The Pulmonary Epithelium in Health and Disease

Edited by

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Preface

The past two decades have seen extraordinary advances in our understanding of the role of the pulmonary epithelium in airway health and disease. The traditional view of the epithelium as predominantly a physical barrier that also plays a role in ion and water transport has been supplanted by one in which the epithelium is now also considered to be a central regulator of airway inflammation, structure and function. In light of the dramatic changes in our awareness of the complexity of epithelial cell functions, it seemed particularly timely to produce a book to comprehensively address our current understanding of epithelial cell biology. In particular, I wished to focus not only on the epithelium as a regulator of normal airway function, but also to highlight the important roles of the epithelium in host defense, and the contributions of aberrant epithelial biology to the pathogenesis of inflammatory airway diseases.

The first two chapters of this volume are designed to provide an update on the basic structure of the epithelium, including information on the cell types that comprise the epithelium at different levels of the airway, and on the capacity of specific cell types to serve as progenitor cells for new growth. In addition, the remarkable recent increases in our understanding of the molecular components of the structures that are critical for the cell-cell, and cell-matrix, adhesion necessary to maintain epithelial structure are discussed, along with the complex roles of epithelial adhesion molecules in regulating not only epithelial function but also the interactions of the epithelium with other cell types and pathogens. The subsequent two chapters focus on the role of the epithelium as a target for damage by a variety of agents, and on the process of epithelial repair. Fragility of the epithelium is a hallmark of asthma, and there is growing recognition that a chronic damage/repair cycle may play a role in the pathogenesis of this disease. Although ion transport has long been recognized as a major function of the epithelium, our understanding of the complexity and regulation of epithelial ion transport, and of the consequences of dysregulation of these events, has improved considerably in recent years, and our current knowledge is detailed in Chapter 5.

Perhaps no facet of our awareness of epithelial cell function has grown as rapidly as our understanding of the role of the epithelium in host defense, the focus of the next block of chapters. As may be expected from its location at the airway surface, the epithelium plays a critical role in protection of the host from inspired pathogens and irritants. In the larger airways, the tightly regulated process of mucociliary clearance provides the initial defense to prevent pathogens from contacting the epithelial surface, and defects in ciliary beat, or abnormal mucus composition, underlie several airway diseases that are characterized by increased susceptibility to repeated infection. In the distal airways, where mucociliary clearance is absent, surfactant plays a critical role in reducing surface tension at the airway surface. Of equal importance, however, is the role of surfactant in host defense. Not only
does it coat particulates and microbes, facilitating clearance via cough, but it is now clear that several of the protein components of surfactant have broad ranging direct antimicrobial actions. If microbes can evade these initial defenses and come into contact with the epithelium, they are detected by a range of recognition molecules. These include specific receptors as well as broad-ranging “pattern recognition molecules”. Depending upon the specific nature of the ligand to be recognized, these molecules can be intracellular or expressed on the cell surface. Once microbial pattern recognition or specific receptor engagement occurs, epithelial cells respond by generating a wide range of defense molecules. These include direct antimicrobials, as well as molecules that serve to recruit and activate inflammatory cells that contribute to host defense. Finally, in this section, a major area of new investigation is the ability of the epithelium to play a major role in immunoregulation, in particular to provide an important link between innate and specific immunity.

The past decade or so also has seen marked improvements in our understanding both of the interactions of specific inhaled stimuli with the epithelium, and of the consequences of such interactions on airway function. The next set of chapters, therefore, deal with the interaction of four major classes of inhaled stimuli that affect epithelial function. Respiratory viruses not only cause upper airway diseases but also play a major role in triggering exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Such effects are initiated via interactions with the epithelium. Similarly, epithelial responses to bacteria play a major pathogenic role in diseases from pneumonia, to cystic fibrosis to COPD. In our modern environment, pollutants are major exacerbators of a range of airway diseases. Finally, while the interactions of allergens with cells such as mast cells, basophils and lymphocytes obviously play a major role in allergic diseases, a growing body of literature demonstrates that interactions of allergens, particularly those with endogenous proteolytic activity, with the epithelium not only contribute to direct inflammatory effects but also play a critical role in permitting access of allergens to target cells in the underlying airway tissue.

There is now no doubt that the epithelial cell plays a major role in regulating the inflammatory and structural status of the airway. The epithelium has wide ranging synthetic and metabolic capacities. It can maintain normal airway status via its ability to inhibit or degrade a range of proinflammatory molecules but, upon repeated exposure to stimuli, can also generate a wide range of mediators that can contribute to, and exacerbate, chronic airway inflammation. Recurrent epithelial damage and repair can also cause repeated interactions between the epithelium and other structural cells, such as fibroblasts/myofibroblasts, leading to chronic reactivation of the so-called “epithelial mesenchymal trophic unit”. This can lead to marked structural changes in the airway, such as the hallmark changes in asthma collectively referred to as airway remodeling.

The final set of chapters deals with the interactions of inhaled medications with the epithelium. Given the wide ranging properties discussed above, and the alterations of epithelial function in airway diseases, several of the beneficial actions of inhaled medications, including glucocorticoids, β2-adrenergic agonists and muscarinic receptor antagonists, in diseases such as asthma and COPD may well be mediated via alterations of epithelial cell function. Last, but not least, there is growing interest in inhaled delivery of drugs, not only as a means to exert local effects in the lung, but also as a means of systemic delivery for drugs, particularly those that cannot survive oral delivery. Preserving the molecular integrity of a formulation and delivering it to the appropriate target in the lung are critical for effective therapy, and some of the recent advances in this regard are discussed in the final chapter.
Each of the chapters in this text were written by leaders in their field. Production of a text of this comprehensive nature would not have been possible without their commitment. I would like to take this opportunity to extend my sincere thanks to all of the contributors for devoting their valuable time and expertise to this volume.

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1 Pulmonary Epithelium: Cell Types and Functions

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1.1 Introduction

The pulmonary airway tree branches in a dichotomous fashion, with repeated bifurcation stemming from the trachea. The conducting airway include the regions that do not undergo gas exchange, beginning with the trachea, which divides into two bronchi. These primary airway then branch into a series of intra-pulmonary bronchial and bronchiolar airway. Both the diameter and the length of each airway branch decrease progressively from the trachea to the periphery, where the terminal bronchioles are the most distal conducting airway (Magno and Fishman, 1982). In rodents, these bronchioles lead directly to alveolar ducts, whereas in humans and monkeys, a region of transitional respiratory bronchioles with characteristics of both bronchioles and alveoli exists between the bronchioles and the alveoli of the gas exchange area (Tyler, 1983).

The entire pulmonary tree is lined by a continuous layer of epithelial cells. The relative distribution and abundance of the epithelial cell types vary significantly, not only between species, but also within the various airway regions of each species. The pulmonary epithelium is important for maintaining the normal functions of the respiratory system, which include acting as a barrier to various insults (Widdicombe, 1987b); facilitating mucociliary clearance (Sleigh et al., 1988); secreting substances such as surfactant proteins, mucus, and antimicrobial peptides for airway surface protection (Widdicombe, 1987a); repairing and regenerating epithelial cells to restore normal airway function (Evans et al., 1976); and modulating the response of other airway components, such as airway smooth muscle cells and inflammatory cells (Flavahan et al., 1985; Holtzman et al., 1983, Breeze and Wheeldon, 1977). As many as 49 cell types have been recognized (Breeze and Wheeldon, 1977). While many of these are intermediate or differentiating cells, at least 10 to 12 morphologically and functionally unique epithelial cell types can be identified throughout the pulmonary structure (Breeze and...
Wheeldon, 1977). They are: long and small ciliated, basal, non-ciliated secretory (goblet, Clara, surface serous, submucosal serous, and submucosal mucous), pulmonary neuroendocrine (PNE), brush, and alveolar type I and type II cell types (Figure 1.1). It is important to differentiate between these cell types, as well as to highlight the often significant species differences that may limit the experimental comparisons between various animal models and human subjects. In this chapter, we will attempt to address both of these issues while focusing on a few main mammalian systems – human, monkey, rabbit, rat, and mouse.

The mature mammalian airway can be divided by function and structure into three regions: (1) the cartilaginous proximal airway, comprising the trachea, bronchi and submucosal glands; (2) the non-cartilaginous distal bronchioles, comprising the bronchioles, terminal bronchioles, transitional bronchioles, and respiratory bronchioles; and (3) the gas exchange region, comprising the alveolar ducts and alveolar sacs. For each region, we will discuss its epithelial makeup, the characteristic features and physiological functions of each cell type present, any known variations between species, and the role of stem and progenitor cell populations.

1.2 Epithelial cell types and functions in the cartilaginous proximal airway region

The epithelial cells of the proximal airway can be broadly separated into the surface epithelial cells of the tracheal and bronchial regions and the cells of the submucosal glands. We will first address the cell types of the tracheal and bronchial epithelium.
1.2 THE CARTILAGINOUS PROXIMAL AIRWAY REGION

The epithelial cells lining the luminal surface of the proximal airway can be further grouped into ciliated cells, non-ciliated secretory cells, and basal cells. A characteristic pseudostratified two-layered epithelium persists throughout the major bronchi, while a multi-layered structure is seen in the more distal, narrow bronchi, which have fewer cartilage rings and more submucosal glands. Ciliated cells and secretory cells attach to the basal lamina via desmosome adhesions and to one another via tight junctions at the luminal surface. The underlying basal cells lie in contact with most of the basal membrane (Breeze and Wheeldon, 1977; Jeffery, 1983). Pulmonary neuroendocrine cells (PNECs) are found as single cells or in clusters throughout the proximal airway. In small animals, they are more prominent at the laryngotracheal junction and the bifurcations of intrapulmonary bronchi (Tateishi, 1973), while in humans, the PNECs are more frequently found in the smaller conducting airway (Johnson et al., 1982). Tracheas and bronchi from various animals reveal species-specific epithelial cell linings (Jeffery, 1983; Plopper et al., 1983c), with the most striking variations in the distribution of secretory cells (Plopper et al., 1983d).

Unique to the proximal cartilaginous airway is the existence of submucosal glands (SMGs). These glands are contiguous with the surface epithelium and are characterized by a variable proportion of ciliated cells, mucous cells and serous cells (De Poitiers et al., 1980). In contrast to human and monkey airway, where submucosal glands are the major secretory structure of the trachea and bronchi, SMGs in rats and mice are very scarce and limited to the upper trachea (Plopper et al., 1986; Widdicombe et al., 2001).

1.2.1 Surface epithelial cell types and functions in tracheal and bronchial regions

Ciliated cells

Ciliated cells are covered with cilia and are roughly columnar in shape, with little variation in morphological appearance between species. Ciliated cells are attached to the basal lamina via desmosomes and extend to the luminal surface, where they are interconnected via tight junctions (Rhodin, 1966). The cytoplasm of these cells is relatively electron-lucent due to their lack of secretory products or mucus granules. Many mitochondria are found in the apical region of the cell, just below the row of basal bodies to which the cilia are attached. Approximately 200–300 cilia are found on the luminal surface of each cell, with approximately half as many microvilli and fine cytoplasmic processes interspersed among them (Watson and Brinkman, 1964). In humans, the cilia are 0.25 micrometres in diameter and range from 6 micrometres in length in the proximal airway to 3.6 micrometres in seventh generation airway (Serafini and Michaelson, 1977). Their structure is comparable to that of other ciliated epithelia in plants and animals. Each cilium is anchored to the cell cytoplasm by a basal body through an axoneme. The axoneme is composed of nine microtubule doublets that formed an outer ring around a central pair of microtubules, with nexin links and radial spokes binding them together (see Chapter 6). Along each outer microtubule there are extrusions referred to as outer dynein arms (odas) and inner dynein arms (idas), both members of the dynein ATPase superfamily. Odas control the cilia beating frequency through a cAMP-dependent phosphorylation mechanism (Satir, 1999), while idas control the form of cilia beating (Brokaw and Kamiya, 1987; Friedmann and Bird, 1971). Mucociliary clearance is the major function of ciliated cells. Cilia are bathed in the watery sol phase of airway secretions and extend into the gel phase, where specialized barb-like
structures on the tips of the cilia alternatively grab and release the mucus during the active and relaxation strokes of cilia beating, thereby propelling the mucus with a rowing-like action (Jeffery and Reid, 1975).

**Proliferation potential** Traditionally, ciliated epithelial cells were considered to be terminally differentiated cells that did not divide, presumably originating from either basal or secretory cells (Inayama et al., 1989; Johnson and Hubbs, 1990). Recent reports, however, have suggested the involvement of ciliated cells in the restoration and regeneration of bronchiolar epithelium (Lawson et al., 2002; Park et al., 2006b). In the naphthalene injury model, Park et al. (2006b) demonstrated that ciliated cells sequentially undergo morphological transitions from squamous to cuboidal to columnar forms as the bronchiolar epithelium is restored, showing remarkable plasticity and differentiation potential. Lawson et al. (2002) also concluded that ciliated cells play a critical role in the repair of distal airway injury. Tyner et al. (2006) recently demonstrated the transdifferentiation of ciliated cells to mucous (goblet) cell metaplasia in allergic mouse airway. This transdifferentiation depends on IL-13 expression and a persistent EGFR signalling. This result further supports the theory of plasticity of ciliated airway epithelial cells. Further study is needed with isolated ciliated cells to reaffirm such a potential.

**Basal cells**

The ovoid basal cells form a monolayer along the basement membrane and are responsible for the pseudostratified appearance of the epithelium. Basal cells have large, indented nuclei that fill most of the cell. The cytoplasm contains many ribosomes, a small Golgi zone, a few mitochondria glycogen granules, a short profile of rough surface endoplasmic reticulum, and occasionally lysozymes. Basal cells are connected to the basement membrane through hemidesmosomes and provide the foundation for the attachment of ciliated and non-ciliated columnar cells to the basal lamina (Frasca et al., 1968; Breeze and Wheeldon, 1977; Rhodin, 1966). Due to their centrally located position, basal cells not only play a role in the attachment of columnar epithelium to the basement membrane, but also have the potential to function as a regulator of inflammatory response, transepithelial water movement, and oxidant defence (Evans et al., 2001).

**Proliferation and stem cell potential** One important feature of basal cells is their capacity to repopulate all the major epithelial cell types found in the trachea, including basal, ciliated, goblet and granular secretory cells (Hong et al., 2004b, 2004a; Inayama et al., 1988). Many studies have demonstrated the potential of basal cells to act in a stem cell or transient amplifying cell capacity in the upper airway. A study of 50 human bronchial biopsies with immunohistochemical staining against the proliferation agent Ki-67 revealed a population of cells that were positive for Clara cell secretory protein (CCSP) but showed no other Clara cell-specific features. This population turned out to be Ki-67 antibody-negative, but the CCSP-negative basal cells were candidate stem cells of the bronchial specimen (Barth et al., 2000). In another study of human trachea and bronchi using the same immunohistochemical staining, basal cells and parabasal cells composed large percentages – 51 and 33 per cent, respectively – of the proliferating compartment (Boers et al., 1998). Parabasal cells are located just above the basal cells and considered to be intermediate cells. The high representation of basal and parabasal cells within the proliferation compartment of normal human conducting-airway
epithelium supports the theory that cells at or near the basement membrane are likely to be the progenitor cells or transient amplifying cells of the airway surface (Hajj et al., 2007). In the mouse trachea, a subset of cells with high keratin 5 (K5) promoter activity residing in the submucosal gland were found to be bromodeoxyuridine label-retaining cells (LRC), which are regarded as stem cells due to their long-lasting proliferation capacity (Borthwick et al., 2001). Hong et al. (2004a) demonstrated that CCSP-expressing (CE) cells play a critical role in the renewal of bronchiolar airway. They suggested, however, that in the absence of Clara cells, basal cells may serve as secondary progenitor cells in the upper airway. Using chemically-injured mice with Clara cell ablation, they found that the cytokeratin-14 expressing basal cells were capable of restoring normal bronchial epithelium and suggested that basal cells may serve as an alternative multipotent progenitor cell in the bronchial airway (Hong et al., 2004b). Debate about the role of basal cells as the primary progenitors in the upper airway continues, especially since several animal injury models have shown that secretory cells, rather than basal cells, exhibit hyperproliferation after mechanical or toxic gas exposure (Johnson et al., 1990; Evans et al., 1989, Basbaum and Jany, 1990).

Non-ciliated secretory cells

The most striking interspecies difference in tracheobronchial epithelial cell types is in the distribution of non-ciliated secretory cells. In humans, ciliated cells predominate and are interspersed with mucus-secreting (goblet) cells, with approximately five ciliated cells for every goblet cell (Rhodin, 1966; Frasca et al., 1968). The goblet cells become less frequent in the bronchioles, as the airway becomes smaller and ciliated and Clara cells prevail (Lumsden et al., 1984). The major secretory cell type in sheep, monkeys, and cats is either the mucous goblet cell or the small mucous granule cell (Mariassy et al., 1988a; Plopper et al., 1989). In rats, the predominant secretory cell is the serous cell, whereas in rabbits and mice, the Clara cell is the only type of secretory cell in the entire conducting airway (Plopper et al., 1983a). In addition to the secretory cells of the surface epithelia, many major secretory cell types are found in the submucosal glands and will be discussed separately.

Goblet cells

Goblet cells have a relatively dense, electron-opaque cytoplasm due to the numerous mucous granules located in the apical region of the cytoplasm. The nucleus is generally compressed at the cell’s basal side. The mucous granules give the cell its typical goblet shape, with a wide, enlarged apical portion and a narrow tapered basal cytoplasm. The granules in human goblet cells are electron-lucent, approximately 800 nanometres in diameter, and usually contain mucins that are acidic due to the presence of sulfate or sialic acid (Lamb and Reid, 1969; Spicer et al., 1971, Mariassy et al., 1988b).

Under healthy conditions, goblet cells, along with submucosal glands, secrete high molecular weight mucous glycoproteins that allow the surface fluid to properly trap and remove particles, thus protecting the epithelial surface. Proper regulation of mucin secretion at the airway surface is crucial to normal functioning, as overproduction can clog the airway and underproduction can impair mucociliary clearance.

Goblet (mucous) cell metaplasia in lung disease

Goblet cell hyperplasia or metaplasia is a common phenomenon associated with airway inflammatory diseases, including asthma,
COPD (chronic obstructive pulmonary disease), and chronic bronchitis (Vestbo et al., 1996; Aikawa et al., 1992; Fahy, 2002; Groneberg et al., 2002). Goblet (or mucous) cell hyperplasia usually refers to an increase in goblet cells in the airway regions where goblet cells exist normally, such as the proximal airway of humans. Goblet (mucous) cell metaplasia, on the other hand, refers to an increase in goblet (mucous) cells in airway regions that normally contain few or no goblet cells, such as in mouse or rat airway. Both cases result in increased mucin secretion at the airway surface, thus compromising airway functions. Adler and colleagues revealed that myristoylated alanine-rich C kinase (MARCKS) is a key molecule regulating mucin exocytosis, a process also involving cooperative interaction between protein kinase C (PKC) and PKG (Park et al., 2006a; Singer et al., 2004). The use of a therapeutic agent developed in conjunction with this study may be a means of controlling mucus secretion. Using transgenic mice and an OVA-sensitized murine model, investigators have linked Th2 cytokine-mediated inflammation to goblet cell metaplasia based on studies involving IL-4, IL-9, and IL-13 (Temann et al., 1997; Kuperman et al., 2002; Vogel, 1998; Wills-Karp et al., 1998). Among these Th2 cytokines, IL-13 was shown to be the most potent. Studies of mice with intratracheal IL-13 instillation consistently showed increased goblet cells in the mouse airway. Additionally, goblet cell metaplasia induced by CD4 T cells and IL-9 was shown to be stimulated through a common IL-13 mediated pathway (Whittaker et al., 2002). Despite these findings, evidence to support IL-13 as the direct mediator of the expression of gel-forming mucin by goblet (mucous) cells is still lacking. In vivo studies may be complicated by the presence of cytokine networks and the inflammatory response upon the administration of cytokines, while in vitro studies may provide a more direct measurement of the effects of cytokines on airway epithelial cell types. Chen et al. (2003) have shown that IL-13 and various Th2 cytokines have no stimulatory effects on either MUC5AC or MUC5B expression in well-differentiated human airway epithelial cultures, while IL-6 and IL-17 can directly stimulate mucin gene expression. This data suggests that the transformation of airway epithelial cells into goblet cells may be a multi-step process that is controlled by different sets of cytokines.

Clara cells

For large animals such as sheep, monkeys and humans, Clara cells are concentrated in the distal conducting airway and bronchioles, while in hamsters, rabbits, and mice, the predominant non-ciliated cells throughout the entire conducting airway have the same ultrastructure features as Clara cells (Plopper et al., 1987; Matulionis, 1972, Jeffery and Reid, 1975). A detailed discussion of Clara cells will be presented in section 1.3, ‘Epithelial cell types and functions of the non-cartilaginous distal bronchioles’.

Surface serous cells

Serous cells on the surface airway epithelium morphologically resemble the serous cell type of the submucosal gland. They are the predominant secretory cells in rat surface epithelium (Jeffery and Reid, 1975) and have also been found sporadically in human small bronchi and bronchioles (Jeffery, 1983). In contrast to goblet and mucous cells, they have discrete electron-dense granules in the apical cytoplasm that are approximately 600 nanometres in diameter and contain neutral mucin. A detailed description of serous cell function is presented in section 1.2.2 ‘Epithelial cell types and functions in the submucosal glands’.
Pulmonary neuroendocrine cells (PNECs)

PNECs are found throughout the conducting airway of most species. They exist either individually or in clusters as neuroendocrine bodies (NEBs). In the rabbit, the NEB is a large intraepithelial organoid that is composed almost exclusively of PNECs. In other species, such as the rat, PNECs in the NEB are interspersed with Clara-like cells (Scheuermann, 1987; Sorokin et al., 1989; Sorokin and Hoyt, 1982). The number of PNECs and NEBs increase from the main bronchi to the terminal bronchioles, with denser populations found around bifurcating regions, such as the bronchioalveolar portals and various airway branching points (Hoyt et al., 1982a, 1982b). Mature PNECs are spindle-shaped, with their basal surface facing the basement membrane and a thin apical process extending toward the epithelial surface (Hage, 1980). The most prominent feature of these cells is the presence of abundant argentophilic vesicles with granular cores concentrated at the base of the cells (Hage, 1980; Capella et al., 1978). As a result, PNEC secretion is polarized and directed toward adjacent cells or structures underlying the basement membrane (Hoyt et al., 1982a). The secretory products of the granules vary between different species and have been immunocytochemically identified as bioactive amines and peptides, including serotonin, calcitonin, gastrin-releasing peptide (GRP), calcitonin gene-related peptide (CGRP), chromogranin A, and cholecystokinin (Becker et al., 1980; Wharton et al., 1978; Sunday et al., 1988; Cadieux et al., 1986; Siros and Cadieux, 1986). The two best-characterized peptides are GRP and the mammalian form of bombesin, CGRP. These peptides, which exert direct mitogenic effects on epithelial cells and exhibit many growth factor-like properties, are thought to be involved in normal fetal lung development, including branching morphogenesis (Li et al., 1994). Additionally, NEBs may play a role as hypoxia-sensitive airway chemoreceptors (Lauweryns and Cokelaere, 1973; Lauweryns et al., 1983) and are involved in regulating localized epithelial cell growth and regeneration (Reynolds et al., 2000b).

Proliferation potential

PNECs are generally believed to be terminally differentiated and mitotically inert cells (Gosney, 1997). Sunday and his colleague (Sunday and Willett, 1992), however, suggested that PNEC hyperplasia in the hamster model is a result of the differentiation from proliferative stem cells or from immature PNECs. Others showed that repair from airway injury is associated with PNEC hyperplasia and that proliferation contributes to this hyperplastic response (Ito et al., 1994; Stevens et al., 1997). A study investigating the role of PNEC-derived neuropeptides in lung development suggested that PNECs are involved in the regulation of epithelial renewal (Pan et al., 2002). Further evidence for this theory is found in the inverse relationship between the epithelial mitotic index at each epithelial location and its distance from the closest NEB (Holt et al., 1990). Recently, several studies have demonstrated that NEBs provide a microenvironment for progenitor cells in the adult airway by showing that the NEB niche of normal and injured lungs supports the maintenance of at least two epithelial cell variants – one with an intermediate phenotype between Clara and PNEC cells, and the other with a Clara cell variant with little or no immuno-reactive CYP-2F2 protein (Reynolds et al., 2000b, 2000a). Further studies using the same naphthalene injury model demonstrated that PNECs are not stem or progenitor cells in the distal airway. Rather, they provide a niche that regulates the expansion of the CCSP-expressing stem cell population in mouse distal airway (Hong et al., 2001).
**Brush cells**

Brush cells are named for the closely packed microvilli that protrude like a brush from their luminal surface. Although they have been identified throughout the conducting airway of many species, their presence is infrequent and has not been convincingly shown in humans (Meyrick and Reid, 1968; Jeffery and Reid, 1975). While their function is not well-defined, some speculated functions include roles in periciliary fluid absorption (Jeffery, 1987), chemoreception (Luciano et al., 1968) and ciliogenesis (Rhodin and Dalhamn, 1956).

### 1.2.2 Epithelial cell types and functions in the submucosal glands

Submucosal glands are found in the upper airway of higher mammals such as humans, monkeys and sheep (Goco et al., 1963; Choi et al., 2000). They occur at a frequency of approximately one gland per square millimetre in the trachea of healthy humans and are abundant down to about the tenth generation bronchiole (Ballard et al., 1995). In small animals such as hamsters, rats and mice, submucosal glands are infrequently expressed and exist only in the uppermost portion of the trachea (Borthwick et al., 1999; Widdicombe et al., 2001).

Each submucosal gland consists of multiple tubules that feed into a collecting duct, which narrows into a ciliated duct that is continuous with the airway surface (Meyrick et al., 1969). The tubules may be inter-connecting and are lined with mucous cells in their proximal regions and serous cells in the distal acini (Meyrick et al., 1969). The secretory products of these two cell types are essential for proper airway mucociliary clearance. In fact, malfunctioning of serous and mucous cells may be the primary cause of many airway diseases, including chronic bronchitis, asthma, and cystic fibrosis (Salinas et al., 2005; Rogers, 2004; Knowles and Boucher, 2002).

**Serous gland cells**

Like surface serous cells, serous gland cells are pyramidal in shape, with electron-dense secretory granules in the apical region and a basally-located nucleus. The mitochondria are long and ovoid and are concentrated in the base of the cell, with a few found among the secretory granules. While most of the rough endoplasmic reticulum is at the cell base, free ribosomes are abundant throughout the cytoplasm. The Golgi apparatus is well-developed and supranuclear, often with dilated lamellae and many associated vesicles. Multivesicular bodies are also seen occasionally. Osmiophilic material is organized either into an irregularly shaped body or an irregular dense region within an electron-dense secretory granule. A large pale secretory granule containing focal condensations of osmiophilic material surrounded by a membrane is found in the apical half of most serous cells (Meyrick and Reid, 1970). Serous cells have been described as ‘immobilized neutrophils’ due to their role in the secretion of water, electrolytes, and compounds with antimicrobial, anti-inflammatory, and antioxidant properties (Basbaum et al., 1990). Serous cells are the predominant sites of cystic fibrosis transmembrane regulator (CFTR) expression in the human bronchus (Engelhardt et al., 1992a). Located distal to mucous cells, they facilitate mucociliary transport by helping remove the mucous glycoprotein produced by submucosal gland mucous cells and maintaining the airway surface liquid (ASL) volume (Inglis et al., 1997). CFTR malfunction in the serous cells can result in defective mucus clearance, which has been implicated as the
primary cause of cystic fibrosis (CF) disease (Knowles and Boucher, 2002; Joo et al., 2002; Yamaya et al., 1991).

**Mucous gland cells**

Like the surface goblet cells of the surface epithelium, mucous cells of the submucosal gland are columnar in shape, with a basally-located nucleus. The rest of the cell is packed with secretory granules of moderate electron density (Meyrick and Reid, 1970). The major function of mucous cells is to secrete mucin in the form of the mucous glycoprotein MUC5B, which is different from the MUC5AC produced by surface goblet cells (see Chapter 7). Together, these glycoproteins make up the gel phase on the apical surface of airway epithelial cells. As previously discussed in conjunction with the goblet cell, overproduction of MUC5AC and MUC5B is a common phenomenon in asthma, COPD and chronic bronchitis (Rogers, 2004, 2000; Rose et al., 2001).

**Stem cell niche at or near submucosal glands** Aside from playing a significant role in airway diseases, the submucosal gland may also provide the microenvironment for a subset of stem cells in the upper airway. Randel et al. discovered a high keratin-expressing subpopulation of cells residing in the submucosal gland ducts of murine trachea that were co-localized with label-retaining cells (LRCs). In mice 95 days post-injury, LRCs were localized to the gland ducts in the upper trachea and to systematically arrayed foci in the lower trachea, especially at the cartilage–intercartilage junction (Borthwick et al., 2001). This suggests that the submucosal gland may provide a protective niche for stem cells (Engelhardt, 2001; Borthwick et al., 2001).

**1.3 Epithelial cell types and functions of the non-cartilaginous distal bronchioles**

In most small laboratory animals such as rats, hamsters and mice, the distal bronchioles consist of several generations of non-alveolized bronchioles and a single, short alveolized bronchiole that connects to the alveolar duct. The lining epithelium is composed of simple cuboidal cells, with approximately equal numbers of ciliated cells and non-ciliated Clara cells (Widdicombe and Pack, 1982; Plopper et al., 1983b). In higher mammals such as humans and monkeys, however, there are several generations of both non-alveolized and alveolized (respiratory) bronchioles (Castleman et al., 1975; Tyler, 1983). The non-alveolized bronchioles are lined with ciliated cells and non-ciliated secretory cells, while the alveolized bronchioles are scattered with alveolar type I and type II cells amongst simple cuboidal cells.

**Clara cells**

Although there are significant inter- and intra-species variations in their ultrastructural characteristics, Clara cells are generally ovoid or columnar in shape, with a centrally-located nucleus, prominent Golgi, and abundant organelles including agranular and granular endoplasmic reticulum. Their most prominent features are the membrane-bound electron-dense secretory granules. While the granules do not contain glycoprotein, Clara cells are metabolically active. CC10 (or CCSP) is a secreted protein homologous to uteroglobin
that may be important in regulating the inflammatory response and is used as a Clara cell marker (Plopper et al., 1980c, 1980a, 1980b; Widdicombe and Pack, 1982; Singh et al., 1990). The surfactant protein SP-B is another secretory product of Clara cells that may be involved in host defence activity (Phelps and Floros, 1991). These cells also produce proteins with inhibitory effects on proteases; one such example is the antileukoproteases found on the surface of human airway (Simionescu and Simionescu, 1983; Yoneda and Walzer, 1984). Furthermore, Clara cells have the capacity to metabolize xenobiotics through their cytochrome p450 monoxygenase activity, a function that renders them susceptible to injury by lipophilic compounds (Baron et al., 1988).

**Stem cell niche at the bronchioalveolar region**  The most important property of Clara cells is their ability to act as stem cells. Clara cells have long been considered to be progenitor cells for the terminal bronchioles (Evans et al., 1976, 1978). Repopulation studies of specific epithelial cell types in vitro and in vivo suggested that basal cells and bronchiolar Clara cells have stem and progenitor cell capabilities in the regeneration of the trachea, bronchi, and bronchioles (Nettesheim et al., 1990). In the study of normal human lungs obtained from autopsy, triple sequential histochemical staining was used to elucidate the contribution of Clara cells to the proliferation compartment. Using MIB-1 as a proliferation marker, anti-CC10 for the identification of Clara cells, and a PAS stain marker for goblet cells, Clara cells were found to be absent in the proximal airway epithelium, while their contribution to the proliferation compartment in the respiratory bronchioles was 44 per cent. This demonstrated that Clara cells play an important role in the normal maintenance of the human distal conducting airway epithelium (Boers et al., 1999). Recent studies using naphthalene-injured mice have suggested that a subset of naphthalene-resistant Clara cells in the bronchiolar epithelium acts as a stem cell population. In mice whose Clara cells were ablated by naphthalene, a population of variant Clara cells that were cytochrome p450 2F2 negative and resided in discrete pools associated with neuroepithelial bodies (NEBs) were found to exhibit multipotent differentiation and to regenerate the bronchiolar epithelium (Reynolds et al., 2000a, 2000b). The associated neuroendocrine cells are thought to provide a niche that regulates the expansion of Clara cell secretory protein (CCSP)-expressing cells (Hong et al., 2001). In a study searching for cells contributing to the renewal of terminal bronchioles after Clara cell depletion in mice, CCSP-expressing cells that were localized to the bronchioalveolar duct junction (BADJ) were also identified as the predominant proliferative population in initial terminal bronchiolar repair. These cells included a population of label-retaining cells, characteristic of a stem cell population. Furthermore, immunohistochemical co-localization studies involving CCSP and the NEB-specific marker, calcitonin gene-related peptide, indicate that BADJ-associated CCSP-expressing stem cells function independently of NEB microenvironments. These studies identify a BADJ-associated, NEB-independent, CCSP-expressing stem cell population in terminal bronchioles and support the theory that region-specific stem cell niches exist to maintain epithelial diversity after injury (Giangreco et al., 2002). Identified at the bronchioalveolar duct junction, bronchioalveolar stem cells (BASCs) retain characteristics of regional stem cells such as LRC accumulation, self-renewal, and multipotency in clonal assays. BASCs are believed to maintain the Clara cell and alveolar cell populations in the distal airway. Interestingly, Clara cells and alveolar cells of the distal lung and their transformed counterparts give rise to adenocarcinoma. This work also points to BASCs as the putative origin cells for this subtype of lung cancer (Kim et al., 2005).