ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF BRONCHIOALVEOLAR LAVAGE CELLS IN NONSPECIFIC INFLAMMATION OF THE LUNGS

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An important place in evaluation of the state of the respiratory portion of the lungs during life is occupied by bronchoalveolar lavage followed by cytological investigation of the bronchoalveolar washings [1-3, 8]. Bronchoalveolar washings give a cytological picture of the population of inflammatory and immunoeffector cells that is close to the actual picture *in situ* [10, 11].

Considerable changes in the endopulmonary cytogram are observed in the lungs in various pathological conditions [10, 13]. Quantitative and qualitative changes in composition of the cell populations of respiratory tissue reflect the time course of physiological and pathological processes in the lungs [4].

The aim of this investigation was to continue the quantitative analysis of endopulmonary cytograms by making an ultrastructural and autoradiographic study of lavage cells in chronic nonspecific inflammatory diseases of the lungs.

EXPERIMENTAL METHOD

Bronchoalveolar washings were studied from 43 subjects aged from 22 to 61 years (18 women and 25 men). Chronic bronchitis was present in 24 patients, chronic pneumonia and lung abscesses in 12, acute pneumonia was the clinical diagnosis in 4 cases, and sarcoidosis in 3. The technique of obtaining material during bronchoscopy was described by the writers previously [5]. The total number of cells in 1 ml of washings was counted. After repeated centrifugation films were prepared from part of the residue, stained by the Pappenheim–Kryukov method, and the cell composition was counted. Part of the residue from all patients was fixed in 4% paraformaldehyde solution, postfixed in 1% OsO_4 solution, and embedded in epoxide resins. In 5 cases unfixed samples were incubated in parallel experiments at 37°C in medium 199 containing 100 μCi/ml of ^{3}H-uridine (specific activity 26.6 Ci/mmole) by the method in [6], after which the fragments were washed with cold medium 199 and phosphate buffer (pH 7.4), fixed in 4% paraformaldehyde solution, postfixed in 1% OsO_4 solution, and embedded in epoxide resins. Semithin sections cut from the blocks were stained with azure II, and ultrathin sections were stained with uranyl acetate and lead citrate. Semithin sections of lavage cells incubated with ^{3}H-uridine were prepared light-microscopic autoradiography [7], then stained with azure II, after which the density and labeling index of the lavage cells were counted.

EXPERIMENTAL RESULTS

Representatives of different cell populations were found in the lavage fluid obtained from the patients: macrophages, neutrophils, eosinophils, lymphocytes, alveolocytes, bronchial epithelial cells, plasma cells, and mast cells. Their relative numbers and the total number

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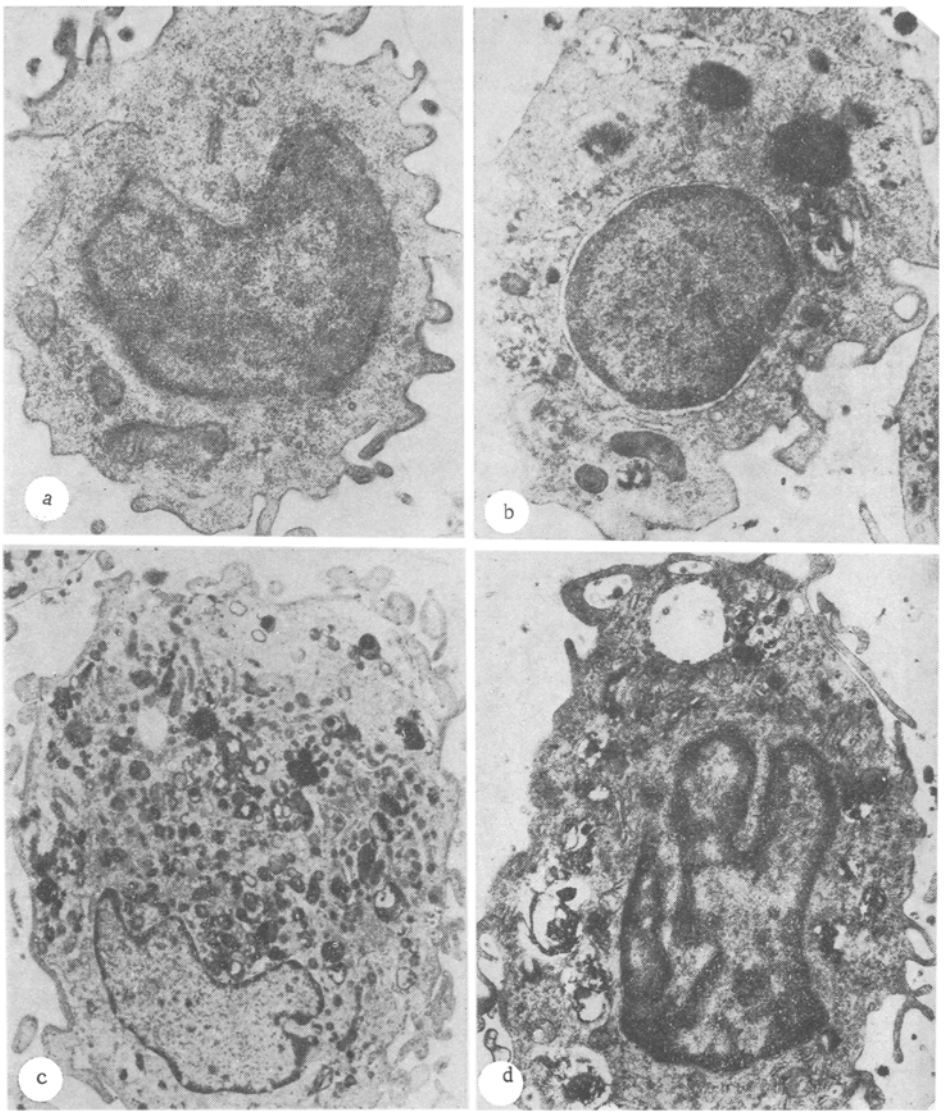

Fig. 1. Alveolar macrophages (AM) of bronchoalveolar washings from patients with chronic inflammation of the lungs. a) AM of form 1 with bean-shaped nucleus and few organelles; 25,000×; b) AM of form 2. Small and large lysosomes, phagosomes, and profiles of dilated tubules of rough endoplasmic reticulum visible in cytoplasm; 20,000×; c) AM of form 3. Cytoplasm contained many lysosomes, phagosomes, and pinocytotic vesicles, heterogeneous in phagosomes; 10,000 ×; d) AM of form 4 with lobular nucleus and many structure; 15,000 ×.

of cells in 1 ml of aspirated fluid varied considerably, depending primarily on the phase of the inflammatory process in the lungs. In acute pneumonias and exacerbations of chronic inflammation the number of cells varied from 0.12×10^6 to 5.1×10^6. In the absence of any clinical features of exacerbation of the process, the number varied from 0.11×10^6 to 0.38×10^6.

Outside the exacerbation phase, the endopulmonary cytogram was dominated by macrophages (80-96%), lymphocytes accounted for 2-4%, neutrophils for 2-13%, and eosinophils for 0-5%. Exacerbation of the inflammatory process was accompanied by a reduction in the number of macrophages to 10-70%, an increase in the number of neutrophils to 22-88%, and a decrease in the number of lymphocytes sometimes to 1%. Moderate lymphocytosis in the lavage fluid (up to 11-13%) was found in all patients with sarcoidosis.

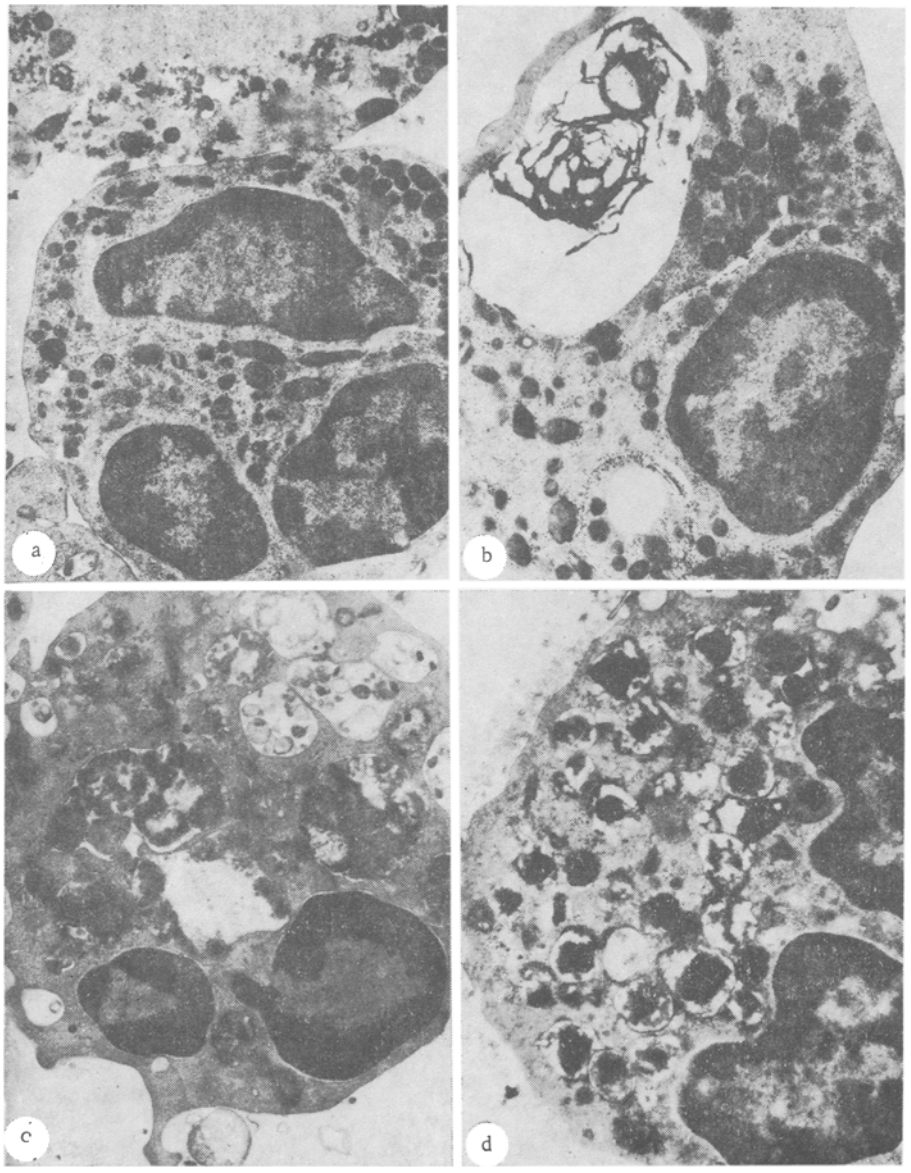

Fig. 2. RNA synthesis in cells of bronchoalveolar washings from patient with chronic inflammation of the lungs; incubation of cells with ^{3}H-uridine, semithin sections, stained with azure II, 1,000 ×. a) Alveolar macrophages (AM) of forms 2 and 3 (in center) with high density of label mainly above nuclei. Polymorphonuclear leukocyte (above) with a few grains of silver above its nucleus; b) intensive incorporation of ^{3}H-uridine mainly in nuclei of AM of forms 2 and 3. A few grains of silver above lymphocyte; c) AM of form 4 (top left) with high level of RNA synthesis, AM of form 5 (bottom left), and polymorphonuclear leukocytes with a few grains of silver above their nuclei; d) AM with different levels of RNA synthesis.

Ultrastructural analysis showed marked heterogeneity of the macrophage population in the washings. We distinguished several basic forms of these cells. Cells with small cytoplasmic processes, and with a bean-shaped nucleus with a distinct layer of heterochromatin, a moderately developed rough endoplasmic reticulum, a small lamellar complex, mitochondria, and single round lysosomes, were classed in form 1 (Fig. 1a). These lysosomes were a little larger than the azurophilic granules of monocytes and were located near the lamellar complex. Because of their structure, these cells can be regarded as an intermediate form between monocyte and macrophage. Cells of form 2 (Fig. 1b) were distinguished by a well-developed rough endoplasmic reticulum, many mitochondria and ribosomes, a distinct lamellar complex, and solitary primary lysosomes and phagolysosomes. The nuclei of these macrophages were rich in euchromatin and contained easily distinguishable nucleoli and, frequently, numerous nuclear pores. Cells of this form thus have structural features of active protein synthesis. Macrophages of form 3 were characterized by numerous convoluted and round lysosomes (Fig. 1c),

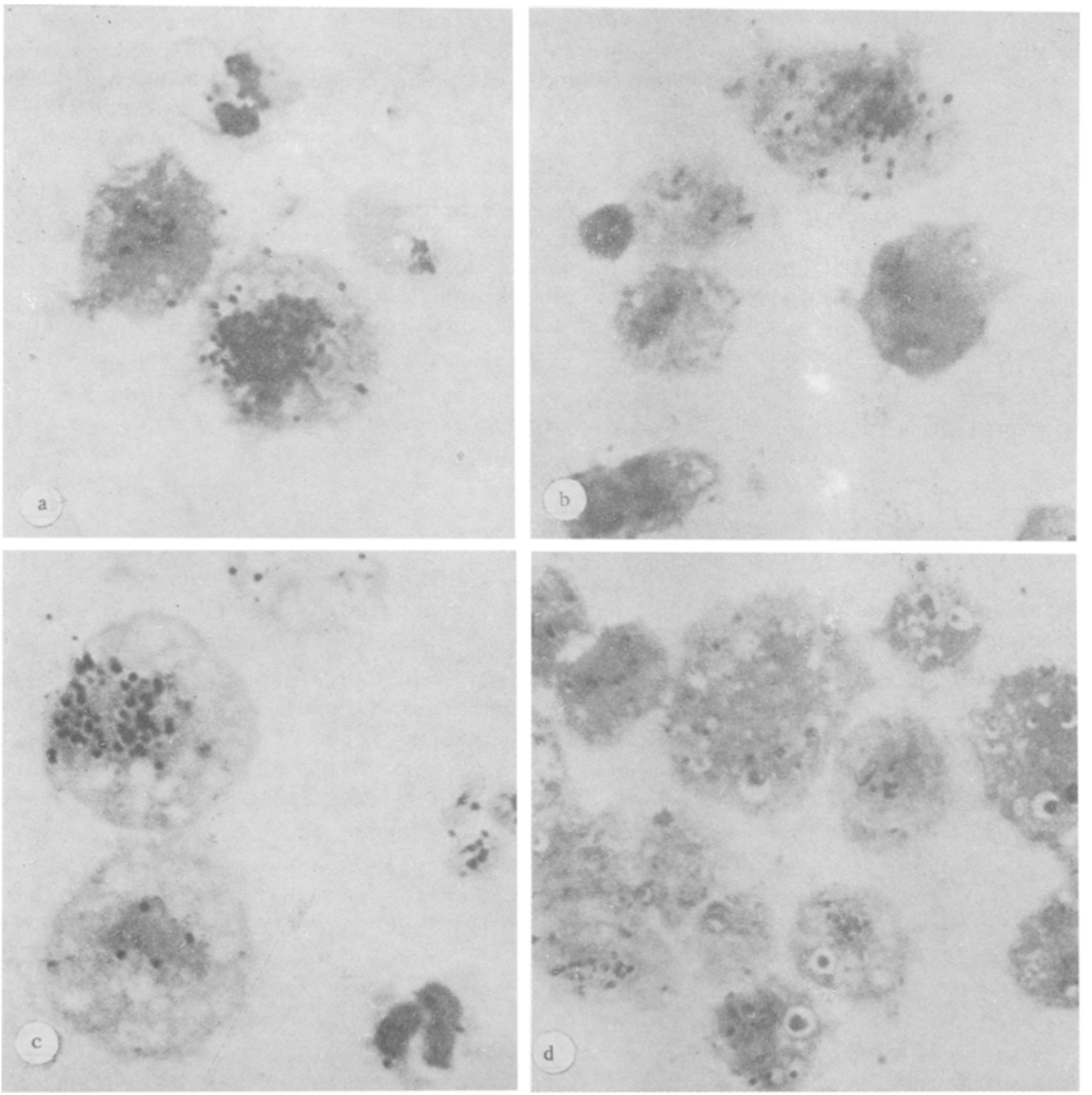

Fig. 3. Neutrophils in bronchoalveolar washings from patient with chronic inflammation of the lungs. a) Polymorphonuclear neutrophil with no signs of phagocytosis and with typical granules in its cytoplasm. Fragment of cytoplasm of neighboring neutrophil contains phagosomes and phagolysosomes; 15,000 ×; b) neutrophil with phagocytic vacuole, with lamellar structures in its center; 25,000 ×; c) neutrophil with many large phagosomes and phagolysosomes and a few primary lysosomes; 20,000 ×; d) neutrophil with many phagolysosomes; 15,000 ×.

which were either uniformly distributed throughout the cytoplasm or concentrated in its peripheral zones. Sometimes large lysosomal structures and phagolysosomes were visible. The cytoplasm of macrophages of this form has a rough endoplasmic reticulum that was less well developed than in the previous form. Macrophages of form 4 had features of active phagocytosis (Fig. 1d). The cytoplasm of these cells contained lysosomes and phagolysosomes, which were heterogeneous in structure, shape, and size. Some cells of this form contained infrequent profiles of the rough endoplasmic reticulum and solitary lysosomes, whereas others preserved a quite considerable number of ribosomes, tubules of the endoplasmic reticulum, and primary lysosomes, and their lamellar complex showed hyperplasia. Macrophages of the last type (form 5) showed signs of degeneration. The cell nucleus was translucent, and the cytoplasm contained many small and large vacuoles and concentrations of phagocytosed material, and the intracellular organelles were partially destroyed.

The autoradiographic investigation showed differences in metabolic activity of the different forms of macrophages described above. The highest density and labeling index with ^{3}H-uridine were observed in young and activated (forms 1, 2, and 3) macrophages (Fig. 2a, b). High incorporation of the isotope was found in certain cells belonging to form 4 (Fig. 2c). Cells with depressed phagocytic function and degenerating macrophages (forms 4 and 5) synthesized RNA less actively and some of them did not incorporate ^{3}H-uridine (Fig. 2d).

Ultrastructural analysis showed that many neutrophils had features of active endocytosis. The neutrophils were polymorphonuclear, with predominance of heterochromatin, characteristic granules (Fig. 3a), and also phagosomes and phagolysosomes, which are heterogeneous in size, shape, and density (Fig. 3a, b). Large phagosomes were found in some neutrophils (Fig. 3c, d). These cells changed their shape to become rounder, their primary lysosomes disappeared, and later they underwent degeneration and destruction.

Autoradiographic analysis showed that compared with the macrophages, neutrophils incorporated much less ^{3}H-uridine (Fig. 2a, c). A few grains of silver (3 or 4) were found above the nuclei of cells possessing phagosomes, but there were more grains (5-7) above nuclei of neutrophils with no features of phagocytosis. Degenerating neutrophils did not contain the label.

Eosinophils of the washings preserved the characteristic structure of cells of this type and contained specific cytoplasmic granules. These cells were discovered more often in patients with bronchial asthma and chronic bronchitis with an asthmatoid component, although in these no ultrastructural differences could be observed.

The structure of the lymphocytes in the washings was heterogenous; activated and inactivated forms were observed. The lymphocytes incorporated ^{3}H-uridine least intensively; the number of grains of silver above each cell did not exceed 3-5. Labeled lymphocytes were more numerous in patients with sarcoidosis. In some cases type 2 alveolcytes with features of intracellular edema and destruction of organelles were found in the washings.

The alveolar macrophage is the most important and specialized cell from the point of view of continuation of the population of cells of the monocyte-macrophage system. These cells have many features in common with macrophages of other tissues, but they have definite differences in their structure and metabolism. Numerous investigations have yielded evidence on the morphology of macrophages obtained from animals, but only relatively little information is available on the human alveolar macrophage.

Analysis of the experimental data showed that, depending on the phase of the inflammatory process in the lungs, changes took place not only in the relative numbers of the different cell populations in the washings, but also in the qualitative composition of each population. These qualitative changes could be seen most clearly in respect of the principal cell of the lavage fluid, namely the macrophage.

During this ultrastructural and autoradiographic study of cells of the bronchoalveolar washings from patients with chronic specific inflammation of the lungs the time course of activation of the macrophage population could be observed: the appearance of young forms intensification of protein synthesis in them (synthesis of lysosomal enzymes, probably), stimulation of phagocytic function, and subsequent degeneration of some cells. Consequently the structural and metabolic heterogeneity of the macrophages can be taken to reflect the successive stages of their development from precursor cells, through activation of protein synthesis, to cells with a fully developed lysosomal cycle, and subsequent phagocytosis. The process of activation of the macrophagal system, as experimental investigations have shown, can take place quite rapidly and can be induced by several different factors [9, 12, 14-16].

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MORPHOLOGY OF THE ORAL MUCOSA IN RATS EXPOSED TO HIGH CONCENTRATIONS OF PHOSPHORUS

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Attempts have recently been made to study the most general features of injury caused to cells, tissues, and organs by exposure to elementary phosphorus and its inorganic compounds [1, 2, 4]. The study of the morphology and function of the oral mucosa under these conditions is particularly interesting because of the known fact that phosphorus compounds are retained by the mouth fluids [3].

The aim of this investigation was to study the morphology of the oral mucosa in rats after long-term exposure to the atmosphere of a phosphorus factory.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 120-140 g. The animals were kept in the furnace room of a phosphorus factory. Rats in the experimental groups were poisoned for 4 h daily, 5 days a week. Control animals were kept in an animal house away from the factory. The animals were decapitated 1, 2, 3 and 4 months after the experiment began. The mucous membranes of the cheek, gum, and hard palate were dissected from underlying tissues, fixed in 10% neutral formalin solution, and embedded in paraffin wax. The animals' tongues were washed initially in the fixing solution, then fixed and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin and with picrofuchsin. Pieces of mucosa from the cheek, gum, and hard palate, and the tip of the tongue were fixed in 4% paraformaldehyde solution, postfixed in 2% OsO_4 solution, dehydrated with alcohol, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-1 ultrotome, stained, and examined in the GEIL 7A electron microscope.

EXPERIMENTAL RESULTS

The structural integrity of the epithelium of the oral mucosa was preserved 1 month after the experiment began. In some cases thickening of the epithelium of the buccal mucosa was observed along the line of contact of the teeth. A moderate degree of hyperkeratosis was observed on the dorsum of the tongue, and in some cases the proliferating stratum corneum sometimes reached as far as the apices of the filiform papillae. The surface of the tongue

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