of regolith; A) alveolar lumen; PC) pulmonary capillary; AC) alveolocyte; E) erythrocyte; CF) collagen fibrils; LC) lymphatic capillary.

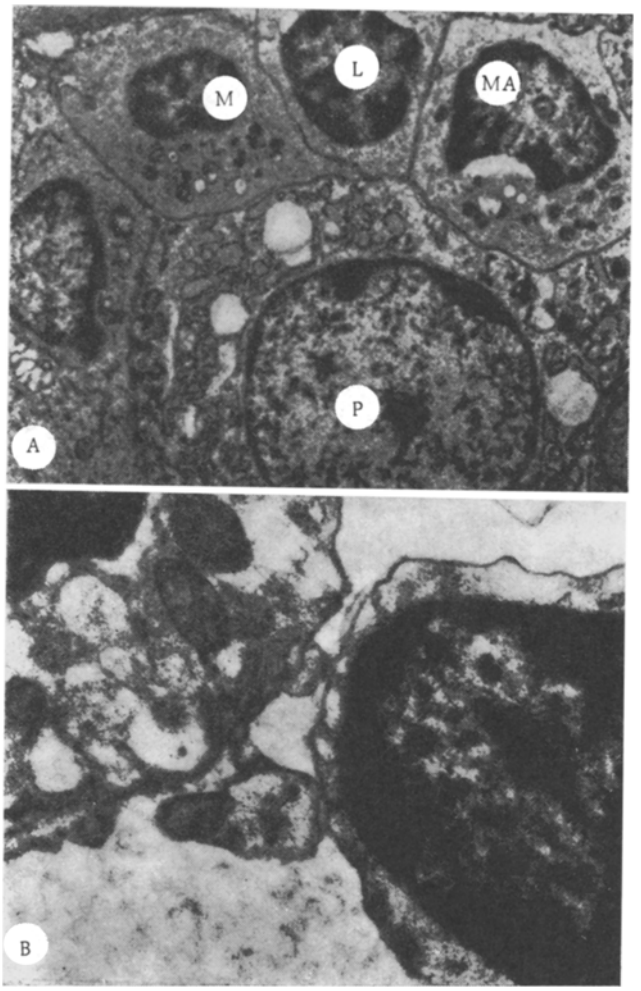

Fig. 2. Cell contacts in peribronchial reticulohistiocytic tissue. A) Cell rosette, plasma cell (P) in center, monocyte (M), lymphocyte (L), and macrophage (MA) at periphery; 3000 ×. B) Tight junction between membranes of lymphocyte and macrophage, 15,000 ×.

Electron-microscopic investigations revealed secondary lysosomes in the phagocytic macrophages. The remaining structures of the cytoplasm also showed signs of an active functional state, including the cell membrane. Particles measuring 300-500 nm detected in the macrophages often lay freely in the hyaloplasm, whereas smaller particles (from 10 to 150 nm) were seen in the composition of the phagolysosomes in groups of a few or as whole conglomerates (Fig. 1A). After phagocytosis of a large quantity of regolith, the macrophages were destroyed. At later stages of the investigation particles of regolith were found only in solitary macrophages. Because of the high phagocytic activity of the alveolar macrophages, intensive clearance of the respiratory portions of the lungs from dust particles took place in the early period of the experiment.

Particles of lunar soil measuring 0.5 μ in diameter penetrated freely from the respiratory portion through the air-blood barrier into the interstitial tissue of the alveolar and interlobular septa and were even found in nerve endings. A marked increase in thickness of the air-blood barrier took place 3 months after the beginning of the experiment on account of five-sevenfold growth of the basal layer. This process is the morphological manifestation of developing diffuse pneumosclerosis (Fig. 1B).

In connection with migration of the particles, changes occurred both in the alveolar epithelium and in the nonrespiratory structures of the acini. Activated fibroblasts, producing collagen fibrils, were found in the interlobular connective tissue. Examination of

the ground substance of the connective tissue in the scanning microscope revealed twisted collagen bundles of varied thickness. In areas of developing fibrous tissue, coarse and chaotically interwoven collagen fibers left only small slit-like spaces for tissue fluid to circulate (Fig. 1C). Migration of regolith particles in the connective tissue of the interlobular septa and the outer membranes of the bronchi and blood vessels led to gradual clearing of the particle carriers from tiny satellite subparticles located on their surfaces and to the wide distribution of these subparticles in the lungs. These satellites, increasing the surface area of the penetrating agent several times over, and with their small size, at the same time had a powerful cytotoxic action against the cells responsible for their phagocytosis, especially if they contained quartz. Degenerating macrophages enriched the ground substance of the connective tissue with breakdown products and induced intensive collagenogenesis. The harmful action of the regolith particles also was manifested during their contact with collagen fibrils in the intercellular space. Under these circumstances the transverse striation disappeared from fibrils adjacent to the particles and necrotic zones were formed. As the pathological process developed, disturbances of the function of the lymphatic capillaries and postcapillaries gradually progressed. The cells which had performed phagocytosis, blood cells, and free particles of regolith, accumulating here, blocked the filtration openings of the terminal segments of the capillaries, as a result of which fibrous tissue was formed around the terminal portions of the lymphatic system (Fig. 1D). Examination of the tracheobronchial lymph nodes 3 months after injection of the regolith demonstrated the presence of many particles in the form of nest-like accumulations, brought here by the lymph flow and macrophages. Particles found by scanning electron microscopy usually did not exceed 0.4 μ in diameter and were leaf- or needle-shaped. Larger dust particles were not found in the lymph nodes (Fig. 1E).

Blocking the terminal portions of the lymphatic component of the microcirculation by cell detritus and regolith particles also led to the formation of perivascular and peribronchial reticulohistiocytic tissue. The cell composition of the latter was somewhat specific in character. The reticulohistiocytic tissue contained lymphocytes, monocytes, eosinophilic leukocytes, polyblasts, plasma cells, and also fibroblasts. Some of them formed cellular organizations of the type known as rosettes (Fig. 2A). As a rule the rosettes consisted of a central undifferentiated polyblast cell, surrounded closely by lymphoid cells and monocytes. Besides rosettes, paired cellular organizations with tight cytoplasmic junctions also were observed: an eosinophil with a large lymphocyte or macrophage, a plasma cell and macrophage (Fig. 2B).

This investigation of the action of lunar soil on the lungs showed that in the intensity of formation of fibrous tissue and of replacement of the respiratory portions of the lungs by fibrous tissue the fraction of lunar soil can be included in the category of weakly fibrogenic dusts by comparison, for example, with quartz, the most aggressive of the known industrial dusts.

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