LIGHT-OPTICAL AND ELECTRON-MICROSCOPIC STUDY OF ALVEOLAR BRUSH CELLS OF THE RAT LUNG

L. N. Filippenko

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In semithin sections through rat lung tissue stained metachromatically with toluidine blue 52 alveolar brush cells (ABC) were discovered. During a light-optical study the distinguishing features of these cells were the pyramidal shape of their body, the basal position of the nucleus, the darker staining of the cytoplasm than in other alveolar cells, and the presence of microvilli on the small free surface of the cell. For every 21 type II and 15 type I alveolar cells there was one ABC. Of the total number of ABC 41.1% are located at junctions between neighboring alveolar cells, 32.7% on the alveolar wall facing the cavity of one alveolus, 16.8% near the entrance to the alveoli, and 9.4% facing the lumen of two adjacent alveoli simultaneously or near Cohn's pores. Parallel electron-microscopic investigations revealed a granular cytoplasmic reticulum in ABC of an unusual type for other alveolar cells; it consisted of blocks of 5 to 8 apparently confluent tubules, bundles of fibers microtubules, and vacuoles in the apical cytoplasm. The structural organization of the ABC, their topography, and the frequency with which they were found in the alveoli of rats are evidence that these cells are chemoreceptor in nature.

KEY WORDS: *alveolar brush cells; ultrastructure; chemoreceptors.*

During an electron-microscopic study of the respiratory epithelium of the rat lung Meyrick and Reid [8, 9] first found cells which differed in their structure from respiratory (type I) and large (type II) alveolar cells. They had characteristic microvilli, they contained bundles of fibrils and granules of glycogen, and on this account they resembled the brush cells of the epithelium covering the air passages [8, 11]. As these workers point out, brush cells are infrequent in the alveoli, and under the light microscope in semithin sections it is impossible to make out any structural features whereby these cells can be distinguished from the ordinary (especially type II) alveolar cells. That is evidently why the discovery of alveolar brush cells (ABC) under the electron microscope has so far been accidental in character, and because of the extreme laboriousness of the process, only a few isolated studies have yet been made [1, 4, 7, 14]. The ultrastructural organization of ABC has still been inadequately studied, and the topography and frequency of discovery of these cells in the alveoli remain unclear. There is thus a need for analysis of a large quantity of material, obtained not only by electron-microscopic investigation, but also by the use of light-optical methods of examination of ABC, primarily for the purpose of sharpening the pyramidal block before section cutting on the ultratome. Accordingly, further attempts were made to discover these cells in semithin sections stained metachromatically with toluidine blue. The writer used similar sections previously with success for the study of the morphological features of type II alveolar cells [4], and this made the task of the present investigation much easier — working out the morphological criteria for reliable identification of ABC during light-optical and electron-microscopic studies, and determining the topography and relative percentage of these cells in rat alveoli.

Laboratory of Geographic Pathology of the Baikal-Amur Trunk Railroad Zone, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 592-596, November, 1978, Original article submitted March 30, 1978.

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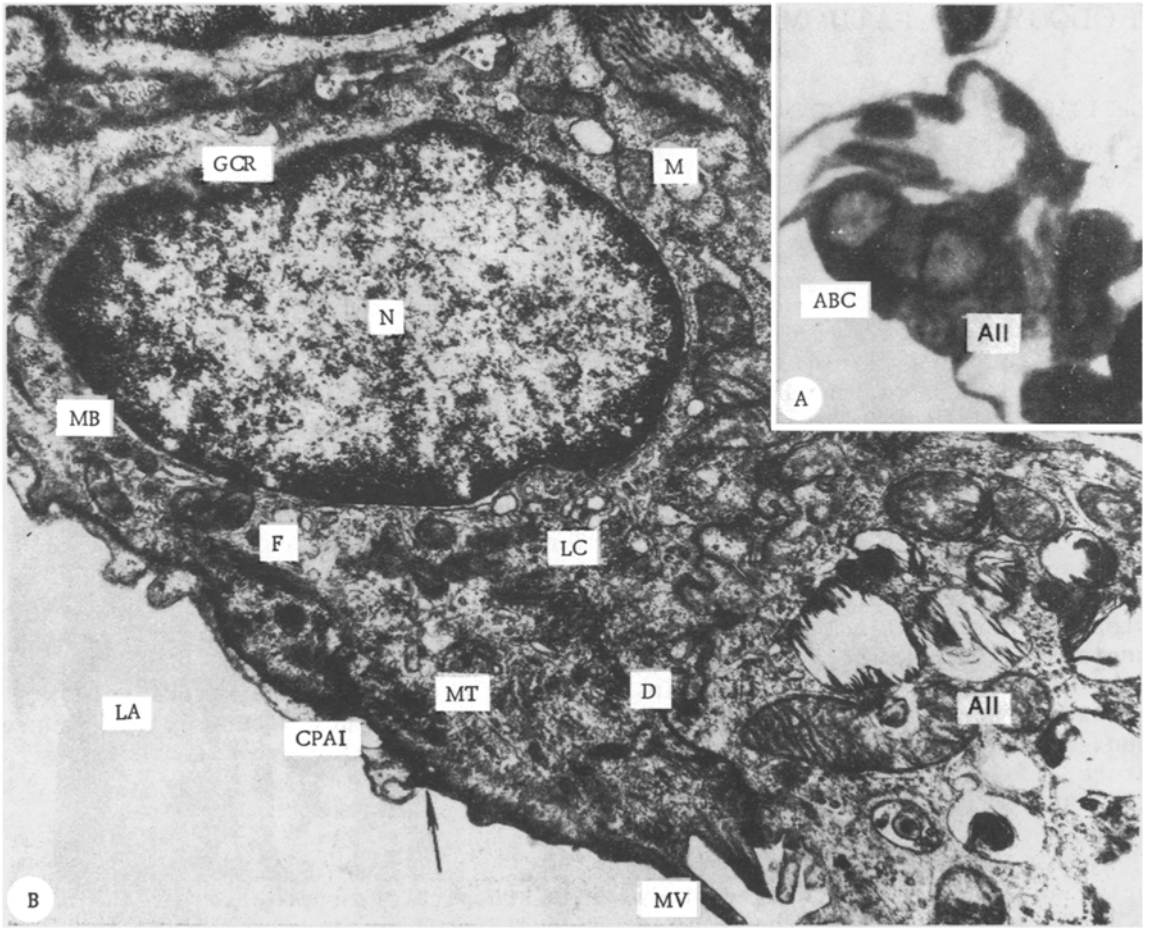

Fig. 1. ABC of intact rat: a) general view of cell in semithin section stained with toluidine blue (900×); b) same cell in ultrathin section; arrow indicates zone of contact with cytoplasmic process of type I alveolocyte (CPAI). Magnification: a) 2700×; b) 23,400×. ABC) Alveolar brush cell; AII) type II alveolar cell; LA) lumen of alveolus; N) nucleus, GCR) granular cytoplasmic reticulum; M) mitochondrion; MB) multivesicular body; F) fibrils; LC) lamellar complex; MV) microvilli; D) desmosome; MT) microtubule.

EXPERIMENTAL METHOD

The lungs of four noninbred male rats weighing 140-200 g were fixed under pentobarbital anesthesia with 3.6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, by perfusion through the pulmonary artery [13] and pieces of tissue were subsequently postfixed with 1% OsO_4. The material was stained with uranyl acetate, dehydrated in acetone and propylene oxide, and embedded in Epon-Araldite. Semithin sections 2 μ thick were cut on a Leitz rotation microtome, specially adapted for cutting with glass knives. The sections were stained metachromatically with toluidine blue [2] and examined under the MBI-15 microscope. After acute trimming of the block under direct vision, some of the ABC were subjected to parallel examination in the JEM-100V electron microscope.

EXPERIMENTAL RESULTS

The parallel study of ABC in semithin and ultrathin sections of lung tissue revealed some characteristic features of these cells that were equally marked at the electron-microscopic and light-optical levels of investigation (Fig. 1). The ABC shared the same basement membrane with other alveolar cells and had a characteristically pyramidal-shaped body. On metachromatic staining of the section with toluidine blue the nuclei of these cells appeared bluish-violet, the nucleoli red, and the cytoplasm purple, usually darker in shade than the type I and II alveolar cells (Fig. 1a). Polarity was distinctly observed in the structural organization of ABC. In the basal part of the cell lay an oval nucleus with

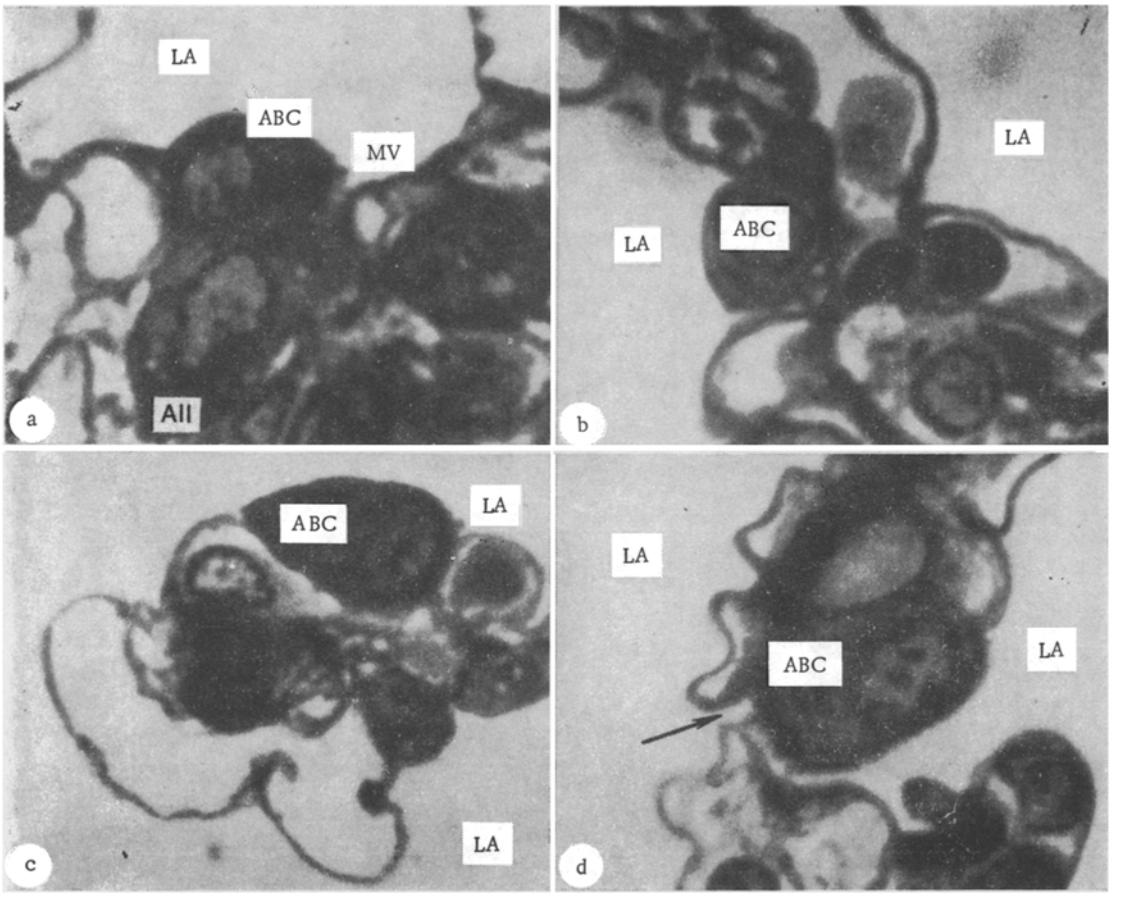

Fig. 2. Morphological features and topography of ABC detectable in semithin sections. a) ABC located on alveolar wall and facing lumen of one alveolus; b) at junction of neighboring alveolar wall; c) at entrance to alveolus; d) near Cohn's pore (arrow). Ocular 10, objective 100. Magnification: a, b, c, 3800×; d, 4600×. Remainder of legend as in Fig. 1.

axes 2 and 5 μ long, as a rule with one nucleolus, and with masses of chromatin concentrated at the periphery of the nucleoplasm (Fig. 2). In the apical zones of the cell nearly all the cytoplasmic structures were concentrated, and microvilli were present on the surface (Fig. 2a, d). The latter could be seen only in a certain plane of section, for most of the apical plasalemma of ABC was covered by closely applied cytoplasmic processes of type I alveolar cells. Usually in one ultrathin section of the cell there were 4 to 10 microvilli. They were cylindrical in shape and contained bands of fibrils and microtubules, crossing into the cytoplasm (Fig. 3a). The length of the microvilli in different ABC varied from 0.55 to 1.10 μ and their width from 0.23 to 0.34 μ.

The cytoplasm of ABC was crossed by bundles of fibrils and microtubules lying in different planes of section through the cell, and evidently forming its supporting framework (Figs. 1b, 3a). The mitochondria were oval in shape, 0.4-0.6 μ long, and contained usually 3 to 6 cristae (Fig. 3b). The granular cytoplasmic reticulum consisted of separate short, lightly dilated tubules, which could merge with each other to form small blocks of 5-8 cisterns (Fig. 3b). The lamellar complex was represented by a contact zone of flat tubules, vacuoles, and vesicles (Fig. 3c). Over the whole cytoplasm of the cell polysomes were scattered, and multivesicular bodies (Fig. 1b) and lysosome-like structures (Fig. 3d) could be seen. Single vacuoles 0.1-0.2μ in diameter were present in the apical zones of the cytoplasm (Fig. 3a). The ABC made contact with the types I and II alveolar cells through zones of obliteration (zonulae occludentes) and desmosomes (Fig. 1b). The distinctive features of the ultrastructure of ABC observed in the present investigation were on the whole similar to those described by other workers for these cells [1, 7, 8, 14].

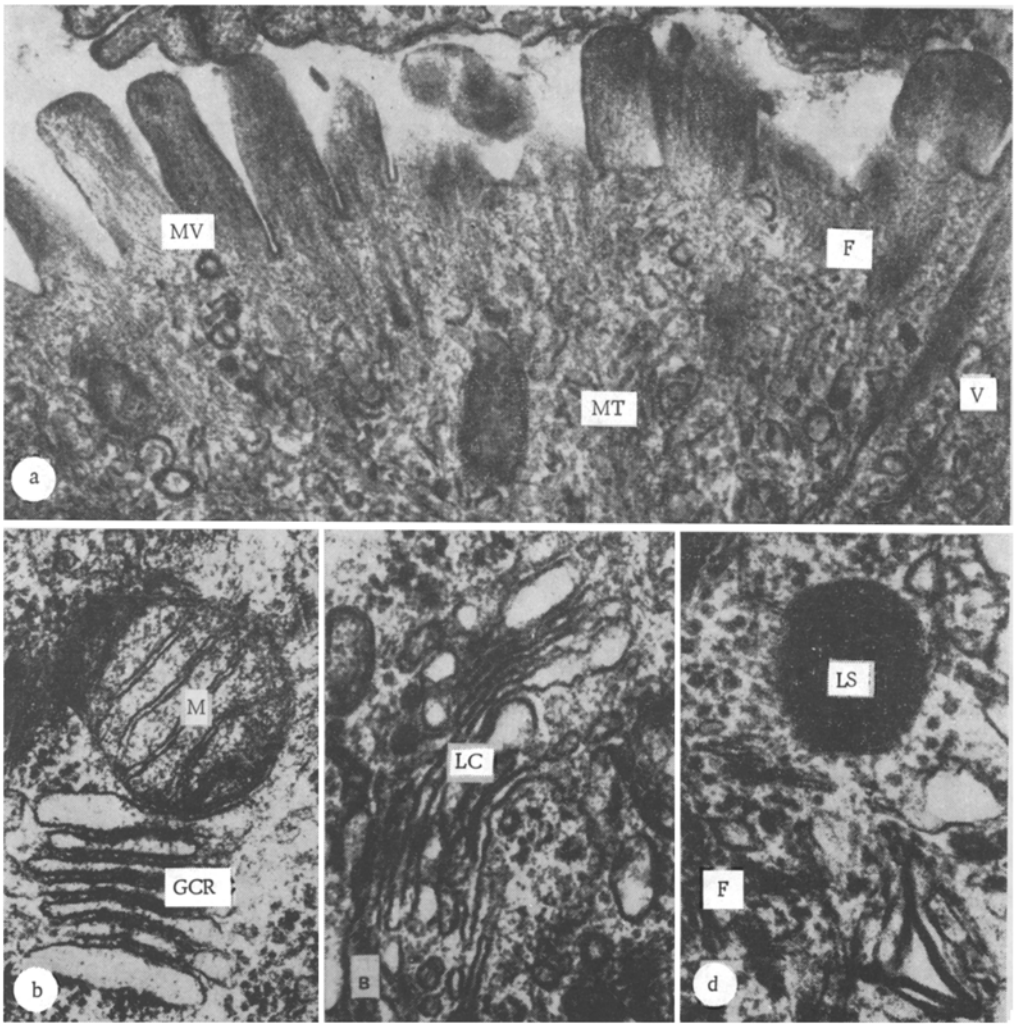

Fig. 3. Ultrastructrual features of ABC. a) microvilli on free surface of cell, vesicles (V) can be seen in apical cytoplasm; b) granular cytoplasmic reticulum consisting of several apparently confluent tubules; c) lamellar complex in perinuclear zone; d) lysosome-like structure (LS). Magnification: a) 45,000×; b, c) 60,000×; d) 88,000×. Remainder of legend as in Fig. 1.

In the present experiments, of 1924 alveolar epithelial cells identified in semithin sections there were 1092 type II and 780 type I alveolar cells and 52 ABC, or 56.7, 40.6, and 2.7% respectively. These figures are close to the results of an electron-microscopic analysis of the cell composition of the rat alveolar epithelium undertaken by Meyrick and Reid [8].

The study of the topography of ABC showed that 41.1% of the cells of this type are located at alveolar "angles" (junctions between neighboring alveolar walls; Fig. 2b), 32.7% on the alveolar wall taking the lumen of one alveolus (Fig. 2a), 16.8% at the entrance to an alveolus (Fig. 2c), and 9.4% faced the lumen of two neighboring alveoli simultaneously or were near Cohn's pores (Fig. 2d). No clearly marked preferential localization of ABC in the subpleural alveoli could be observed, as other workers have described [8, 9].

The characteristic features of ABC whereby they can be distinguished under the light microscope from other alveolar cells are thus the pyramidal shape of the cell body, the basal position of the nucleus, the presence of microvilli on the apical surface, and the dark staining of the cytoplasm on metachromatic staining of the sections with touidine blue, whereas during electron-microscopic analysis other features are the presence of a small area of free cell surface, the abundance of fibrils and microtubules penetrating the microvilli and the

whole cytoplasm of cell, the presence of vesicles in the apical cytoplasm, and the unusual shape of the granular cytoplasmic reticulum, compared with that of other alveolar cells, in the form of blocks consisting of several apparently confluent tubules.

The function of the ABC and their interaction with other alveolar cells have not been finally settled. According to some workers, these cells have adsorptive properties, and the presence of microvilli and vacuoles in the apical cytoplasm makes them similar to cells of the intestinal epithelium [5, 11, 12]. Some authors who found sensory nerve endings near the epitheial brush cells of the lungs concluded that cells of this type are chemoreceptors [6, 10]. This conclusion is supported by their extremely low frequency compared with other epitheliocytes. According to counts in the present experiments, in the alveolar epithelium of intact rats there is only one ABC for every 21 type II and 15 type I alveolar cells. Meanwhile, the number of ABC may increase with an increase in the functional load of the lung. For instance, in the solitary residual lung after left-sided pneumonectomy in rats the frequency of discovery of these cells under the electron microscope increased considerably; this corresponded to the period of sharp hypertrophy of the alveoli and correlated with an increase in the reserves of alveolar surfactants [4]. On this basis it was postulated that ABC can adsorb the liquid of the alveolar extracellular lining and can respond to changes in its qualitative composition, thus taking part in the regulation of the surface tension of the alveoli [3]. By its position at the alveolar "angles," at the mouths of the alveoli, and near the Cohn's pores, the same cell may perhaps control the composition of the liquid phase (hypophase) of 2 or 3 neighboring alveoli. The "confluent" tubules of the granular cytoplasmic reticulum may be a special form of that structure, producing definite mediator proteins.

Consequently, the ABC are an independent type of alveolar cell which can take part in the regulation of the synthetic activity of cells belonging to the surfactant system of the lung.

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