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POLYFUNCTIONAL ROLE OF THE ALVEOLAR BRUSH CELLS IN THE RAT LUNG

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An investigation by scanning and transmission electron microscopy revealed an increase in the number of vacuoles in the apical part of the cytoplasm in the alveolar brush cells of the regenerating rat lung, hyperplasia of the lamellar complex, and activation of the protein-synthesizing apparatus. Immature surfactant material and secretory granules with an osmiophilic center were found in the cytoplasm of the brush cells. Colchicine, injected intramuscularly into rats six times in the course of the 24 h before sacrifice in a dose of 0.1 mg/100 g body weight, affected neither the number, topography, nor the structure of the bundles of microfibrils present in large numbers in the brush cells. Meanwhile, under the influence of colchicine, some of the microvilli of the alveolar brush cells undergo destruction and resorption. These data on the ultrastructural organization of these cells indicate that they can perform several functions: absorptive, contractile, and secretory.

KEY WORDS: regeneration; surfactant; lung chemoreceptors.

Interest in the study of chemoreceptors of the lung has increased considerably in recent years. According to some observations [7], single Kulchitsky cells or concentrations of them (neuroepithelial bodies), capable of reacting to a change in the gaseous medium through increased secretion of granules containing biogenic amines, are present in the epithelial layer of the bronchi of the mammalian and human lung. No Kulchitsky cells are found in the respiratory bronchioles, alveolar passages or alveoli. Meanwhile, cells described by Meyrick and Reid as "brush cells" [8] are found in the epithelial lining of the air passages and also of the alveoli. The particular features of the ultrastructural organization of these cells, located in the alveoli, or alveolar brush cells (for instance, the presence of numerous vacuoles in the apical cytoplasm, and of microvilli on the cell surface facing the lumen of the alveolus) suggest that these cells, which perform an absorptive function, are alveolar chemoreceptors [3, 6, 8].

The writer has previously postulated principles of autonomous regulation of surface tension within the "surfacton*, the functional analog of the acinus of the lungs, in which the alveolar brush cells play a leading role. This hypothesis is based on the assumption that all cells of the surfacton, including alveolar brush cells, "surfacton,"* the functional with a rich network of feedback [3]. The alveolar brush cells analyze the composition of the hypophase and surfactant and respond primarily to a change in surface tension of the alveoli. The ultrastructure of the alveolar brush cells of the lungs of intact animals has been described several times [3, 6, 8]. However, none of the authors cited states that these cells absorb surfactant material. Yet this phenomenon is evidently the first stage that is essential for the role of alveolar brush cells in the regulation of the intensity of surfactant secretion.

The objects of the present investigation were accordingly: 1) to discover whether alveolar brush cells can absorb surfactant and, in particular, at the time of its active secretion; 2) to study the nature of the microfibrillary apparatus of these cells.

*The term "surfacton" was suggested originally in the Russian literature [3].

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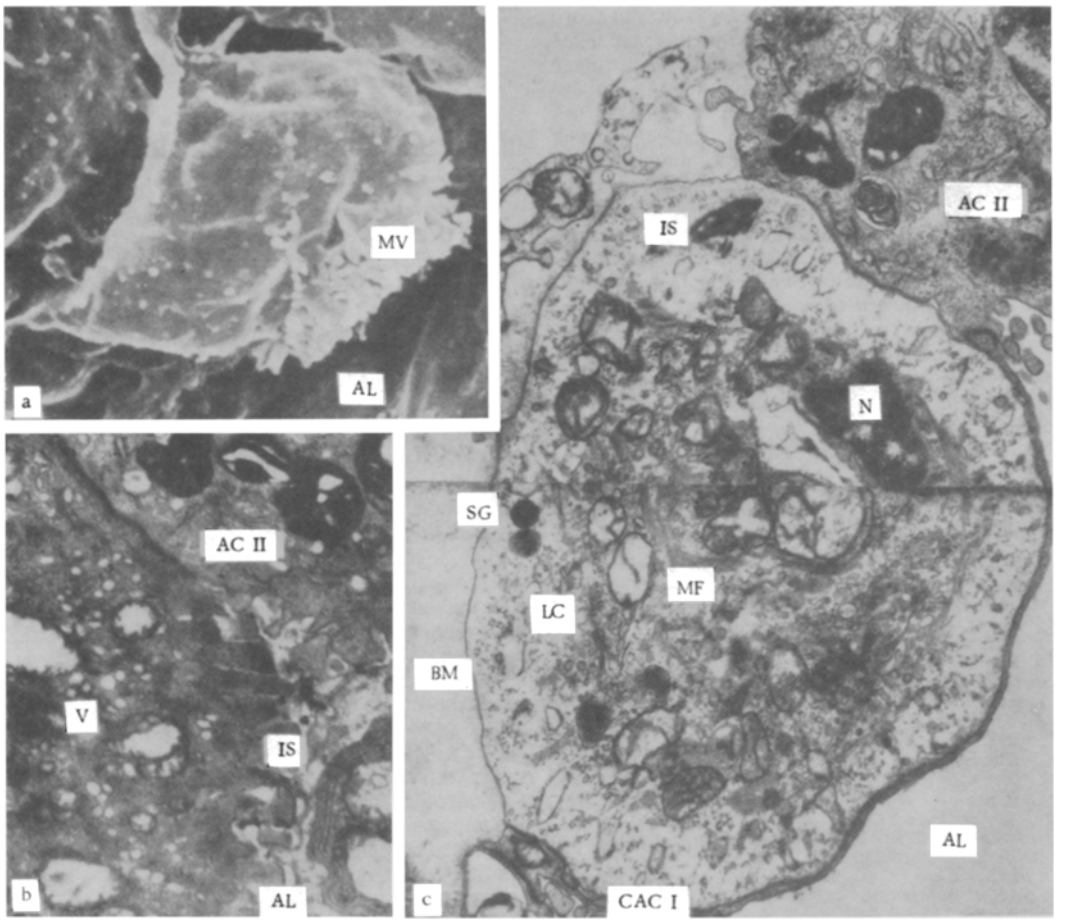

Fig. 1. Alveolar brush cells of regenerating rat lung: a) external appearance of cell. Surfactant can be seen on surface of microvilli. Scanning electron microscopy, 24,000×; b) contact of brush cell with type II alveolocyte secreting surfactant. Osmiophilic granules of immature surfactant distributed on surface and between microvilli of brush cell, 8000×; c) section through basal part of brush cell of regenerating lung of rat receiving colchicine. Immature surfactant visible in cytoplasm, 10,000×. Here and in Figs. 2 and 3: AL) alveolar lumen; ACII) type II alveolocyte; IS) immature surfactant; N) nucleus; MV) microvilli; MF) microfibrils; LC) lamellar complex; V) vacuoles; BM) basement membrane of alveolus; CACI) cytoplasm of type I alveolocyte; SG) secretory granule.

EXPERIMENTAL METHOD

To study the particular features of the ultrastructural organization of the alveolar brush cells at a time of marked hypertrophy of the alveoli and active secretion of surfactant, experiments were carried out on noninbred albino rats weighing 200-250 g, in which 37-63% of the total lung tissue had been removed. The animals were killed on the 4th-6th day after the operation [2, 4].

In another group of experiments, intramuscular injections of colchicine in a dose of 0.1 mg/100 g body weight began to be given to rats 4-5 days after removal of 63% of their lung tissue and also to control rats. During the 24-h period before sacrifice the animals received colchicine six times at intervals of 4 h. Colchicine causes depolymerization of the tubulins of the microtubules but without affecting contractile proteins (G actin, actomyosin) [1]. It was accordingly decided to study the effect of colchicine on bundles of microfibrils, which are present in large numbers in the cytoplasm of the brush cells.

The lungs for transmission electron microscopy were fixed by perfusion with 2.5% glutaraldehyde (pH 7.4) in 0.1M cadocylate buffer through the pulmonary artery, followed by postfixation of pieces of tissue with 1% OsO_4. The material was dehydrated in acetones of increasing concentration and propylene oxide; the tissue was embedded in Epon and Araldite. Sections 40-60 nm thick were stained with lead citrate and examined in the IEM-100B electron microscope.

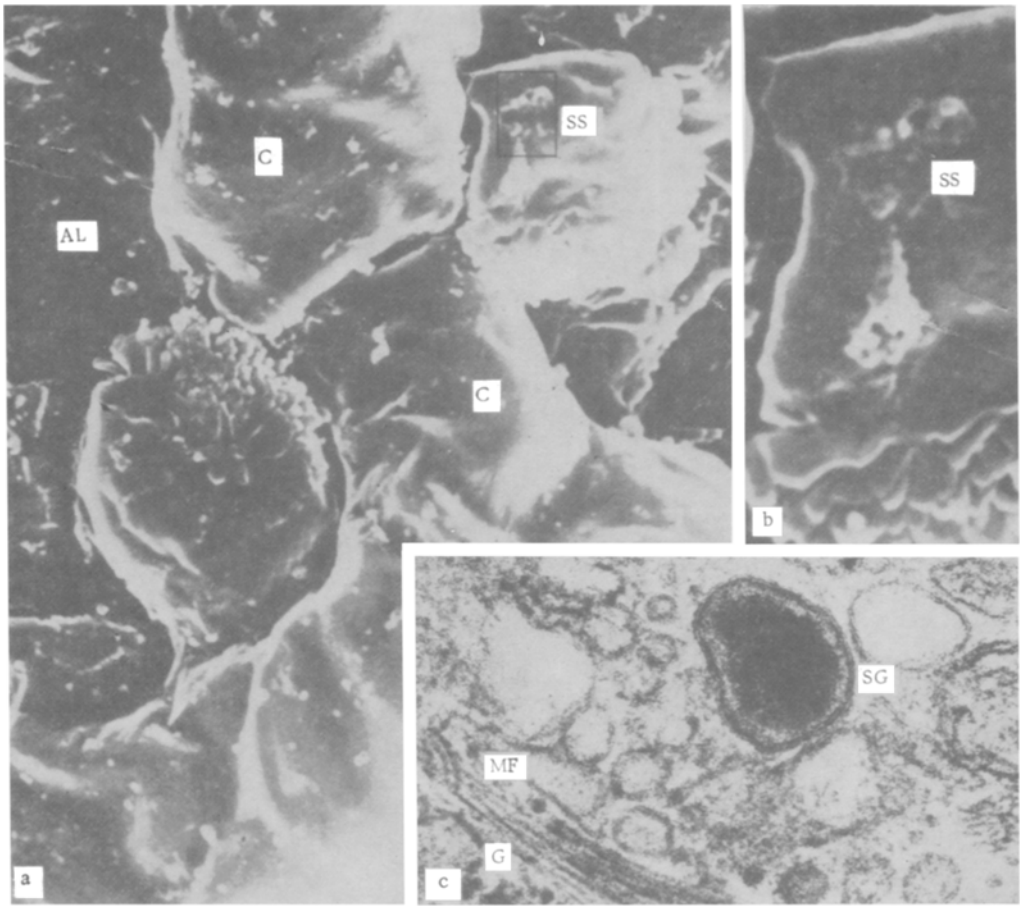

Fig. 2. External appearance of two alveolar brush cells of regenerating rat lung. Scanning electron microscopy: a) "crater" visible on the surface of one cell, 25,000×; b) fragment of Fig. 2a, 50,000×; c) zone of lamellar complex. Secretory granule visible. Fragment of Fig. 3a. Here and in Fig. 3: C) capillaries; SS) site of secretion; G) glycogen.

For scanning electron microscopy the lungs were fixed by intratracheal injection of 2.5% glutaraldehyde solution in 0.1M cacodylate buffer (pH 7.4). The tissue was dehydrated in mixtures of alcohols and acetones of increasing concentration. The critical drying point of the sample was obtained with CO_2 [5]. The specimens were sprayed with gold and examined in the Hitachi S-500 microscope.

EXPERIMENTAL RESULTS

A study of alveolar brush cells by scanning microscopy during active secretion of surfactant by the type II alveolocytes, showed that the external appearance of these cells in the lungs of the experimental animals was almost indistinguishable from that in the controls. They were oval or circular in shape (Figs. 1 and 2). The length of the microvilli was 0.6-1.1 μm, and most of them were 0.2-0.3 μm wide. The surface area of the cell body, excluding the apical part, containing the crown of microvilli, was covered by cytoplasmic veils of several type I alveolocytes. The borders of the veils were closely applied to the base of the microvilli, forming a ridge around them. Where two or three microvilli were covered by the cytoplasmic processes of type I alveolocytes they appeared duplicated in structure (Figs. 1 and 2). Microvesicular bodies, the functional role of which is not yet known, were seen in the cytoplasm of the type I alveolocytes in the zone of the ridge (Fig. 3b).

The surface of the type I alveolocytes, covering the brush cells and facing the lumen of the alveolus, was nodular, and covered with mounds, hollows, and craters. The irregularities of the cell surface that could be observed were evidently due to a change in the configuration of the body of the alveolar brush cells as a result of uneven contraction of its microfibrillary apparatus in different parts of the cell. The appearance of dome-shaped evaginations, of "crater," and holes indicates probably a process of secretion of material, the nature of which is unknown (Fig. 2a, b). Considering the particular features of the shape and topography of the alveolar brush cells, they were described as Actinia-like cells (because of their external similarity to sea anemones).

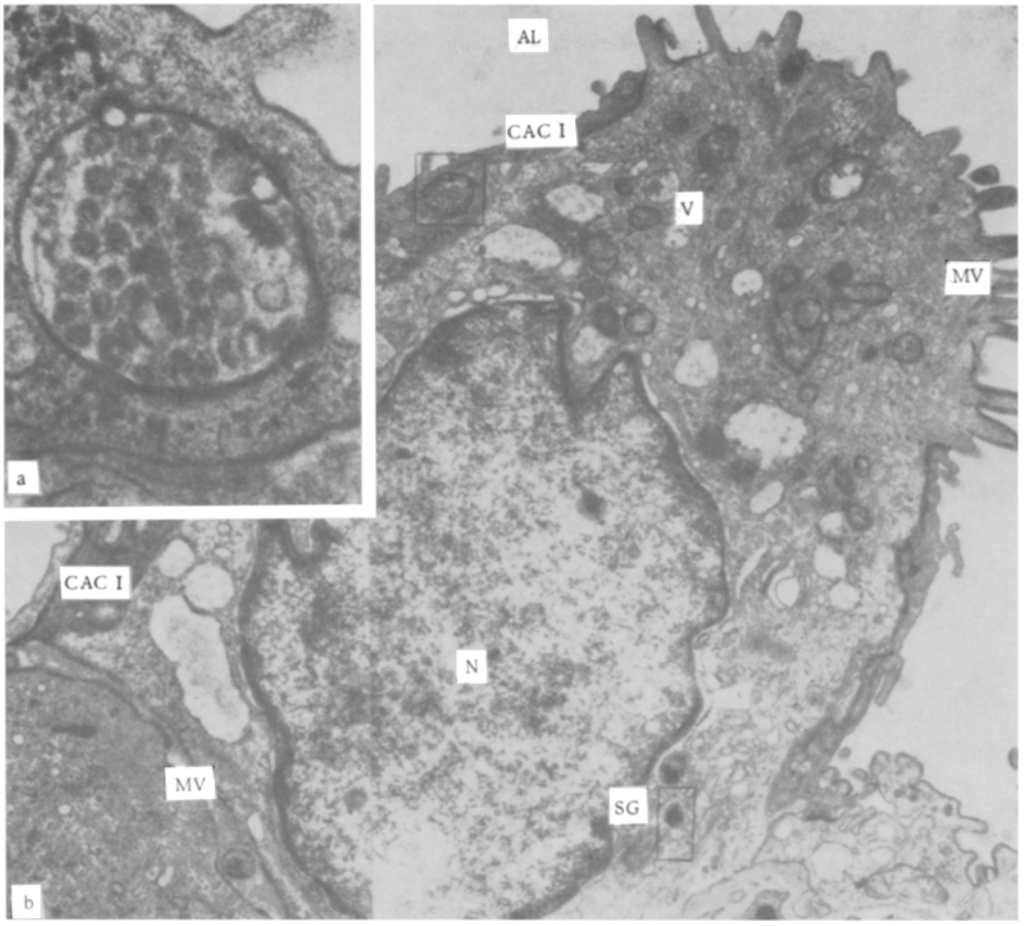

Fig. 3. Alveolar brush cell of regenerating lung of rat receiving colchicine: a) reduction of microvilli; apical part of cell rich in vacuoles; lamellar complex shows hyperplasia and secretory granules can be seen in its zone. Numerous bundles of microfibrils. Cisterns of cytoplasmic reticulum dilated, 7000×; b) microvesicular body in cytoplasmic process of type I alveolocyte. Fragment of Fig. 3a, 50,000×.

Different stages of contact of immature surfactant, present as shapeless granules of osmiophilic material, with the surface of the microvilli could be observed in the regenerating lungs (Fig. 1b). Part of the surfactant was situated deeply between the villi and near their base. The surfactant penetrated into the cytoplasm of the brush cells (Fig. 1c) and could remain there unchanged in appearance. These observations suggest that the brush cells can absorb surfactant. Examination of alveolar brush cells in the lungs of animals receiving colchicine revealed that it affects the structure of the microfibrillary system of the cells (Figs. 2c and 3a), i.e., the microfibrils do not consist of tubulins, but they evidently contain contractile proteins of a different nature. The number of bundles of microfibrils and their dimensions and location in the cytoplasm of the alveolar brush cells were the same in the experimental series as in the control. Meanwhile, some microvilli of the alveolar brush cells underwent destruction and resorption as a result of destruction of the microtubules contained in them (Fig. 3a). The same round structures (remnants of destroyed villi) could be seen in the vacuoles of the apical part of the cell and on the surface of the cell actually at the site of resorption of the microvilli. Destruction of the microtubules in the cytoplasm was reflected in the state of the cytoplasmic reticulum of the alveolar brush cells: its cisterns were dilated and amorphous contents of average electron density appeared in them.

Single secretory granules surrounded by a membrane and containing an electron-dense center could be seen in the brush cells in the lungs of the experimental animals (Figs. 2c and 3a). These granules were located in the zone of the lamellar complex and were similar in their ultrastructural organization to granules of Kulchitsky cells containing and secreting serotonin [7]. Indirect evidence that the alveolar brush cells can secrete was given by the well-marked hyperplasia of the lamellar complex and activation of the protein-synthesizing

apparatus in the cells of the regenerating lung (Figs. 1c and 3a). Scanning electron microscopy showed that the brush cells are in fact capable of secretion (Fig. 2a, b).

The electron-microscopic data thus show that alveolar brush cells can perform several functions: absorptive, contractile,and secretory. The microfibrillary apparatus of the alveolar brush cells contains proteins that differ from the tubulins of microtubules.

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ELECTRON-MICROSCOPIC AND ELECTRON-AUTORADIOGRAPHIC CHARACTERISTICS OF EMBRYONIC LUNG CELLS IN ORGAN CULTURES

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Electron-microscopic and electron-autoradiographic investigations of embryonic mouse lung cells were undertaken during the early stages of organotypical culture. The fate of undifferentiated cells could be traced by electron autoradiography.

KEY WORDS: Electron-microscopic autoradiography; histological differentiation; ultrastructural differentiation.

The relationship between differentiation at the histological (tissue, organ) and subcellular levels, and in which they occur have not yet been adequately studied [1, 6, 8, 9, 11], and this is an obstacle to our understanding of the mechanisms of morphogenesis taking place under physiological (regeneration etc.) and pathological (tumor growth etc.) conditions. Organ cultures of embryonic tissues, in which morphogenetic processes similar to those in vivo are preserved, constitute a convenient model with which to study this problem [3, 5, 7]. The object of the present investigation was to study the degree of differentiation of cells in the developing embryonic lung under conditions when histological differentiation of the tissue had not yet reached the postnatal level.

EXPERIMENTAL METHOD

The lungs of 18-19-day line A mouse embryos were used for organ culture. The technique of organotypical culture was described previously in detail [3]. Organ cultures were investigated 24, 48 and 72 h after the beginning of culture. Thymidine-^{3}H (specific activity 24 mCi/mmole) was added to the organ culture in a dose of 5 mCi/ml medium 24 h after the beginning of culture. The thymidine-^{3}H was washed out of part of the material after 1 h and of the rest after 24 h. Pieces of lung tissue were fixed in 2.5% glutaraldehyde solution in cacodylate buffer, pH 7.4, postfixed in 1% OsO_4 solution, dehydrated, and embedded in Epon 812. Sections 10 μm thick were prepared by the method of Rengol'd et al [4], coated with Ilford L-4 emulsion, and exposed for 14 days at 4°C. After development of the autoradiographs, pieces of tissue containing the label were selectively

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