Airway mucosa: secretory cells, mucus and mucin genes

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ABSTRACT: The airway mucosa is lined by a continuous epithelium comprised of multiple cell phenotypes, several of which are secretory. Secretions produced by these cells mix with a variety of macromolecules, ions and water to form a respiratory tract fluid that protects the more distal airways and alveoli from injury and infection. The present article highlights the structure of the mucosa, particularly its secretory cells, gives a synopsis of the structure of mucus, and provides new information on the localization of mucin (MUC) genes that determine the peptide sequence of the protein backbone of the glycoproteins, which are a major component of mucus.

Airway secretory cells comprise the mucous, serous, Clara and dense-core granulated cells of the surface epithelium, and the mucous and serous acinar cells of the submucosal glands. Several transitional phenotypes may be found, especially during irritation or disease. Respiratory tract mucins constitute a heterogeneous group of high molecular weight, polydisperse richly glycosylated molecules: both secreted and membrane-associated forms of mucin are found.

Several mucin (MUC) genes encoding the protein core of mucin have been identified. We demonstrate the localization of MUC gene expression to a number of distinct cell types and their upregulation both in response to experimentally administered lipopolysaccharide and cystic fibrosis.

The epithelium lining the airways of the normal mammalian lung consists of many morphologically distinct cell types with different, but sometimes overlapping, functions. In disease, the integrity of the epithelium may be compromised, such as in symptomatic asthma [1]. In bronchitis, there are increased numbers of surface mucus-secreting cells and enlargement of submucosal glands in bronchi, and mucous metaplasia in bronchioles [2]. In cystic fibrosis, altered movement of ions and macromolecules through and between cells can lead to defective transepithelial flux of water and drying of airway secretions [3].

The airway wall comprises epithelial, lymphoid, muscular, vascular and nervous elements interspersed in a pliable connective tissue support, arranged as: 1) a lining mucosa of surface epithelium supported by a reticular basement membrane and a poorly-defined elastic lamina propria (or subepithelium), in which there are bronchial blood vessels, nerve bundles and free cells (including fibroblasts and mononuclear cells); 2) a submucosa, in which lie the bulk of the mucus-secreting glands, muscle and cartilage plates; and 3) a relatively thin adventitial coat.

Surface epithelium and secretory cells

The airway epithelium includes the surface epithelium, which lines all airways (nose to alveolus) and which is continuous with that forming the tubulo-acinar submucosal mucus-secreting glands, which develop from the surface [4, 5]. The stratified squamous epithelium lining of much of the larynx gives way to one which is pseudostratified, ciliated and columnar when the trachea is reached. Where it is "pseudostratified", all cells rest on the basement membrane, but not all reach the airway lumen (fig. 1). In humans, this type of epithelium persists throughout the major bronchi, becoming simple cuboidal towards the periphery. Ciliated cells...
predominate, interspersed with mucus-secreting (goblet) cells, which are regularly found in the tracheobronchial tree (fig. 2) but rarely in bronchioles of less than 1 mm diameter [6].

A variety of cell types is recognized in airway surface epithelium: at least eight different epithelial cell types have now been delineated, depending on species [7, 8]. In addition, cells involved in the immune response and its reactions may migrate through the epithelial reticular basement membrane: some of these remain within the surface epithelium, whereas others are in the process of passing through to the luminal surface [9]. The terminal processes of sensory nerve fibres, whose cell bodies lie deep beneath the epithelium, pierce the epithelial basement membrane and lie surrounded by epithelial cells, where they initiate airway reflexes, such as bronchoconstriction and cough [10]. These endings may be inappropriately stimulated in conditions such as asthma and chronic bronchitis. Secretions produced by airway epithelial cells mix with a variety of macromolecules, ions and water, whose passage from vascular compartment to airway lumen is largely controlled by the epithelium itself (this will be the subject of a forthcoming article in this series, by J. Widdicombe). Thus, respiratory tract fluid comprises a mixture of mucous-glycoproteins, glycosaminoglycans, proteins/peptides, lipids, antiproteases/antioxidants, ions and water, whose origin is both from epithelial and vascular sources. The secretory cells will now be considered in more detail.

**Mucous cell**

In human trachea, the normal mean density of surface mucous cells (fig. 2) is estimated at 6,000–7,000 cells·mm⁻² surface epithelium [11]. By electron microscopy, the mucous cell has electron-dense cytoplasm containing electron-lucent, confluent granules of about 800 nm diameter (fig. 3). Most contain high molecular weight glycoprotein, which is acidic due to sialic acid or sulphate groups located at the ends of the oligosaccharide side-chains, which branch from a backbone of protein [12]. Secretion of the correct amount of mucus with an optimum viscoelastic profile is important in the maintenance of normal mucociliary clearance [13]. Alterations in the predominant histochemical type of mucus have been associated with airway irritation, carcinogenesis, chronic bronchitis and cystic fibrosis (see below), but no novel glycoprotein moiety has been identified. The numbers of mucus-secreting cells increase in chronic bronchitis and, experimentally, in animal models of bronchitis, following inhalation of sulphur dioxide [14], tobacco smoke [15, 16], or intratracheal instillation of endotoxin (lipopolysaccharide (LPS)) [17, 18]. Their increase in number and extension to the peripheral bronchioles is one characteristic of small airways disease [19]. The mucous cell is clearly capable of division and may show stem cell multipotentiality [20].

The solubility and viscosity of mucus varies considerably with ionic strength, and divalent cations, such as calcium, cause mucus to form a rigid cross-linked gel, which may be difficult to clear by mucociliary action or cough. In cystic fibrosis (CF), high calcium and sulphate content is reported in tracheobronchial secretions [21]. In biopsy studies, mucous cells from patients with CF have been shown to contain significantly raised intracellular calcium and sulphate levels and lower potassium levels than those of patients with chronic bronchitis [22]. The clinical significance of these results is as yet unclear. In asthma, there is controversy as to the extent of goblet cell hyperplasia in bronchi and metaplasia in bronchioles. The blockage of airways by highly tenacious plugs in fatal asthma appears to be the result of a mixture of inflammatory exudate, fibrin and epithelial-derived mucins.
Serous cell

Serous cells have electron-dense cytoplasm, much rough endoplasmic reticulum and, in contrast to mucous cells, discrete electron-dense granules of about 600 nm diameter (fig. 4). Morphologically, serous cells of the surface epithelium resemble those present in the submucosal glands. They have been described in surface epithelium only in the rat, cat, young hamster and human foetus [23]. They have also been described in human small bronchi and bronchioles [24]. Many contain neutral mucin, and there is evidence that some may also contain a nonmucoid substance, probably lipid [25].

Clara (nonciliated bronchiolar) cell

Clara cells in humans are restricted in location to the terminal bronchioles, where they typically bulge into the airway lumen and contain electron-dense granules of about 500–600 nm diameter, ovoid in humans but irregular in most other species [26] (fig. 5). The function(s) of this cell type is as yet unclear. It has the capacity to metabolize xenobiotics, may produce a carbohydrate (hypophase) component of surfactant [27], or an antiprotease [28, 29], and is known to have ion-absorbing and secreting properties [30]. Furthermore, the Clara cell acts as the stem cell of small airways, where basal and mucous cells are normally sparse: both ciliated and mucous cells may develop from the Clara cell subsequent to its division and differentiation [20].

Dense-core granulated (DCG) cell (synonyms: endocrine, Kulchitsky and Feyrter cell)

Argentaffin-positive and argyrophilic cells have been identified within the surface epithelium by light microscopy. By electron microscopy, DCG cells are infrequently found, generally basal in position, but often with a thin cytoplasmic projection reaching the airway lumen [19, 31]. Single cells and clusters of such cells may also be associated with nerve fibres (i.e. so-called neuroepithelial bodies or neurite-receptor complexes) [19, 32]. The cytoplasm of DCG cells usually contains large numbers of small (70–150 nm) spherical granules, each with an electron-dense core surrounded by an electron-lucent halo (fig. 6). Granule subtypes have been described and the cells may contain biogenic amines [33] or peptides, such as bombesin [34], which, when released, may influence vascular and bronchial smooth muscle tone, mucous secretion and ciliary activity. The location of the cell...
in surface epithelium and its cytoplasmic content make it a prime candidate for sensing hypoxia in the airway lumen: as a consequence, vasoactive substances may be released, which cause local vasoconstriction and shunting of blood to better ventilated zones of the lung.

Transitional cells

Abnormal epithelium may include a number of cells, each of which shows features transitional to two or more morphologically well-defined cell types [19], for example:

Serous-mucous cells may be found rarely in normal specific-pathogen free (SPF) rats, but are frequent in rats made "bronchitic" by inhalation of cigarette smoke. They may also be found in areas of grossly normal human epithelium in lungs resected for carcinoma. The cells contain secretory granules of the mucous type, with electron-dense cores resembling serous granules.

Clara-mucous cells may be found, experimentally, after irritation by sulphur dioxide [19], or multiple injections of the beta-adrenergic agonist, isoprenaline sulphate. The transitional cell retains the protruding apex, abundance of smooth endoplasmic reticulum and many of the electron-dense granules of the Clara cell but, in addition, has many large mucous granules.

DCG-mucous cell transitional forms have been found by histochemistry in the gut and by electron microscopy both in gut and bronchi [19].

Basal-mucous-squamous cell tracheobronchial epidermoid metaplasia is a change from an epithelium which is pseudostratified, mucus-secreting and ciliated to one which is stratified and keratinized. McDowell and Trump [35] have presented evidence and argued convincingly that such an epidermoid change arises subsequent to division of mucous cells, rather than arising directly from existing basal cells. Experimentally, carcinogens, mechanical trauma and vitamin A deficiency may each induce changes in mucous cells, leading to squamous cell metaplasia with or without stratification and keratinization [36].

Ciliated-secretory cells have been identified after injections of isoprenaline sulphate or exposure to LPS in rats, and, occasionally, in resected human lung [19] (fig. 7). The cell retains the electron-lucent cytoplasm of the ciliated cell with apical microvilli and ciliary basal bodies, but cilia are absent and there are secretory granules in the cytoplasm. The long slender microvilli, which normally project between cilia of ciliated
cells, are associated with an acidic surface mucosubstance, probably a glycosaminoglycan, which may be an important component of mucosubstance [25, 37–39]. The microvillus border and associated pinocytotic vesicles may play a role in ion translocation and fluid absorption, and thereby control the depth of the periciliary fluid layer in which the cilia beat. The extent to which this cell surface mucosubstance may be released and contribute to respiratory tract fluid is presently unclear.

**Submucosal glands**

The submucosal glands in humans are relatively numerous and in the lower respiratory tract are found wherever there is supportive cartilage in the airway wall, *i.e.* from larynx to small bronchi. The volume, distribution and histochemical composition of gland cells shows considerable species variation [7, 40]. It has been estimated that some 4,000 glands are present in the human trachea [41]. Developing from surface epithelium in utero, each gland unit is of the tubulo-alveolar type, and in humans may be composed of four regions whose lumina are continuous: 1) a relatively narrow ciliated duct in continuity with the surface epithelium; 2) an expanded collecting duct of cells of indeterminate morphology or of eosinophilic cells (also referred to as "oncocyes") packed with mitochondria; 3) mucous tubules and mucous acini; and 4) serous acini [42]. The movement of ions and water from the vascular compartment to the airway lumen is regulated both by surface epithelium and submucosal glands [43]. In the latter, it is suggested that watery, serous secretions pass from the outermost regions of each gland into the mucous tubules and mix, and that the ionic balance of the mixed secretion may be adjusted in the collecting duct before its discharge through the ciliated duct to the bronchial lumen. Discharge is aided by contractile myoepithelial cells, which form a basket-like structure around the outer aspects of the acinus.

Both synthesis of the intracellular section and discharge are influenced by nerves, whose terminals lie adjacent to (in humans) or pierce (as in the cat) the secretory tract of cells of indeterminate morphology or of eosinophilic cells, associated with an acidic surface mucosubstance, probably a glycosaminoglycan, which may be an important component of mucosubstance [25, 37–39]. The microvillus border and associated pinocytotic vesicles may play a role in ion translocation and fluid absorption, and thereby control the depth of the periciliary fluid layer in which the cilia beat. The extent to which this cell surface mucosubstance may be released and contribute to respiratory tract fluid is presently unclear.

### Secretory Cells

**Mucin genes**

With the development of molecular biological techniques, the complementary deoxyribonucleic acid (cDNA) sequences of mucin genes can now be obtained and the amino acid sequences of the mucin peptide core deduced. At the present time, there are at least eight human mucin genes (*MUC1* to *MUC4*, *MUC5AC*, *MUC5B*, *MUC6* and *MUC7*), one mouse mucin gene (*MUC1*), one hamster mucin gene (*MUC1*), three rat mucin genes (*RMUC7S*, *MUC2* mucin gene and a *MUC2* homologue, and *RMUC176*). Several other mammalian mucin genes from bovine submaxillary gland, canine tracheobronchial and porcine submaxillary gland have also been discovered. These discoveries have accelerated and intensified research into the extent of mucin gene expression in distinct airway conditions, and yet the specific role of mucin genes is at present unclear.
Mucin gene expression in experimental animals

The specific mechanisms by which pathogens (and their derivatives) and irritants induce upregulation of mucin genes are unknown: they probably act either by increasing the transcription rate or decreasing the rate of degradation of mucin messenger ribonucleic acid (mRNA). The isolation of a number of "mucin" genes has added a new dimension to the use of animal models to investigate the pathogenesis of hypersecretory disease.

JANY et al. [59] observed human MUC2 gene expression in SO2-exposed rats. These authors used rats exposed to 400 ppm SO2 for 3 h·day⁻¹, 5 days a week, for 1–3 weeks, and found the airways contained increased numbers of goblet cells and visible mucinous secretions in the airway lumen. In parallel, they applied Northern blot analysis, using the total ribonucleic acid (RNA) extract of the airway lumen. In three surgical specimens of human bronchi from lungs resected for carcinoma. The HAM1 antisense radiolabelled probe localized to occasional goblet cells of the surface epithelium and to submucosal gland ducts, and was applied, and localized to occasional goblet cells in the epithelium both of adult main bronchi and bronchioles, and was restricted to the surface epithelium of large airways after 19 weeks of gestation. In contrast, MUC1 gene expression was detected by 12.5 weeks of gestation and was more peripherally distributed. DOHRMAN et al. [62] compared the localization of MUC2, MUC3 and lysozyme mRNA transcripts in three surgical specimens of human bronchi from lungs resected for carcinoma. The HAM1 antisense radiolabelled probe localized heavily to cells in the bronchial surface epithelium thought to represent 20–30% of the goblet cell phenotype. These authors also reported that MUC2 gene was weakly and diffusely expressed in submucosal gland acini both of the serous and mucous phenotype. In contrast, the lysozyme antisense probe used, showed strong labelling of serous gland acini.

Our own findings [63] in the nasal mucosa demonstrate that MUC2 mRNA transcripts are present in: 1) serous and mucous acini of submucosal glands; 2) ciliated and basal cells of the surface epithelium; and 3) occasionally, mononuclear inflammatory cells (fig. 8a and 8b).
and b). The percentages (mean±SEM) of serous and mucous acini showing positivity for MUC2 gene expression in four samples surgically resected from non-CF subjects were: 25±2% and 27±3%, respectively. Compared with the non-CF subjects, the mean percentage of acini showing MUC2 gene expression in the four placebo-treated CF subjects was significantly higher for serous acini (80±13%; p<0.05, t-test), but not for mucous acini (53±17%; p=0.38). In CF and non-CF groups, where present, MUC2 positivity was strongly expressed and constituted approximately 84% of the cell area in serous acini, whereas it was less obvious and confined to the perinuclear area of cells in mucous acini. A significantly greater proportion of the surface epithelium was positive for MUC2 mRNA transcripts in the CF subjects (89±1%) than in the surgically resected tissues of the four non-CF subjects (19±4%) (p=0.02). These novel findings in the nasal mucosa are in the process of being extended to studies of bronchial biopsies obtained from subjects with asthma and chronic bronchitis.

Summary

The airway epithelium is comprised of many interacting structural components and inflammatory cells. Several morphologically distinct secretory cell types are present in the surface epithelium and submucosal glands. The secretions produced are, in part, high molecular weight, richly glycosylated, complex molecules, whose protein backbone consists of variable numbers of amino acids encoded by several "mucin" genes. The relative proportions of epithelial cell phenotypes and their secretory component alters in disease, and this may involve a metaplastic change where the characteristics of several morphological phenotypes may be found in one cell. In concert with this, there is altered expression of mucin genes and those which encode the enzymes responsible for mucin glycosylation. In airway disease, there may be mucosal damage, altered viscoelasticity of secretions and failure of clearance, with the enhanced likelihood of bacterial colonization leading to epithelial destruction. Further research in this area is required to improve our understanding of the function of mucin genes and of both the beneficial and potentially harmful effects of mucous hypersecretion.

References

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