NHLBI Workshop Summary

The Mysterious Pulmonary Brush Cell

A Cell in Search of a Function

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Brush cells, also termed tuft, caveolated, multivesicular, and fibrillovesicular cells, are part of the epithelial layer in the gastrointestinal and respiratory tracts. The cells are characterized by the presence of a tuft of blunt, squat microvilli (~ 120–140/cell) on the cell surface. The microvilli contain filaments that stretch into the underlying cytoplasm. They have a distinctive pear shape with a wide base and a narrow microvillous apex. The function of the pulmonary brush cell is obscure. For this reason, a working group convened on August 23, 2004, in Bethesda, Maryland, to review the physiologic role of the brush (microvillous) cell in normal airways and alveoli and in respiratory diseases involving the alveolar region (e.g., emphysema and fibrosis) and airway disease characterized by either excessive or insufficient amounts of airway fluid (e.g., cystic fibrosis, chronic bronchitis, and exercise-induced asthma). The group formulated several suggestions for future investigation. For example, it would be useful to have a panel of specific markers for the brush cell and in this way separate these cells for culture and more direct examination of their function (e.g., microarray analysis and proteomics). Using quantitative analysis, it was suggested to examine the number and location of the cells in disease models. Understanding the function of these cells in alveoli and airways may provide clues to the pathogenesis of several disease states (e.g., cystic fibrosis and fibrosis) as well as a key for new therapeutic modalities.

Keywords: airway epithelium; alveolar epithelium; microvilli; third pneumonocyte

Many cells throughout the body have developed "microvillous" appendages for various tasks, including sensing fluid flow (through renal distal tubules), absorption, chemosensing, or as a repair process for ciliated epithelial cells after injury. Because of the presence of "microvilli" on these cells, some confusion has arisen in the nomenclature of the brush cell. For example, in the kidney proximal tubules, there are no brush cells, although the lining cells do exhibit an apical brush border. Pleural mesothelial cells do not have brush cells, but these mesothelial cells do have a network of thin and often branching microvilli. The focus of this article is on the pulmonary brush cell first described in the airway epithelium (1) and later in the alveolar lining where it was called a third pneumonocyte (2). Identification of brush cells has relied primarily on electron microscopy; they have a distinctive pear shape with a wide base and a narrow microvillous apex. Brush cells have been identified in the respiratory tract from nose to alveoli. Thus far, these cells have not been identified in the alveolar lining of humans, except under disease states (3–5). The function(s) of brush cells is uncertain. Pathologically, increased numbers of airway brush cells have been described in a human infant with desquamative interstitial pneumonitis (3) and in the immotile cilia syndrome (4, 5), but whether this is a true event or whether the increased number reflects an abnormality of lung development is not certain.

A working group convened on August 23, 2004, in Bethesda, Maryland. The purpose of the workshop was to review the physiologic role of the brush (microvillous) cell in normal airways and alveoli and in respiratory diseases involving the alveolar region (e.g., emphysema and fibrosis) and airway disease characterized by either excessive or insufficient amounts of airway fluid (e.g., cystic fibrosis, chronic bronchitis, and exercise-induced asthma). A better understanding of the function of the brush cell may provide a key to designing new therapies for such diseases. For example, if the cell were found to regulate fluid and solutes across the respiratory mucosa, therapeutic strategies might be suggested that could optimize the fluidity of the surface secretions.

ORGAN DISTRIBUTION OF BRUSH CELLS

Brush cells, also termed tuft, caveolated, multivesicular, and fibrillovesicular cells, were first described in the rat airway by Rhodin and Dalhamn (1). Since that time, brush cells have been found in the respiratory epithelium, including nasal cavity, larynx, trachea, and bronchi, and the gastrointestinal tract, including pancreas and submandibular glands (6) and taste buds (7). They have also been identified in the epithelium of the testicular ductuli efferentes (8). These cells are sparse but are easily distinguished from other epithelial cells by the presence of a tuft of blunt, squat microvilli on the cell surface. The microvilli contain filaments that stretch into the cell's cytoplasm, often forming rootlike structures. The cells in the lung and airways differ from those in the gastrointestinal system in that they lack the distinctive terminal web that lies immediately beneath the microvillous border. There is species variation in occurrence of airway brush cells. They have been identified within the airway epithelium of cat, pig, cow, rat, mouse, rabbit, guinea pig, ferret (9), and sheep (B.M., unpublished observations), but not in monkey or dog. Thus far, these cells have not been identified in the alveolar lining of humans, although they have been identified in airways under disease states (3–5).

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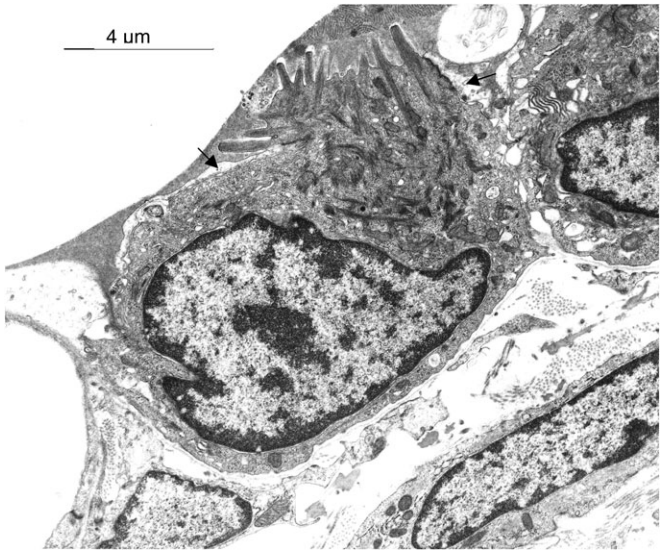

Figure 1. Electron micrograph of an alveolar brush cell from a rat exposed to hypoxia. The cell exhibits microvilli that project into the alveolar space. The microvilli contain filamentsthat extend into the supranuclear portion of the cell. The cell is covered along its alveolar surface by type I pneumonocytes (at *arrows*) to the base of the microvilli (B.M., unpublished observations).

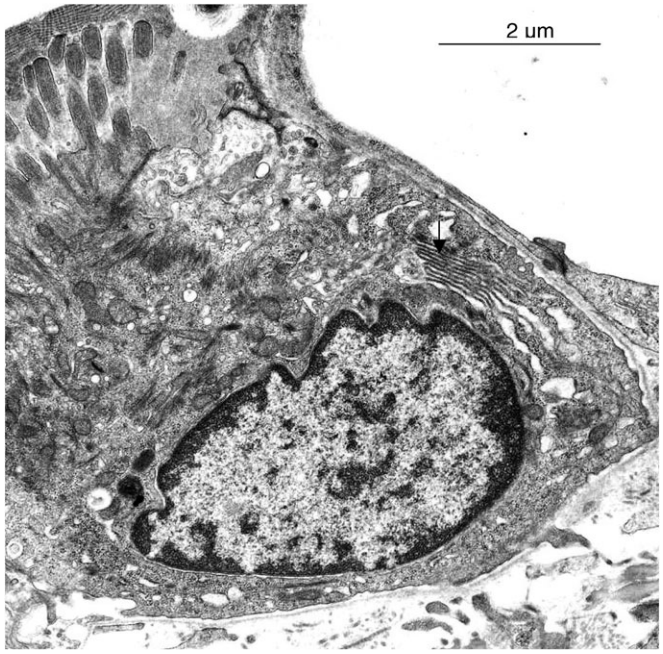

Figure 2. Part of an alveolar brush cell showing parallel arrays of smooth endoplasmic reticulum (*arrow*; B.M., unpublished observations).

REVIEW OF THE THREE TYPES OF PNEUMONOCYTES IN THE LUNG

The lung is a highly metabolic and synthetic organ composed of more than 40 cell types. Many of these specific cell types serve several but poorly defined functions. The alveolar surface of the lung is immense. Its area has been calculated to be approximately 140 m^2, and approximately 93% of this area is covered by type I pneumonocytes. The capillary surface area is slightly less, 126 m^2. Furthermore, it has been calculated that approximately 300 million alveoli contribute to the respiratory region of the lung, each supplied by 1,000 capillary segments. The total number of cells that constitute the alveolar lining is approximately 19×10^9 (10).

Two cell types are generally recognized to line the alveoli: the type I and II pneumonocytes. The type I cells are large squamous cells having a diameter of approximately 40 μm and constituting approximately 35% of the alveolar lining cells. In regions of gas exchange, this cell may measure as little as 0.1 and 0.2 μm in thickness. The major functions of this cell type are gas exchange and fluid transport that is dependent on the type 1–specific membrane channel aquaporin 5 (11). Although other functions are likely for these cells, they have proven difficult to maintain in culture, thereby limiting a more complete understanding of their role. However, recent studies of type I and II cells have shown that expression of several genes seems limited to the type I cell, such as fibroblast growth factor receptor activating protein 1, purinergic receptor P2X7, interferon-induced protein, and Bcl2-associated protein (11).

Type II pneumonocytes are a cuboidal cell with a diameter of approximately 8 to 10 μm, thereby covering only 3 to 5% of the alveolar surface. This cell is usually found in the angles of alveoli. One of its characteristic features is the osmiophilic membrane-bound lamellar body that is extruded onto the alveolar surface as the surface tension-lowering agent, surfactant. Major functions for this cell include production of surfactant proteins as well as production and secretion of the antiprotease α_1-antitrypsin (12) and cytokines, such as interleukin 1β, interleukin 8, and tumor necrosis factor α (13). Type II cells also maintain alveolar homeostasis by acting as a progenitor cell to replace injured type I cells (14) and regulate fluid balance by conducting solutes and fluids across transmembrane channels (15).

A third cell type, the alveolar brush or type III pneumonocyte cell, has also been described to form part of the alveolar lining (Figure 1). Initially described in the rat (2, 16, 17), it has also been found in other species, such as hamsters (18), bullfrogs (19), and striped snakes (20). The cell may be columnar or flasklike in shape, and its most salient feature is the broad, squat microvilli (0.5–1 μm in length and 150–180 nm in width) on its surface that extend into the alveolar space either perpendicularly or parallel to the alveolar wall. Approximately 120 to 140 microvilli may be found on each cell. The microvilli contain filaments that stretch into the cytoplasm forming a long rootlike structure. Other features include the presence of glycogen and numerous vesicles in the apical cytoplasm. Occasional parallel arrays of smooth endoplasmic reticulum have also been identified toward the base of the cell (Figure 2). The cell may lie adjacent to either a type I or a type II cell. When adjacent to a type I cell, the alveolar surface of the brush cell is covered by a flange of cytoplasm from the type I that reaches to the base of the microvilli. A tight junction between the type I cell and brush cell occurs at this site. When adjacent to type II cells, the brush cell has been shown to form numerous interdigitations with the type II. An unmyelinated nerve fiber has been tentatively identified near the basement membrane of the cell (21). Aggregation of smooth endoplasmic reticulum in the supranuclear region has also been described in the brush cells of the striped snake (20).

DISTRIBUTION OF LUNG BRUSH CELLS

In the upper airways, brush cells have been reported to comprise from 1 to 7% of the epithelium (2, 9, 17, 27). The frequency of the alveolar brush cell has been reported to vary from 5 to 10% of the alveolar epithelial cells (2) to as few as 0.5% (21). It is likely that their number varies between strain and species. The

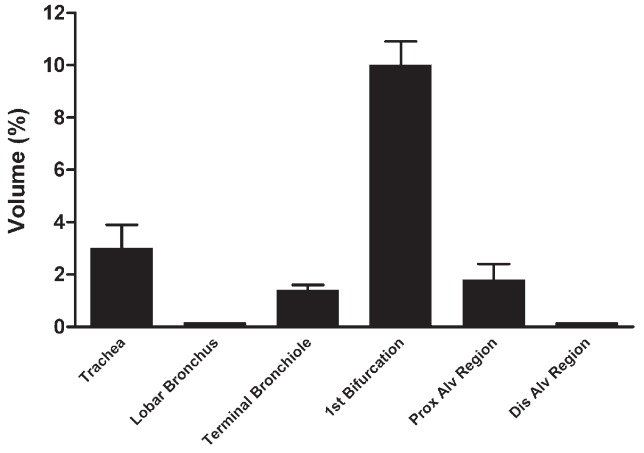

Figure 3. Distribution of brush cells in the rat lung. Data are mean ± SEM. Alv = alveolar; Dis = distal; Prox = proximal. (Modified by permission from Reference 17).

cells have been identified throughout the alveolar region, including the alveoli immediately beneath the pleura (2, 22). In a detailed study of their distribution from alveolus to trachea, Chang and coworkers (17) reported that the number of brush cells in the rat was greatest at the first bifurcation of the alveolar ducts where they comprised 10% of the epithelial volume (Figure 3). They also found that they comprised 3% of tracheal epithelial volume, 1.4% of terminal bronchioles, and 1.8% of the distal alveolar region (Figure 3). Few brush cells were identified in the lobar bronchi, suggesting a differential localization of brush cells along the airways.

Several factors have been claimed to alter the number of alveolar brush cells. Increased numbers of brush cells have been described in bleomycin-induced interstitial pneumonitis in the rat (21), after pneumonectomy (22) and after chronic exposure to intermittent low-frequency noise (26), perhaps suggesting that they may be associated with epithelial regeneration. No formal quantitation was applied in these studies. Increased numbers of brush cells have also been described in a human infant with desquamative interstitial pneumonitis (3) and in immotile cilia syndrome (4, 5). No systematic studies of brush cells and their distribution in human lung are available.

PROPOSED FUNCTIONS FOR LUNG BRUSH CELLS

No function, as yet, has been attributed to the alveolar or any brush cell with certainty. Villin and fimbrin have been demonstrated in the microvilli and their rootlets in brush cells in the epithelium of duodenum, gastric cardia, submandibular gland, and trachea. Their presence is likely linked to formation of cross-linked bundles of actin filaments that serve as a cytoskeletal scaffold for the apical microvilli (8), thereby suggesting that the microvilli are capable of limited movement. Cytokeratin 18 has also been identified in the supranuclear bundles of intermediate filaments in alveolar brush cells, indicating the squamous nature of these cells (23). In addition, the mitochondria of the alveolar brush cell have been shown to exhibit strikingly less cytochrome oxidase activity than the type I and II cells (24). The gastric brush cell has been shown to specifically bind an antibody against rat hepatic fatty acid–binding protein (25), suggesting that, at least at this site, the cell is involved in fatty acid metabolism. Furthermore, they have been shown to express α-gustducin, the α-subunit of the trimeric G-protein complex that was believed to be specific for the tongue (7).

At present, the function of pulmonary brush cells can only be guessed. The increased surface area provided by the microvilli and the presence of apical vesicles have been suggested to be linked to an absorptive function for these cells, although, at least for the brush cells of the gastrointestinal epithelium, that has been shown not to be the case. In addition, the small number of brush cells in the lung makes it unlikely for them to be an effective competitor to ciliated cells or alveolar type II pneumocytes for the task of fluid transport. The finding of unmyelinated nerves in association with the airway brush cell (27) and possibly with the alveolar brush cell (21) suggests a chemoreceptor function for these cells. A similarity of the brush cell to the "taste receptor cells" has also been noted (7). On the basis of their high concentration at first alveolar duct bifurcations, Chang and colleagues (17) suggested that they may play a role in detoxification or act as a sensor for alveolar fluid or alveolar air tension. Another possible function for brush cells that arises from their location in the lung is immune surveillance. Although brush cells have a sparse distribution, they appear to be strategically located. First alveolar duct bifurcations have been shown to be a primary site of deposition for particulate matter and gaseous pollutants (28, 29). A high presence of brush cells at the alveolar first bifurcation coupled with the fact that these cells possess an enlarged apical surface in the form of a brush border greatly increase the probability of their contact with air pollutants and infectious agents. The increase in number of brush cells after unilateral pneumonectomy (22) and in bleomycin-induced interstitial pneumonitis (21) suggests that these cells play a role in cell regeneration. Hijiya and colleagues (21) also proposed that the cells may act as a regulator of capillary resistance and perfusion during hypoxia.

What is the future of the alveolar brush cell? How can we elucidate its function? In the past, studies of this cell were hampered by our lack of ability to isolate these cells for culture. However, Hofer and Drenckhahn (8) and Kasper and colleagues (23) showed that it was possible to clearly distinguish the alveolar brush cell from the types I and II cells at light microscopic level using colocalization of cytokeratin 18 and villin as markers. Similarly, villin and fimbrin could be used to isolate brush cells from the airway epithelium. Future studies with such markers and cell-sorting techniques should enable us to determine the function of this fascinating cell in the lung and perhaps point to roles in the pathogenesis of lung diseases, such as asthma, cystic fibrosis, pulmonary hypertension, and fibrosis.

WORKING GROUP RECOMMENDATIONS

To discern if the respiratory brush cells have an important normal physiologic or immunologic role in the lungs, or a pathologic one in certain lung diseases, several suggestions for future investigation were formulated. These include the following:

1. A rigorous consensus definition of the brush cell is needed, discarding other descriptors, such as microvillous and caveolated. A "tuft" cell may be a preferable name.
2. Morphology by transmission electron microscopy has been the standard for identifying brush cells, but searching for specific markers would be helpful. Staining with a variety of antibodies to markers such as cytokeratin, villin, and fimbrin and viewing by light microscopy is suggested, but whether such markers would be affected in other cell types during the development of disease is not clear.
3. With the development of a distinct marker for brush cells, it may be possible to isolate these cells and apply techniques such as microarray analysis or proteomics to further our understanding of their function. In addition, antigenic screening might be attempted by immunizing mice with a

population of these cells and creating a monoclonal antibody.

4. Further identification of brush cells needs to be made in distal airways and alveolar tissue in normal and diseased tissue, such as cystic fibrosis.
5. Because of their high density in the area of the bronchoalveolar junction, a location prominently involved with trapping inhaled particles or airway pollutants, brush cells might be involved in chemosensing or antigen presentation and thus be involved in innate immunity. This should be investigated.

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