

Brush-like cells within bronchial epithelia of chicken lung (*Gallus gallus*)

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Summary. The secondary and primary (mesobronchus) bronchi of chicken lung are lined by a typical respiratory epithelium: pseudostratified columnar ciliated with goblet cells. Up to date, four constituting epithelial cell types have been identified: ciliated, mucosecretory, basal and endocrine cells. In this study a putative new epithelial cell type, the brush-like cell, is described. The avian brush-like cells have only been found in the bronchial epithelia but never in the gas-exchange areas. They are scattered among the other epithelial cells, mainly ciliated cells, and their number is extremely low. The characteristic morphological feature of these cells is an apical protruding cytoplasm with microvilli. This cell type is similar to that found in the lung of some mammalian and non-mammalian species. The functional role of these cells is not yet clear; they could carry out absorptive processes.

Key words: Brush-like cells, Bronchi, Lung, Chicken

Introduction

The first descriptions of the brush cells were performed by Rhodin and Dalhamn (1956) on the rat tracheal epithelium and by Meyrick and Reid (1968), who observed this new cell type in the alveolar epithelial lining of the rat lung, among the type I and II pneumocytes. Later, brush cells were found in the lung of other mammalian species (mouse, hamster, pig, guinea pig, calf), including man (Luciano et al., 1968; Baskerville, 1970; Hanna and Nagata, 1970; Sorokin, 1970; Weibel, 1973; Inoue and Hogg, 1974; Andrews, 1974; Allan, 1978; Kennedy et al., 1978; Pastor et al., 1985; DiMaio et al., 1988, 1990; Ito and Kanisawa, 1990). In some mammalian species, brush cells are only observed in the epithelial lining of conducting airways (trachea, bronchi and bronchioles); and in other species

(rat, hamster and pig) they only appear in the respiratory epithelia, then being called type III pneumocytes. The different location of brush cells, in the conducting or in the respiratory areas, seems to be species-specific (Penney, 1988).

The presence of this cell type in non-mammalian vertebrate lungs has only been described in some amphibian species (Gomi et al., 1987; Goniakowska-Witalinska and Lauweryns, 1991) and reptiles (Gomi, 1982; Sheuermann and Timmermans, 1982). The number of brush cells is extremely low in both cases and they have only been found in the respiratory epithelia, but never in bronchial-type epithelia. Therefore, they have been considered alveolar brush cells or type III pneumocytes.

So far, no references on the occurrence of brush cells in the avian lung have been reported in the reviewed literature. Thus, the aim of the present study is to describe this epithelial cell type in the chicken lung by means of light and transmission electron microscopy.

Materials and methods

Ten specimens of *Gallus gallus* (Warren strain), ranging in age from 10 days after hatching to cockerels, were used. The animals were anaesthetized with ether. Their lungs were removed and flooded with fixative.

Small pieces (1mm³) of the lungs were fixed in 4% glutaraldehyde in 0.1M sodium cacodylate and 0.25M sucrose buffer, pH 7.2, at 4 °C for 5 h, and postfixed in 1% phosphate-buffered osmium tetroxide, pH 7.2, at 4 °C for 2.5h. The pieces were then dehydrated with an ethanol series and embedded in epon 812 as usual.

Semithin, 1 µm-thick, sections were cut and transferred onto glass slides, and the plastic entirely removed with sodium methoxide (Mayor et al., 1961) or with aged saturated sodium hydroxide in ethanol (Lane and Europa, 1965). Deplasticized sections were stained with borated methylene blue and then used to select areas for electron microscopy.

For the ultrastructural investigation, suitable ultrathin sections were selected, then double stained with uranyl acetate and lead hydroxide and examined with a

Zeiss EM10CR electron microscope.

Results

The primary (mesobronchus) and secondary bronchi of the chicken lung are lined by a columnar

pseudostratified ciliated epithelium. In all light and electron microscopic studies performed so far, the constituting cell types described in these epithelia have been four: ciliated, mucosecretory, basal and endocrine cells.

Now, we have detected a new epithelial cell type in

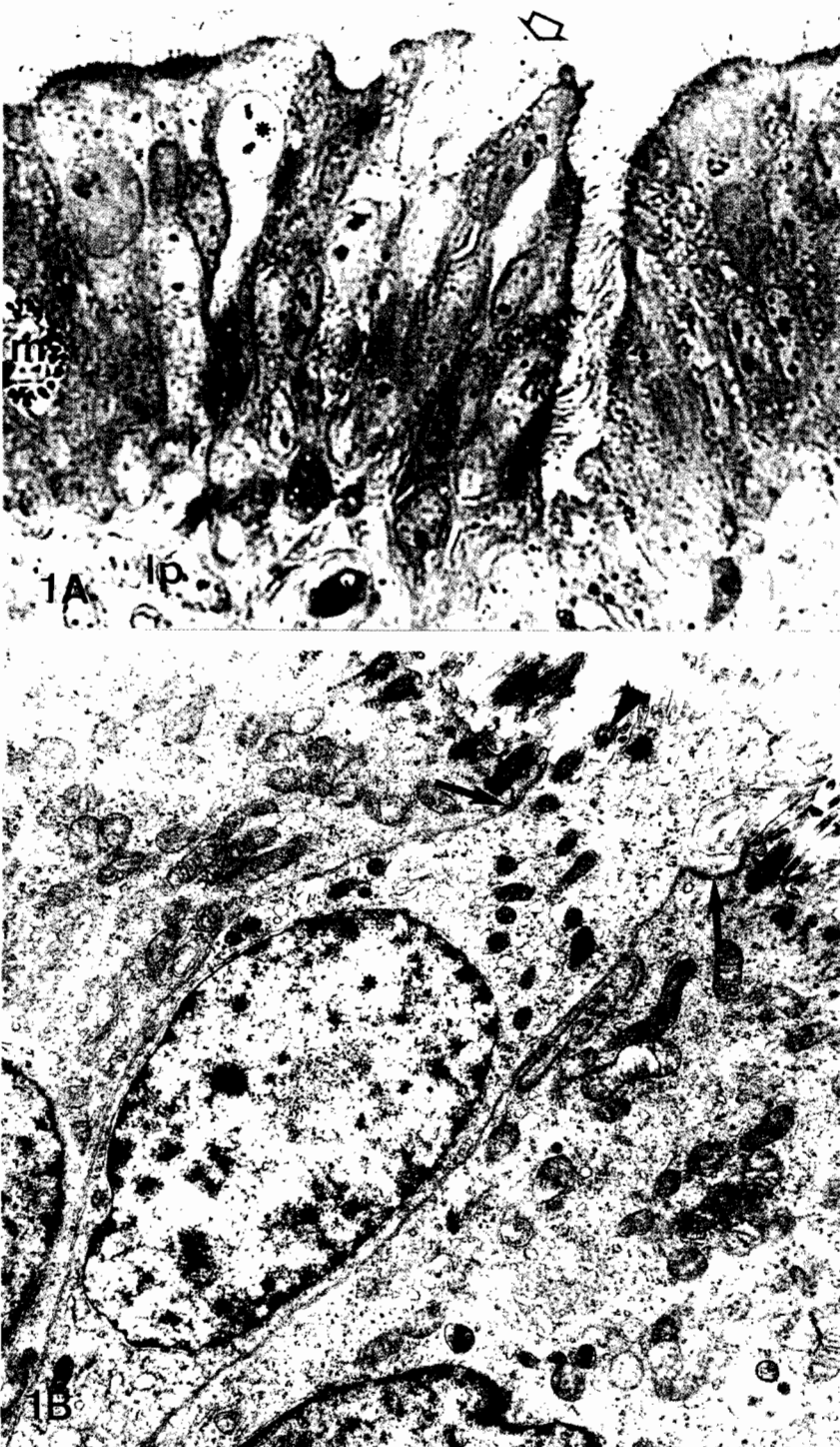


Fig. 1. Serial semithin (A) and thin (B) sections of the mesobronchial epithelium showing the same brush cell (*). Part of another brush cell (open arrow) is observed in A. Arrowhead points out a cilium in the brush cell. Arrows point out junctional complexes in the neck-like apical cytoplasm. m: mast cell; lp: lamina propria. A, Methylene blue; x 1,500; B, x 15,000

Brush-like cells in chicken lung

the primary and secondary bronchial epithelia of adult chicken lung; the brush-like cells, according to the nomenclature used in mammalian studies. These cells were observed both in semithin sections stained with methylene blue by light microscopy and in ultrathin sections by electron microscopy (Fig. 1). This cell type was only located on the surface areas of bronchial epithelium but never in the crypts or glands. The brush cells were very scarce, even less frequent than endocrine cells.

At light microscopic level, the brush-like cells were observed among the ciliated ones. These cells were columnar and were characterized by the presence of a clear cytoplasm and nucleus; moreover, the cell apex protruded over the epithelial surface and did not present cilia (Fig. 1A). The nucleus showed one or two nucleoli. The supranuclear cytoplasm presented some small dense granules (Fig. 1A).

At electron microscopic level, the brush-like cells were easily distinguished by their apical cytoplasm, which protruded into the bronchial lumen and had thin, short microvilli, with an average height of $0.7\mu\text{m}$ and a width of $0.08\mu\text{m}$. Occasionally, these cells presented one or two cilia (Fig. 1B). The cytoplasm showed a low electron density and contained some free ribosomes, vesicles and tubules of smooth endoplasmic reticulum, a scantily developed Golgi complex and small dense mitochondria, which were more abundant in the supranuclear region, corresponding to the dense cytoplasmic granules observed by light microscopy (Fig. 1). The nucleus was round or oval in shape, euchromatic, with small heterochromatic granules attached to the nuclear envelope and scattered in the karyoplasm, and it contained one or two poorly developed nucleoli. In the apical region there were junctional complexes attaching these cells to the neighbouring ones. Some lateral interdigitated infoldings of the cell membrane reinforced the cell attachment (Fig. 1B). No relationship with intraepithelial nerve endings was observed.

Discussion

The first time that we observed the brush-like epithelial cells in the primary and secondary bronchial epithelia by electron microscopy, we considered them as immature stages of the differentiating ciliated or mucin-producing epithelial cells. Then, these cells were also identified under the light microscope (in semithin sections) by their protruding apical cytoplasm devoid of cilia. Later, after reviewing the literature about the pulmonary epithelial cells in other vertebrate lungs, we began to consider the chicken brush-like cells as a new bronchial epithelial type, different from both ciliated and mucosecretory cells whose maturing stages show dissimilar morphological features to those observed in brush-like cells. The analysis of images of these cells obtained from different aging specimens and the comparative study with the similar cell type described in other vertebrate lungs, allows us to confirm the presence

of this new cell type in the chicken lung.

Up to date, the pulmonary chicken brush-like cells have only been found in the epithelia lining the primary and secondary bronchi, and they do not appear in other locations, such as the parabronchial (tertiary bronchial), atrial or air capillary epithelial linings. For this reason, the presence of brush-like cells has not been pointed out in the detailed studies on the parabronchi and associated structures of chicken (López et al., 1986; López, 1995) and of different avian species performed by Klika et al. (1996, 1997, 1998) and Sheuermann et al. (1997). Thus, the avian brush-like cell location is in agreement with that described in some mammalian species; so, in man, calf, mouse and guinea pig this cell type is present in conducting airways (trachea, bronchi and bronchioles), but not in the alveolar epithelium (Hanna and Nagata, 1970; Inoue and Hogg, 1974; Allan, 1978; Reid and Jones, 1979). On the contrary, in rat (Meyrick and Reid, 1968; Jeffery and Reid, 1975; Dormans, 1985), turtle (Sheuermann and Timmermans, 1982), snake (Gomi, 1982) and bullfrog, *Rana catesbiana*, and *Bombina orientalis* (Gomi et al., 1987; Goniakowska-Witalinska and Lauweryns, 1991) brush cells have only been found in the respiratory epithelium. In these cases, the brush cells are called type III pneumocytes (Pastor et al., 1985; Penney, 1988, among others).

The low number of these cells in the lung of the studied vertebrate species, as well as the general cytoplasmic features of this cell type, are similar to those found in chicken lung. The protruding apical cytoplasm has also been described in *Bombina orientalis*. The presence of immature and mature lamellar bodies observed in *Bombina orientalis* brush cells, related to a putative surfactant production, is not observed in chicken and other vertebrate species.

In rat, a more or less close association between brush cells and intraepithelial nerve endings has been reported. In some cases, this association appears as synaptic junctions (Luciano et al., 1968; Weibel, 1973; Hijjiya, 1978; Chang et al., 1986), and this fact allowed the postulation of a chemoreceptor function for these cell type.

In chicken, intraepithelial nerve endings are present in the bronchial epithelia; some of them constitute "reciprocal synapsis" with endocrine cells, described by ourselves among other authors (Cook and King, 1969; King et al., 1974; Walsh and McLelland, 1974; López et al., 1983a,b; López, 1995). These neuroendocrine structures have been considered to be the pulmonary carbon dioxide chemoreceptors postulated by physiological studies (Burger et al., 1974; McLelland and Molony, 1983). Moreover, in bronchial epithelia of chicken there also are intraepithelial nerve endings not associated to brush cells or any particular cell type (López et al., 1983b). Thus, the chemoreceptor function in avian lung seems to be more related to endocrine cells than brush cells.

Nevertheless, the presence of some ciliary structure in the chicken brush-like cells could be interpreted as a

receptor feature; since, as is well known, modified ciliary structures appear in some sensory cell types (olfactory cells and outer segments of photoreceptors, for example), although Schmidt-Nielsen (1990) comments: "it is difficult to determine the importance of the ciliary structure in sensory reception, whether of light or of chemical molecules".

In some cases, an absorptive function has been attributed to the brush cells (Meyrick and Reid, 1968; Allan, 1978; Reid and Jones, 1979; Chang et al., 1986). This function could be involved in detoxication tasks (Chang et al., 1986) or in the control of fluid balance in the lung (Meyrick and Reid, 1968). The presence of microvilli and vesicles of smooth endoplasmic reticulum in the apical cytoplasm of chicken brush-like cells could represent the morphological basis of absorptive processes.

Concerning the precise function of the bronchial epithelial brush-like cells in chicken lung, the hypothesis postulated by other authors both in mammalian and non-mammalian species, can also be extrapolated to our case. So, though the brush-like cells could play a receptor role, according to that discussed in the previous paragraphs, the absorptive function seems to be the most probable. Undoubtedly, further studies will be required to clarify these items.

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Brush-like cells in chicken lung

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