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Immunological functions of the pulmonary epithelium

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Immunological functions of the pulmonary epithelium. A.B. Thompson, R.A. Robbins, D.J. Romberger, J.H. Sisson, J.R. Spurzem, H. Teschler, S.I. Rennard. ©ERS Journals Ltd 1995.

ABSTRACT: The mature pulmonary epithelium forms a continuous lining to the airspace. Recent data suggest that this specialized epithelium may also contribute to host defence *via* interactions with inflammatory cells. Pulmonary epithelial cells can serve as part of the local immune system, providing structures and functions crucial for the maintenance of normal pulmonary function.

This article will briefly review the morphology and development of the pulmonary epithelial cells, their function with regard to host defence, alterations of the pulmonary epithelium associated with airway diseases, and potential therapeutic implications for the treatment of respiratory diseases.

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In health, the mature pulmonary epithelium forms a continuous lining to the airspace. Airway epithelial cells line the airway lumen and extend into the submucosal glands of the larger airways. Airway epithelial cells serve functions which contribute to defending the lungs against inhaled insults. These functions include provision of physical and functional barriers to and clearance of environmental agents, modulation of the inflammatory response to noxious stimuli, and regulation of the cellular activities necessary for responding to injury [1]. The alveolar compartment is likewise lined by epithelial cells. In the alveoli, the epithelial cells are specialized to serve the function of gas exchange. However, recent data suggest that the alveolar epithelium may also contribute to host defence *via* interactions with inflammatory cells. The pulmonary epithelium, thus, serves as part of the local immune system, providing structures and functions crucial for the maintenance of normal pulmonary function. Alteration of the structure and function of the pulmonary epithelium plays an important role in the pathogenesis of respiratory diseases.

This section of the series, "Pulmonary Immune Cells in Health and Disease", will briefly review the morphology and development of the pulmonary epithelial cells, their function with regard to host defence, alterations of the pulmonary epithelium associated with airway diseases, and potential therapeutic implications for the treatment of respiratory diseases.

Morphology of the airway epithelium

The mature airway is a complex structure lined by a continuous layer of epithelial cells. The distribution of cell types within the epithelium varies along the airway. Whilst as many as 49 cell types have been recognized in the airway [2], many of these may represent intermediate or differentiating cells, and a smaller number of cell types constitutes the bulk of the epithelium [3, 4]. On the surface of the epithelium of the proximal lower respiratory tract, ciliated cells predominate. Together with basal cells and a small percentage of goblet cells, the ciliated cells form a pseudostratified epithelium, with ciliated cells occupying the majority of the luminal surface and basal cells in contact with much of the basement membrane. The surface epithelium extends into the ducts of the mucosal glands, where it is characterized by a variable proportion of ciliated and mucous cells [5]. The specialized secretory epithelium of the submucosal gland is composed of serous cells and mucous cells. In the distal airway, Clara cells and basal cells predominate, cilia are not present, goblet cells are less numerous, and the epithelium has a more columnar appearance [6, 7]. At the alveolar level, the columnar epithelium gives way to a thin epithelium predominantly comprised of Type I cells, interspersed with Type II cells [8]. Each of the major cell types in the airway epithelium has distinctive histological characteristics.

Ciliated cells are characterized by prominent cilia, which attach by extension of axonemal microtubules (see below) to the basal body, which can be used to cytologically identify exfoliated ciliated cells in cases of ciliacytophoria [9]. Mucociliary clearance is the major function of the ciliated cells. Cilia are bathed in and extend through the thin, watery sol phase of airway secretions, into the gel phase, where specialized barb-like structures on the tips of the cilia [10] grab the mucus

during the active stroke phase of cilia beating, but not during the backward or relaxation stroke phase; and, thereby, propel the mucus with a rowing-like action. Ciliated cells possess 200–250 cilia per cell [2, 11]. In the human, the cilia are 0.25 μm in width, and range from 6 μm in length in the proximal airways to 3.6 μm in seventh generation airways [12]. The axoneme of the cilium contains the elements necessary for the conversion of chemical energy into mechanical force [13]. Dynein complexes, complexes of high molecular weight enzymes with adenosine triphosphatase activity, catalyse hydrolysis of adenosine triphosphate and transduce the energy into mechanical force in the form of ciliary bending [14]. The appropriate orientation of the direction of the beating of the cilia is related to the orientation of the structural elements of the axoneme. The axoneme of the cilium is made up of an outer ring of nine microtubule doublets surrounding a central pair. The microtubules are tethered, and their orientation maintained, by nexin links (between adjacent outer ring microtubule doublets) and radial spokes (between outer ring microtubule doublets and the central pair of tubules). This tethering restricts ciliary bending to a fixed orientation. The cilia in a given cell and its neighbours share a common orientation, and the beating of the cilia is maintained in an effective, metachronous state by cell-cell interactions, presumably, in part, *via* gap junctions.

Goblet cells contain large granules of varying electron density interspersed with few cytoplasmic inclusions. The nucleus is displaced away from the apical surface, and the cell tapers as it extends basally, giving the cell its typical goblet shape [15]. The goblet cell secretory granules are large, about 800 nm in diameter, and contain predominantly neutral mucins and sulphomucins. However, the content of the goblet cells is somewhat variable, with areas of the airway containing goblet cells rich in sialomucins. Goblet cells make up 15–25% of the surface epithelium in the proximal airways, decreasing in number distally [16–19].

Basal cells are flattened, pyramidal-shaped cells with a small cytoplasmic/nuclear ratio. The presence of basal cells underlying the ciliated and goblet cells, in part, lends the airway epithelium its pseudostratified appearance. The surface of the basal cells is rich in desmosomes, which attach to surrounding epithelial cells, thereby anchoring them to the basement membrane [20].

The epithelial cells which line the submucosal glands also have a typical anatomical distribution [3]. The submucosal gland has a duct of variable length lined with both serous and goblet cells, and connects to lobules. The lobules are lined with mucous and serous cells. In health, the ratio of mucous cells to serous cells is about 2:1, and is quite constant throughout the airways [5]. The serous cells tend to occupy the most distal portion of the glands, with the mucous cells lying more proximal to the ducts. This arrangement allows the less viscous secretions of the serous cells to flush out the thicker, more tenacious secretions from the mucous cells. Accompanying the lobules are myoepithelial cells. The myoepithelial cells extend from the serous cell rich terminus of the lobule to the duct, and presumably contract to expel glandular secretions into the airway lumen [21].

Clara cells compose the majority of cells in the bronchiolar epithelium. They are columnar shaped cells, with apical surfaces which bulge into the airway lumen. Ultrastructurally, the cell has a centrally placed, indented nucleus, prominent Golgi apparatus and rough endoplasmic reticulum [22, 23]. Apically, the cell contains membrane-bound, electron-dense secretory granules. The ultrastructure of Clara cells and histochemical studies suggest that the cell is active in the secretion of proteins. Specifically, CC10 is a low molecular weight protein which, although not unique to Clara cells within the lung [24], appears to be the major secretory product of Clara cells [25]. The role of this protein is not well-established, but it has been postulated to have possible anti-inflammatory activities as an inhibitor of elastase [26], or phospholipase A_2 [27]. The Clara cell may also be active in the secretion of surfactant, as surfactant-related glycoprotein has been identified within the cell [28]. In addition to secretion of biologically active substances, Clara cells may participate in clearance of noxious agents *via* the detoxification of inhaled substances [29].

In addition to the more common cell types described above, the normal airway epithelium has a small number of neuroendocrine cells. Neuroendocrine cells typically lie in the basal portion of the epithelium, but usually have thin cytoplasmic projections which extend to the lumenal surface [30, 31]. Neuroendocrine cells increase in number from the largest bronchi to reach maximal density in the bronchioles [32]. More distally, neuroendocrine cells are rarely found in the terminal bronchioles [33]. The cells occur singly and in clusters termed neuroepithelial bodies, and tend to be concentrated at the bifurcations of the conducting airways. The neuroendocrine cell is distinguished by cytoplasmic neurosecretory-like granules. These granules localize to the basal surface of the cell, and range 70–150 nm in size. By electron-microscopy, the granules have an electron-dense core, which is separated by an electron-lucent rim from the organelle's outer membrane [34]. The neuroendocrine cells are a rich source of bioactive secretory products, including bombesin-like activity (gastrin-releasing peptide), somatostatin, endothelin, serotonin, and calcitonin [34].

The role of neuroendocrine cells in the healthy lung is not clear but consideration of the fact that they are part of the neuroendocrine system (as defined by the unique appearance of the secretory granules, and the contents thereof) would suggest regulatory roles for these cells [32, 34]. The localization of the body of the cells to the basement membrane, the basal distribution of their granules, and the contents of their secretory granules, would suggest regulatory roles for secretion, smooth-muscle function, and cell growth modulation. In addition, the close association with neuronal cells, and the degranulation of the cells which occurs in response to hypoxia, hypercarbia, and hyperoxia, has lead to the suggestion that the neuroendocrine cell may act as an

airway chemoreceptor [34]. Moreover, hypertrophy of neuroendocrine cells occurs in association with residence at high altitude [35], and in association with a number of pulmonary diseases, particularly in cigarette smokers [36]. A recently described syndrome of neuroendocrine cell hyperplasia in nonsmoking patients without other lung diseases underscores the potential importance of these cells for normal lung health. Patients with neuroendocrine cell hyperplasia were found to have multiple carcinoid tumorlets, and, importantly, peribronchiolar fibrosis with obliteration of small airways [36].

The epithelial cells which line the alveoli are specialized cells, whose main function is to permit gas exchange. Type I cells are thin, flattened cells, which by their attenuated shape permit efficient gas exchange. Type I cells cover 95% of the alveolar surface and have a paucity of organelles [8]. Type II cells are cuboidal in appearance. Their apical surface is covered with microvilli and their cytoplasm is notable for numerous lamellated inclusions. The lamellated inclusions are composed of lipids (predominantly phospholipids) and proteins [37–40]. They are secreted onto the apical surface of the alveolar epithelium to form surfactant. Surfactant acts to reduce alveolar surface tension and one of its constituents, surfactant protein-A (SP-A) has recently been noted to also have potentially important immunomodulatory activities (reviewed below).

The airway epithelium rests upon a connective tissue substrata consisting of a basement membrane, lamina propria, muscularis mucosa, and submucosa. The lamina propria contains a network of fine capillaries, a reticular connective tissue meshwork, and well-developed elastic tissue [41]. The submucosa consists of a denser extracellular matrix and contains the submucosal glands and the cartilaginous structures which lend support to maintain airway patency. Importantly, within the connective tissue matrix lie stromal cells, fibroblasts and smooth muscle cells, as well as cells which may participate in an inflammatory response, mononuclear cells, lymphocytes, neutrophils, eosinophils, mast cells, and Langerhans' cells. The close proximity of these cellular components of the airway walls to the airway epithelium make them targets for paracrine modulation by epithelial cells.

In the alveoli, the interstitium is thin in support of gas exchange. The close apposition of alveolar epithelial cells and the alveolar capillaries has functional and morphological implications. The very small distance between the Type I cell apical surface and the endothelial surface of the capillary minimizes the distance which gases need to diffuse through tissue [42, 43]. Moreover, the juxtaposition of alveolar epithelial cells with alveolar capillary cells seems to be crucial for the development of the Type I morphology [44].

Development and differentiation of pulmonary epithelial cells

The intrauterine development of the lungs is generally considered as two processes, the development of the conducting airways and the development of the gas-exchange, terminal respiratory units. The bronchial structure is developed by the 16th week of gestation [45]. Until the 16th week, the primordial segmental airways undergo repeated divisions until more than the usual number of airway generations found in the adult lung have been formed [46]. Transformation of distal conducting airways into respiratory bronchioles then ensues, leading to loss of distal generations of conducting airways. At birth, the tracheobronchial structures are complete. In contrast, the development of acinar structures begins late in gestation and continues after birth. Alveolar development begins at 30–32 weeks gestational age and continues to term. Recognizable alveolar structures are present at birth, but differ enough from adult structures that the term "saccules" has been applied to differentiate them from the mature anatomical unit. By two months post delivery, morphologically mature alveoli appear [47]. The number of alveolar structures increases exponentially from weeks 30–32 to term, so that at birth the estimated gas-exchange area is 3–4 m^2. Within this anatomical framework, the pulmonary epithelium develops structurally and provides functions felt to be important for the normal development of the lungs.

The epithelium of the conducting airways first appears as primitive, pluripotent foetal cells, recognizable as epithelial cells by the presence of tight junctions and desmosomes [48]. The airway develops differentiated epithelial cells, at first centrally in the trachea, and then progressively distally. Basal cells, identified morphologically, are found at 10 weeks. They overlie the basement membrane and may serve as a precursor to more differentiated cells, quickly giving rise to ciliated cells and goblet cells in the central airways [48]. More distally, a cell of intermediate appearance has been postulated to be the precursor cell which gives rise to differentiated Clara cells and ciliated cells [48]. Goblet cells first appear during the 13th gestational week in the trachea and proximal bronchi. Their numbers increase with further appearance of goblet cells towards the periphery, but not reaching the proximal bronchioles [41]. Submucosal glands appear at 10 weeks in the trachea and 16 weeks in the bronchi. Fully formed cilia and mucus are found in the airways by 13 weeks. Neuroendocrine cells develop throughout the airways by week 16 [49].

Type I and Type II cells develop from endodermal origins [36]. During both the glandular and canalicular stages, the primordial alveoli are lined by cells identified as epithelial by the presence of gap junctions and tight junctions [50, 51]. Type II cells can be identified by their lamellar inclusions, histochemical reactivity with monoclonal cell-specific and surfactant-related protein-specific antibodies and by *in situ* hybridization with surfactant associated protein messenger ribonucleic acid (mRNA) at about week 24 [52–54]. Fully mature lamellar bodies develop only late in gestation, weeks 30–32. The development of mature lamellar bodies in sufficient numbers to support pulmonary function in the newborn is dependent upon hormonal signalling, especially by glucocorticosteroids [55, 56]. The Type I cells appear in the saccular stage. They can be distinguished by their

attenuated morphology, and appear to be derived from Type II cells [57, 58]. The differentiation of Type I cells may be directed by interactions with pulmonary endothelial cells. In the foetal rat lung, direct contact of alveolar epithelial cells with endothelial cells has been associated with loss of lamellar bodies from Type II cells, attenuation of the cellular morphology, and fusion of the epithelial basal lamina with the endothelial basal lamina [44].

The developing pulmonary epithelium contributes important functions to normal lung development. The pulmonary epithelium is central to the secretion of fluid by the neonatal lung, and is integral to the secretion of surfactant. The neonatal lung is a secretory organ. In foetal lambs, 2–3 $ml \cdot h^{-1} \cdot kg$ of fluid is secreted [59]. The fluid secreted by the lungs flows to the oropharynx by mass movement, as the result of foetal respiratory movements [60], and by contraction of airway smooth muscle [61]. The expelled fluid is added directly to the amniotic fluid or is swallowed. Analysis of the fluid secreted by foetal lambs demonstrates that the fluid is relatively rich in K^+ and Cl^-, and poor in protein, HCO_3^- and Ca^{2+} [62]. The protein content of foetal pulmonary secretions is approximately 10 fold lower than plasma or lymph, suggesting that the epithelium is impermeable to macromolecules early in development. The ionic composition of foetal lung fluid led to the suggestion that fluid secretion in the foetus results from active transport of Cl^- in excess of the uptake of HCO_3^-. Na^+ ion flux was hypothesized to follow the electrical gradient resulting from the transport of Cl^-, and water flux to follow the resulting osmotic gradient. These suppositions have been confirmed in various experimental models of the developing lung.

Explants of first- and second-trimester human foetal lung tissue demonstrate cyclic adenosine monophosphate (cAMP)-stimulated fluid secretion [63, 64]. Using submersion explant culture of foetal rat lung, a model which maintains the early foetal phenotype of pulmonary epithelial cells, fluid secretion has been further characterized [65]. Fluid production was found to be dependent upon Na^+/K^+-adenosine triphosphatase (ATPase) secretion of Cl^-. The cystic fibrosis transmembrane conductance regulator (CFTR) is present throughout the pulmonary epithelium of foetal human lung during the first two trimesters [66]. The presence of CFTR has been further demonstrated in cultures of human foetal alveolar epithelial cells on the cell apical surface. Secretion of Cl^- by the cultured cells was stimulated by raising intracellular cAMP in the presence of amiloride, an inhibitor of Na^+ absorption, indicating that CFTR was active [67].

The importance of sufficient quantities of active surfactant for normal pulmonary function in the neonate is well-established. Analysis of the surfactant-associated proteins in foetal rat and human lungs has raised the possibility that SP-A may have functions in the foetal lung in addition to being a constituent of surfactant. SP-A is only a minor component of the total protein content of lamellar bodies, and the secretion of SP-A is largely independent of the release of lamellar bodies and is constitutive, not responding to secretogogues for lamellar bodies [68]. Moreover, in the ductal foetal rat lung, SP-A is expressed throughout the respiratory epithelium. This is in contrast to surfactant protein-B and C (SP-B and SP-C) which are limited to the distal, prealveolar areas of the ductal system [69]. These observations suggest that SP-A may have functions dissociated from surfactant. Such functions might include the immunomodulatory properties suggested for the molecule in postnatal lungs (see below).

Host defence functions specific to the pulmonary epithelium

On a daily basis, a person inhales approximately 10,000 *l* of air. The nose and upper airway act to filter large particulates, and warm and humidify the inspired air. However, the lower respiratory tract is exposed to large amounts of environmental agents on a daily basis. These agents take several physical forms, particulates, gases, fumes, droplets, or biological matter. In addition, it is felt that some degree of aspiration normally occurs during sleep, further exposing the airways to infectious organisms and noxious physical agents. The pulmonary epithelium has several functions, which act to protect the airspaces and preserve normal respiratory function. These functions can be grouped into three categories: 1) features of the epithelium that contribute to the barrier function of the epithelium and the resulting physiological consequences; 2) the co-ordinated interactions of secretion and ciliary function, leading to effective mucociliary clearance; and 3) secretion of substances, both derived from cells other than pulmonary epithelial cells and synthesized by pulmonary epithelial cells, which target specific environmental challenges to the pulmonary epithelial surface. Integration of all these functions is required to maintain a healthy epithelium. Pulmonary diseases associated with disruption of any of these activities quickly leads to the alteration of others and to impairment in pulmonary function.

Barrier function

The pulmonary epithelium provides a morphological and functional barrier to the environment. Histological studies demonstrate the presence of junctional complexes within the airway and alveolar epithelia [2, 70]. Junctional complexes are composed of three parts: the zonula adherens, desmosomes, and tight junctions [71]. Each of these is believed to have a unique role in the maintenance of epithelial cell interactions. The zonula adherens contains cellular adhesion molecules (CAMs), and contributes to cellular adhesion and recognition. Desmosomes contain a number of proteins, and play a role in epithelial integrity. Finally, the tight junctions, which also are composed of specific proteins, provide a physical barrier function.

The arrangement of the epithelium into a continuous surface by the presence of these junctional complexes leads to important functional consequences. The lumenal cell membrane forms an impermeable barrier to

macromolecules and infectious agents. Moreover, ionic diffusion is greatly limited by the junctional complexes. Airway epithelial cells are polarized by virtue of having an apical surface (lumenal to the level of the tight junctions) and a basolateral surface (all of the cell membrane basal to the tight junctions). Thus, ion, and, consequently, water transportation, which are crucial for maintaining the proper state of hydration of the fluid which bathes the cilia, can occur in a directed manner [72]. Similarly, epithelial cell products can be transported either to the lumenal surface to interact with environmental agents or basally to affect neighbouring or distant cells.

Exclusion of inhaled particulates and molecules from the basal surface of the epithelium, isolates the exposures, protecting cells underlying the epithelium, which are sensitive to environmental agents. The airway submucosa is rich in a neural plexus which, when stimulated by exposure to irritants, mediates the release of mediators which are associated with airway hyperresponsiveness and airway obstruction [73]. Similarly, resident mast cells are stimulated to release the contents of their secretory granules by a wide range of environmental triggers. Mast cell secretory granules contain a large number of bioactive substances, including histamine and proteolytic enzymes, which may exert deleterious effects upon airway function [74]. The airway mucosal barrier function also serves to lessen the exposure of lymphocytes to potential allergens. The importance of the barrier property for epithelial integrity is illustrated by the experimental observation that cultured airway epithelial cells, grown to confluence and having formed tight junctions, are resistant to the effects of human neutrophil elastase on the epithelial surface [75]. In contrast, the same concentrations of elastase cause detachment of epithelial cells which do not have functional tight junctions [75].

Additionally, the airway epithelium provides an effective barrier against invasion by microbes [76]. The importance of the exclusion of microbes by the epithelium is underscored by the pathogenic importance of the attachment of microbes to the epithelium. This is believed to be the initiating event for infections of the lower respiratory tract. Very few bacterial species have developed mechanisms for attachment to normal, intact human respiratory epithelium, notable exceptions being *Mycoplasma pneumoniae* and *Bordetella pertussis* [77, 78]. However, disruption of the epithelial surface compromises the epithelium, through proliferation of differentiating cells and denudement of extracellular matrix, allowing bacterial attachment to occur [79]. Injury of the airway epithelium by infection with viruses, particularly Influenza virus, permits bacterial attachment [79], and is associated with greatly increased incidence of pneumonia [80]. Clinically, bacterial colonization follows disruption of the normal mucosa, such as results from mechanical injury following endotracheal intubation, or due to squamous metaplasia found in chronic bronchitis. In addition to compromising mucociliary clearance (see below), these changes permit adherence of species of bacteria to the airway mucosa which are usually excluded, *e.g. Haemophilus influenza*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* [76]. In chronic bronchitis, bacteria colonizing the tracheobronchial tree are associated with exacerbations of airway disease [81].

In the alveoli, accumulation of oedema quickly leads to impairment of gaseous exchange. Alveolar epithelial cell tight junctions are an important barrier to the ingress of interstitial fluid into the airspace.

Mucociliary clearance

Efficient clearance of inhaled or aspirated particles, including viruses and bacteria, which impact upon the airways, depends upon trapping of the particle in mucus and clearance of the mucus by cough and ciliary activity. As noted above, cilia are not found in the more distal airways. At this level of the airways, particles are cleared by macrophages and cough. Surfactant, produced by Type II epithelial cells and Clara cells, assists in the clearance of particles by coughing, and by changing the surface charge properties, rendering foreign particles "less sticky" and, thus, more easily cleared.

In the more proximal airways, ciliated cells predominate and function to transport airway secretions to the oropharynx, where they can be expectorated or swallowed. The relative importance of cough and mucociliary clearance for the clearance of secretions is not well-defined. Clearly, cough alone is able to remove large amounts of sputum from the lower respiratory tract. However, the importance of mucociliary clearance is demonstrated by the obstructive airway disease, which may result from primary ciliary dyskinesia [82].

The gel phase of respiratory mucus is rich in mucins, which impart to the gel phase the stickiness to trap particulates and the viscoelastic properties characteristic of mucus. Mucins are a group of molecules which consist of high molecular weight ($>10^3$ kDa), complex glycoproteins. The physiochemical properties of the mucins make them sticky, and thus effective for trapping particulates. In addition to nonspecific interactions, mucins present a great number of potential carbohydrate receptors for more specific interactions [83]. A number of species of bacteria, notably *H. influenza*, *S. pneumoniae*, and *S. aureus,* bind avidly to airway mucins [76]. Whilst this may be important as a virulence factor for these species, allowing them a "foothold" in the airway, in the presence of normal mucociliary clearance such an interaction would effectively trap the organisms in the airway mucus and contribute to their clearance. The viscoelastic properties of mucus imparted by the mucins, which determine the ease or difficulty for clearance of airway secretions, are altered by infection and airway inflammation.

The distribution of mucus along the airway surface has been a matter of controversy. Experimental evidence suggests that mucus is distributed as discontinuous globules, which become increasingly confluent in the larger airways, resulting in a nearly continuous film of mucus in the large bronchi and trachea [84]. This distribution would help prevent pooling of secretions as they move up the airways, through airways progressively larger in diameter, but smaller in total surface area.

Table 1. – Factors that alter mucociliary function

Factor	Ciliary motility	Mucus velocity	Source
β-agonists	↑	↑	Hormonal
Bradykinin	↑↑	↑	Airway neurones
Histamine	±	↑	Eosinophils, mast cells
Interleukin-1β	↑	?	Macrophages
Leukotrienes	±	?	Eosinophils
Major basophilic protein	↓	?	Eosinophils
Nitric oxide	↑	?	Airway epithelium
Oxidants	↓	↑	PMNs Macrophages
Substance P	↑	↑	Airway neurones
Tumour necrosis factor-α	↑	?	Macrophages

PMN: polymorphonuclear cells.

Interactions of ciliated epithelial cells and inflammatory cells

Effective mucociliary clearance is dependent on the propulsion of airway mucus by the co-ordinated beating of cilia. The regulation of ciliary motility and mucociliary clearance is complex and not completely understood. However, it is clear that immune effector cells and their products play an important role in altering and regulating mucociliary function (table 1).

Activated polymorphonuclear cells (PMNs) are capable of releasing oxidants, including hydrogen peroxide and superoxide [85], which have been demonstrated to impair ciliary function [86], and which may decrease ciliary motility in conditions associated with airway neutrophilia, *e.g.* chronic bronchitis [87]. Neutrophils can release a number of proteases, such as elastase, which probably damage ciliated cells, and have been shown to arrest mucociliary activity [88]. PMNs can also produce bioactive lipids, such as platelet-activating factor [89], which may impair ciliary motility and decrease mucociliary clearance [90].

Macrophages can synthesize a variety of inflammatory products that have been shown to alter ciliary motility. Macrophages, like PMNs, produce cilia-damaging oxidants and proteases. In contrast to their ability to release factors that impair ciliary functions, macrophages also have a robust capacity to synthesize cytokines, which appear to upregulate mucociliary function. Tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), released from alveolar macrophages, have recently been shown to upregulate ciliary beat frequency related to the release of nitric oxide (NO) in a manner that requires induction of NO synthase (NOS), and is blocked by glucocorticoids [91]. Neural and hormonal mediators, such as β-agonists [92–95], bradykinin [92, 96–98], and substance P [99, 100], which upregulate ciliary beat frequency, also effect NO synthesis. However, in contrast to the response to cytokines, the NO-dependent increase in ciliary beat frequency is rapid and transient. The difference in the temporal response of ciliary motility to NO in these differing circumstances suggests that NO is a fundamental cellular signal for upregulating ciliary motility, regardless of how it is triggered. The rapid transient increase in ciliary motility is likely to occur during "fight or flight" conditions, such as during exercise, whereas the release of the cytokines during macrophage activation may by an important mechanism by which mucociliary clearance is increased during infection, allergies, or other sustained inflammatory conditions.

Eosinophils are laden with substances which, when released, may impact on ciliary motility. Major basophilic protein, which is released from injured eosinophils, is known to impair ciliary motility [101], and probably accounts for the damage that occurs to nasal ciliated cells co-cultured with eosinophils [102]. Interestingly, this effect can be attenuated by adding nedocromil sodium. This suggests that the ciliastasis is a consequence of the inflammatory response. Histamine and leukotrienes, inflammatory mediators released from basophils, mast cells, and platelets during anaphylaxis, can have variable effects on ciliary motility [103]. For example, inhalation of histamine increases tracheal mucus velocity in human volunteers [104], but has no effect on ciliary motility *in vitro* [105].

Activated lymphocytes can alter ciliary activity. Activated lymphocytes are a rich source of cytokines, such as γ-interferon, which can induce NOS in epithelial cells [106]. It is also likely that other lymphocyte-derived cytokines impact on ciliary motility in the airway, especially in conditions associated with chronic inflammation, such as asthma and bronchiolitis.

Thus, the cells and mediators of the immune system can both impair and enhance mucociliary function, impacting upon lung host defences. It is clear that the complex regulation of these cells and release of their mediators is required to maintain normal function. Loss of balance in effector signals leads to injury of this vital host defence function.

Epithelial cell mucous secretions

In addition to providing the mucins integral for trapping particulates and normal mucociliary clearance, the airway epithelium secretes a number of substances with a variety of properties important for host defence of the airways. Airway secretions provide protection against a wide variety of potentially injurious agents, including infection, reactive oxygen species and proteases (table 2).

Antimicrobial activities

The lower respiratory tract is protected against infection by complimentary mechanisms. In addition to the exclusion of microbes by the airway epithelial barrier function, trapping by mucins, and clearance by the mucociliary escalator and cough, the airway epithelium secretes a number of substances with antimicrobial properties [107]. Some of these substances have direct antibacterial activities, such as lysozyme [108], and

Table 2. – Pulmonary epithelial cell-derived substances and host defence

Mucins
Antibiotic Substances
SP-A
Immunoglobulin A
Complement
Lactoferrin
Lysozyme
Antioxidants
SP-A
Lactoferrin
Glutathione
Catalase
Antiproteases
Elafin
Secretory leukoprotease
CC10

SP-A: surfactant-associated protein A.

lactoferrin [109–110]. Others, especially components of the complement system [112], and perhaps SP-A [113, 114], act as opsonins, allowing for efficient phagocytosis. Secretory immunoglobulin A (sIgA) plays a major role in airway host defence, as demonstrated by the susceptibility to infection which may result from sIgA deficiency [115]. Plasma cell-derived IgA is specifically bound by the epithelial cell basal-surface receptor, secretory component. The complex is transported from the basolateral cell surface to the apical surface, where it is secreted [116]. Secretory IgA acts primarily upon viruses, but on bacteria also, coating them and preventing attachment to the respiratory epithelium [117].

Lactoferrin is a major secretory product of the mucous gland serous cells, which has modest direct bacteriostatic properties, but which is presumably more effective as a sink for iron [118]. When bound to lactoferrin, and the closely related serum protein transferrin, iron is made unavailable to most bacterial species, depriving them of this essential growth factor. These epithelial cell products interact with antimicrobial substances from other cell sources, such as defensins from neutrophils, and immunoglobulin G (IgG), which are also present in airway secretions, together forming an effective array of antimicrobial defences.

Antioxidant activities

The airway epithelium is constantly exposed to an oxidant-rich environment. Cigarette smoke [119] and other inhaled exposures such as ozone and NO_2 [120], as well as activation of inflammatory cells [121–123] increases the oxidant burden to which the airway epithelial cells are exposed. Reactive oxidant species are potent initiators of cell injury, and oxidant-mediated cell injury has been identified in a growing number of pulmonary diseases [124]. Airway epithelial cells have both intracellular and extracellular antioxidant activities.

Airway epithelial cells share with other lung cells three major intracellular antioxidant systems, the glutathione redox cycle, superoxide dismutase, and catalase [124]. Superoxide dismutase reduces the superoxide radical to H_2O_2 and catalase reduces H_2O_2 to water. The glutathione redox system maintains a high ratio of reduced glutathione (GSH)/oxidised glutathione (GSSG). Reduced GSH reduces intracellular hydroperoxides, lipid peroxides, and products of lipoxygenase-catalysed reactions. The high intracellular concentrations of GSH and its ubiquitous distribution within the cell, suggest that the GSH redox cycle is of central importance. Exposure of epithelial cells to oxidant stress leads to upregulation of intracellular antioxidant mechanisms [124].

The extracellular epithelial lining fluid is rich in antioxidant activities. Much of the antioxidant activity of the epithelial lining fluid is derived from the serum. However, epithelial cells release substances with specific antioxidant activities. Prominent amongst these is lactoferrin, which is synthesized and secreted by serous cells. Together with serum-derived transferrin and ferritin, lactoferrin avidly binds iron as Fe^{3+}. Lactoferrin and transferrin, and under most conditions ferritin, bind iron in a manner which renders the iron inaccessible for the catalysis of the Haber-Weiss reaction. In this reaction, iron, as Fe^{2+}, is rate-limiting for the formation of hydroxyl or hydroxyl-like radicals, which are potent initiators of oxidant chain reactions. Whilst both lactoferrin and transferrin, unlike ferritin, are resistant to oxidant-induced release of bound iron [125, 126], transferrin releases its bound iron at a pH less than 5. In contrast, lactoferrin is resistant to this effect of acidic environments [127], a property important on mucosal surfaces, where local effects of inflammation may lower the pH. Recent data suggest a role for SP-A as an antioxidant in the lower respiratory tract. SP-A, in physiologically relevant concentrations, has been found to inhibit superoxide production by alveolar macrophages stimulated by phorbol 12-myristate-13-acetate (PMA) or zymosan-activated serum [128]. Primarily intracellular antioxidants, *e.g.* catalase and glutathione, are presumably released from airway epithelial cells and contribute to the antioxidant activity of the epithelial lining fluid [129, 130].

The antioxidant activity of lower respiratory tract epithelial lining fluid has been measured and partially characterized [131]. Deficiencies have been found in association with smoking cigarettes [132]. Moreover, cigarette smoking has been shown to be associated with the presence of abnormal, oxidized substances in the epithelial lining fluid [133]. These observations would suggest that exposure to cigarette smoke may overwhelm airway antioxidant defences, and that oxidants may contribute to airway inflammation in chronic bronchitis.

Antiprotease activities

Pulmonary inflammatory cells, including neutrophils, macrophages, basophils and mast cells, are rich sources of proteases. Activated inflammatory cells release their proteases extracellularly, causing the concentrations of proteases to become quite high in the local surroundings of the activated cells [134]. Cleavage of pulmonary parenchymal proteins by proteases and direct effects on airway epithelial cells have been postulated to be

important pathogenic mechanisms for both emphysema and chronic bronchitis. Normally, the airway epithelium and lung parenchyma are protected from the effects of proteases by the presence of an excess of antiproteases.

The pulmonary antiproteases are largely class specific. Thus, α_1-protease inhibitor, secretory leucoprotease inhibitor (SLPI), and α_1-antichymotrypsin are inhibitors of serine proteases, especially neutrophil elastase, and tissue inhibitors of metalloproteases (TIMP) inhibit metalloproteases. In contrast, α_1-macroglobulin is not class specific, and inhibits proteases from all four of the protease classes, serine, metallo-, cysteine and aspartic, whilst elafin is specific for elastase The lower respiratory tract antiproteases are derived from a variety of sources, including the serum (α_1-protease inhibitor, α_1-antichymotrypsin, and α_1-macroglobulin) and pulmonary macrophages (α_1-protease inhibitor and α_1-macroglobulin). The source of elafin in the lower respiratory tract has not been entirely determined, but it appears to be derived from pulmonary epithelial cells. The Clara cell-like cell line, NCI-H322, and the Type II cell line A549, both secrete elafin [135].

Likewise, SLPI derives entirely from the pulmonary epithelium. It has been presumed to be a predominant inhibitor of neutrophil elastase in the airways, and also inhibits other serine proteases, including mast cell chymase, cathepsin G, trypsin, chymotrypsin. It is derived from glandular serous cells as well as the surface epithelium in the central airways, from Clara cells in the more distal airways, and from Type II cells in the alveoli [135–138]. Furthermore, it is elevated in states of airway inflammation, suggesting upregulation of synthesis [139]. However, the precise role of SLPI is clouded by the observation that the majority of SLPI recovered from the airways of normal individuals is inactivated [140]. The inactivated SLPI is not bound to human neutrophil elastase, nor has it been cleaved into smaller peptides. SLPI is sensitive to oxidative damage by neutrophil products, and, thus, inactivation of SLPI *in situ* could result from interaction with resident neutrophils. SLPI could still play a significant role in the airway, as its release from epithelial cells directly subjacent to neutrophils might result in high, local concentrations, and act as a first line of defence against neutrophil elastase.

Imbalance of lung proteases and antiproteases, favouring increased free protease burden has been documented in inflammatory lung diseases, especially those associated with an influx of neutrophils into the lung. Free neutrophil elastase is not normally measurable in the epithelial lining fluid. However, free neutrophil elastase can be found in some cigarette smokers [141]. In animal studies, the instillation of free elastase into the airways leads to emphysema and goblet cell hyperplasia, suggesting that free proteases may contribute to the morphological changes found in the airway in patients with chronic bronchitis [142]. Proteases have also been demonstrated to be potent secretogogues for airway epithelial cells [143]. Thus, proteases potentially contribute to the hypersecretory state which characterizes chronic airway inflammation *via* both morphological and functional mechanisms.

Regulation of cellular activities

Airway epithelial cells, in addition to having the functions important for host defence, which are described above, participate in the regulation of other airway cells contributing to the host response to environmental stresses. Cells with which pulmonary epithelial cells have been shown to interact include the following: 1) inflammatory cells, important for host defence, but associated with injury if unchecked; 2) airway and bronchial vascular smooth muscle cells, cells which are intrinsic to the airway and vital for airway function; and 3) airway mesenchymal cells, which play a major role in the reparative response to injury. Presumably, these mechanisms, by providing an interface between inhaled environmental constituents and the lung, are important for maintaining normal airway function. However, evidence suggests that in pathological states, stimulation of pulmonary epithelial cells can contribute to disordered lung function.

Inflammatory cells

There is much experimental evidence to suggest that airway epithelial cells interact with inflammatory cells *via* a number of mechanisms (summarized in fig. 1). Airway epithelial cells have the capacity to recruit inflammatory cells to the airways *via* the release of chemoattractants, to direct inflammatory cell migration across the epithelium *via* the expression of cell surface molecules, and to regulate inflammatory cell activity *via* the release of cytokines. Each one of these steps amplifies the inflammatory response, establishing the importance of airway

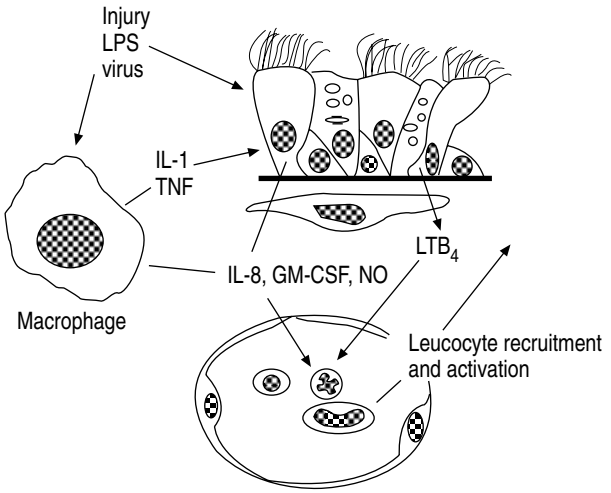

Fig. 1. – Cytokine interactions between airway epithelial cells and inflammatory cells. Inflammatory stimuli, *e.g.* LPS or viral infection, intitiate release of mediators including LTB_4, IL-8, NO, and GM-CSF from epithelial cells, and IL-1 and TNF from macrophages, which further stimulate the epithelial cells. The macrophages and epithelial cells augment the inflammatory response by releasing additional mediators that recruit and activate leucocytes. LPS: lipopolysaccharide; LTB_4: leukotriene B_4; IL-8: interleukin-8; NO: nitric oxide; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL-1: interleukin-1; TNF: tumour necrosis factor.

epithelial cells for the modulation of airway inflammatory diseases. Recently, evidence has accrued to suggest that alveolar epithelial cells may also interact with inflammatory cells.

Recruitment of inflammatory cells

Cultured airway epithelial cells, under basal conditions, release negligible amounts of neutrophil chemotactic activity, but respond to a number of stimuli by upmodulating this activity [144]. Stimuli shown to enhance the release of neutrophil chemotactic activity, *in vitro,* include environmentally-derived stimuli, cigarette smoke extract [145], endotoxin [146], organic dust extract [147], and viruses [148], stimuli derived from cells intrinsic to the lungs, cytokines, substance P, neutrophil proteases, and acetylcholine, and other experimentally important stimuli, calcium ionophore, phorbol myristate acetate, and opsonized zymosan [149]. *In vitro* observations have been confirmed *in vivo* using bronchoalveolar lavage, which have demonstrated low basal activity with significant increases of neutrophil chemotactic activities in states of airway inflammation [150].

Whilst many exposures lead to upregulation of the release of neutrophil chemotactic activity, the regulation of release of neutrophil chemotactic activity is complex and involves opposing pathways with stimulatory and inhibitory activities [151]. Increases of intracellular cAMP are associated with attenuation of release of neutrophil chemotactic activity [152]. Thus, substances which are known to stimulate intracellular cAMP levels for which there are airway epithelial cell receptors, such as β-agonists and vasoactive intestinal peptide, are potential downregulators of chemotactic activity release.

The identity of the neutrophil chemotactic activities and the time course of release following stimulation of the epithelial cell has been partially established. Within the first few hours after smoke or lipopolysaccharide (LPS) stimulation, bronchial epithelial cells release arachidonic metabolites. Whilst species differences are likely, leukotriene B_4 (LTB_4) and hydroxyeicosatetraenoic acids (HETEs) with neutrophil chemotactic activity have been reported [146, 153–155]. Although *in vivo* proof is lacking, it has been suggested that this early release of LTB_4 accounts for the initial attraction of neutrophils into the lower respiratory tract. Subsequent to the initial response of the epithelial cells to a provocative stimulus, macrophage stimulation of epithelial cells by TNF or IL-1 is thought to perpetuate the inflammatory response by the release of chemokines.

Chemokines are low molecular weight peptides, which belong to a superfamily consisting of a number of structurally related cytokines characterized by conservation of a four cysteine motif (table 3) [156]. The chemokine superfamily can be divided into two branches, according to the position of the first two cysteines. The C-X-C branch, where the first two cysteines are separated by an intervening residue, includes such chemokines as interleukin-8 (IL-8), platelet factor-4 (PF-4), neutrophil-activating peptide-2 (NAP-2), neutrophil-activating protein from epithelial cells (ENA-78), and melanocyte growth-stimulatory activity. In general, the C-X-C branch of chemokines preferentially attract neutrophils. The other branch of the chemokine superfamily, the C-C branch, where the first two cysteines are not separated by an intervening residue, includes RANTES (regulated upon activation in normal T-cells expressed and secreted), human monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory proteins-1α and -1β (MIP-1α and -1β). The C-C branch of chemokines preferentially attract monocytes, although several members of both branches of the chemokine superfamily have cell specific chemotactic properties (discussed below).

Table 3. – The chemokines

	C-X-C Subfamily	C-C Subfamily
Target cells	Neutrophils	Monocytes
Major members	IL-8	MCP-1
	PF-4	MIP-1α
	NAP-2	MIP-1β
		RANTES
Known airway epithelial cell product	IL-8	RANTES

IL-8: interleukin-8; PF-4: platelet factor-4; NAP-2: neutrophil-activating peptide-2; MCP-1: monocyte chemotactic protein-1; MIP-1α and β: macrophage inflammatory protein-1α and β; RANTES: regulated on activation, normal T-cells, expressed and secreted.

In the context of the lower respiratory tract, there is abundant evidence that airway epithelial cells can be induced to release IL-8, one of the most potent chemotactic factors for neutrophils [148, 157–159]. Although not demonstrated at the time of this writing, it seems likely that other members of the C-X-C branch might also be released by lung epithelial cells. The release of IL-8 by cultured human lung epithelial cells in response to TNF is directionally specific, suggesting that the epithelial cells can direct inflammatory responses to either their basal or apical surface [160]. Corticosteroids are potent inhibitors of IL-8 mRNA transcription, suggesting that airway epithelial cells are a rational target for the therapy of airway inflammatory disorders [158].

The lung macrophage is largely derived from the peripheral blood monocyte [161]. Release of monocyte chemotactic activity by stimulated bronchial epithelial cells has been demonstrated, and is similar in many respects to the stimulated release of neutrophil chemotactic activity [146, 155]. This is not unexpected, since it is often observed clinically that increased numbers of macrophages and neutrophils may coincide in the lower respiratory tract during chronic inflammation. Furthermore, LTB_4 and some of the chemokines are chemotactic for both monocytes and neutrophils [157]. In particular, RANTES has been identified as a product of cultures of the human lung epithelial cell line, A549. Following stimulation with TNF or IL-1, RANTES peptide is found in the supernatant fluid in association with increases in mRNA [162]. This suggests that RANTES may, at least in part, be responsible for the monocyte chemotactic activity derived from lung epithelial cells. It seems likely that other C-C chemokines might be induced in lung epithelial cells.

Airway epithelial cells have several potential mechanisms for the recruitment of eosinophils. RANTES and LTB_4 are both eosinophil chemoattractants [163, 164]. Airway epithelial cells have also been demonstrated to release platelet-activating factor [165], a potent eosinophil chemoattractant [166].

Chemotactic activity for lymphocytes is also released by airway epithelial cells, and has been suggested to account for the presence of lymphocytes in the airway mucosa and bronchial associated lymphoid tissue (BALT) [167]. Separation of bronchial epithelial cell-derived lymphocyte activity on molecular sieve column chromatography reveals several peaks of chemotactic activity which are remarkably similar to results obtained from keratinocytes [167, 168]. Identification of the exact nature of these lymphocyte chemotactic factors is unknown, but RANTES is a chemoattractant for CD4+ lymphocytes [156], and corresponds in molecular weight and lymphocyte specificity to one of the peaks of chemotactic activity released by bronchial epithelial cells [156, 167].

Cell-cell adhesion molecules

A number of recent investigations have demonstrated the importance of intercellular adhesion molecule-1 (ICAM-1) expression by lung epithelial cells. ICAM-1 is the ligand for the CD11/CD18 leucocyte integrins expressed on the surface of neutrophils, monocytes, lymphocytes, and eosinophils [169, 170]. Cell-cell contact mediated by leucocyte integrin/ICAM-1 interaction is thought to be important in inflammatory cell-induced target recognition and cytotoxicity [171]. Thus, leucocyte integrin/ICAM-1 interactions may play important roles in leucocyte adhesion to epithelial cells, followed by maturation and activation of leucocytes in the epithelial compartment. Cytokines, such as TNF, IL-1 and γ-interferon (γ-IFN) appear to contribute to epithelial cell and inflammatory cell interactions by enhancing ICAM-1 expression on epithelial cells [172].

The potential therapeutic implications of the central role of ICAM-1 in airways inflammation have been documented in a primate model of asthma [173]. In these experiments, infiltration of eosinophils into the airway was correlated with epithelial cell expression of ICAM-1. Intravenous administration of anti-ICAM-1 antibodies attenuated both airway eosinophilia as well as bronchial hyperresponsiveness. In human tissue, ICAM-1 receptors have recently been identified on bronchial epithelial cells from asthmatics, but not from nonasthmatics [174]. Whilst not segregating the role of endothelial cells and epithelial cells, these results provide strong support for the role of ICAM-1 in the recruitment and maintenance of airway eosinophilia in association with asthma.

Alveolar epithelial cells have recently been demonstrated to express ICAM-1. In one set of experiments, freshly isolated rat Type II cells did not exhibit ICAM-1 by immunofluorescence. With continuing culture, ICAM-1 was present in low levels after 24 h, and in abundance after 48 h, as the cells developed a Type I cell phenotype [175]. This suggested that ICAM-1 expression in the alveolar epithelium is a function of Type I cells, an assertion confirmed by immunofluorescence in frozen sections of normal lung. The cultured cells exhibited ICAM-1 activity as the ICAM-1 specific monoclonal antibody, MoAb 1A29, blocked adherence of activated T-lymphocytes to the epithelial cells.

These findings were confirmed in a mouse model of hyperbaric lung injury. Using immunocytochemistry and electron-microscopy, it was demonstrated that in normal lungs ICAM-1 expression was limited to Type I cells and tended to be concentrated around cell junctions [176]. With exposure to hyperoxic conditions, the distribution of ICAM-1 on Type I cells changed, becoming more homogeneous on the apical epithelial surface, and Type II cells expressed large amounts of ICAM-1 on their surface. The ability of human Type II cells to express ICAM-1 has been confirmed by immunocytochemical characterization of Type II cells freshly isolated from histologically normal sections of lung removed during resection for lung cancer [177]. Thus, the expression of ICAM-1 by alveolar epithelial cells is responsive to phlogisitic stimuli, and may direct inflammatory cell recruitment and retention in the alveoli.

Pulmonary epithelial cells are capable of expressing major histocompatibility complex (MHC) antigens [178–181]. Expression of MHC antigens allows cells to directly interact with T-lymphocytes, and makes them potential targets for cytotoxic T-cells and candidates for antigen presentation to helper T-cells. The expression of MHC class II antigens (human leucocyte antigen (HLA)-DR, DQ and DP) is relatively low in normal airway epithelium, but can be increased under certain conditions. In cultured epithelial cells, the most potent stimulation for MHC class II expression appears to be γ-IFN [179, 180], and TNF may potentiate γ-IFN effects [180]. Bronchial epithelial cells that express MHC class II antigens are capable of stimulating allogenic lymphocyte reactions [179]. HLA-DR antigens have been identified on freshly isolated human Type II cells [181], raising the possibility that alveolar epithelium may likewise contribute to the stimulation of lymphocytes.

The presence of MHC antigens on epithelial cells is thought to be important for the pathogenesis of airway disease that occurs after lung transplantation [182, 183]. Similar to the aberrant MHC antigen expression described in rejection of other organs, increased MHC expression on epithelial cells in transplanted lungs is probably responsible for immune rejection resulting in obliterative bronchiolitis [183]. A parallel phenomenon probably occurs during the obstructive airways disease that occurs in relation to graft *versus* host disease after allogenic bone marrow transplantation [184].

The ability to express MHC class II antigens raises the possibility that airway epithelial cells are capable of directly presenting soluble antigens to helper T-cells [185, 186]. Whilst bronchial epithelial cells are capable of stimulating allogenic lymphocyte reactions, they appear to be poor at processing and presenting soluble antigens. Enterocytes from the gut have been demonstrated to be

capable of presenting selected antigens, and keratinocytes are capable of presenting some super antigens [187–190]. Thus, whilst direct experimental evidence for antigen presentation by airway epithelial cells is lacking, there remains the possibility that epithelial cells may play a limited role in this regard.

Modulation of inflammatory cell activity

An important aspect of the regulation of lung inflammatory responses is thought to be the modulation of cellular responses by pulmonary epithelial cells. Cytokines, which are chemoattractants for inflammatory cells, also tend to be stimulators of inflammatory cells. Moreover, both airway and alveolar epithelial cell expression of cell-cell adhesion molecules may contribute to the activation of inflammatory cells [173, 175]. However, certain cytokines may demonstrate both anti- and proinflammatory effects, and epithelial cells may release cytokines with predominantly anti-inflammatory effects. Thus, epithelial cells may produce both the critical proinflammatory or anti-inflammatory activities that control the progress of inflammatory events. These observations have lead to the concept that these structural cells play an important role, contributing to the network of cytokines that control chronic airway inflammation [191]. Investigation of the components of this cytokine network have lead to a recognition of complex intracellular interactions between epithelial cells and inflammatory cells.

Many of the chemotactic factors released by airway epithelial cells have also been demonstrated to activate neutrophils, monocytes (or macrophages), eosinophils and lymphocytes. However, as pointed out by MARTIN *et al*. [192], *in vitro* studies do not always correctly predict *in vivo* results. LTB_4 is known to cause neutrophil degranulation and superoxide production *in vitro* [193]. LTB_4 induced the expected neutrophil accumulation when instilled into the lungs of normal volunteers. However, the anticipated increase in protein and markers of neutrophil activation in bronchoalveolar lavage fluid did not occur. These results have been interpreted to suggest that neutrophil activation *in vivo* may be more complex than previously thought, requiring more than one stimulus, with specific concentration or time dependencies.

Recent investigations demonstrate that the presence of inflammatory cells within tissues might not be entirely attributable to the continual attraction of these cells from the vasculature, but also by inhibition of programmed cell death (apoptosis) [194], and epithelial cell-directed maturation of inflammatory cells. Studies with conditioned medium from cultures of human bronchial epithelial cells markedly enhanced the survival of neutrophils, macrophages and eosinophils [195–197]. The survival-enhancing properties of the conditioned media have been attributed to epithelial cell release of granulocyte-macrophage colony-stimulating factor (CSF), granulocyte-CSF or macrophage-CSF alone or in combination [195–197]. The release of growth factors from epithelial cells can also induce monocytic [198], and mast cell [199] differentiation. GM-CSF release is responsive to IL-1 [200], suggesting a mechanism for upregulation in airway inflammation.

The search for the identification of the endothelial-derived relaxing factor led to the appreciation of NO as a pulmonary vasodilatory agent [201]. However, it has also become apparent that NO may have a number of other effects in the lung in addition to smooth muscle relaxation, including neurotransmission, cytotoxicity for micro-organisms and tumour cells, alteration of enzyme activities, and modulation of ciliary beat frequency (reviewed above) [202]. NO has also been demonstrated to modulate inflammatory cell chemotaxis. In the context of the lower respiratory tract and inflammation, neutrophils, macrophages, and bronchial epithelial cells have all been demonstrated to release NO under stimulated conditions. This suggests that a complex interplay may occur between epithelial cells and inflammatory cells *via* NO production [202].

In addition to recruiting and stimulating inflammatory cells, epithelial cells may participate in the down-regulation of inflammatory cells. Transforming growth factor-β (TGF-β) is present in the epithelial lining fluid of the lung, and is present in the epithelium of injured lung [203, 204]. In addition to important effects on matrix production, TGF-β has anti-inflammatory properties, such as the inhibition of interleukin-2 (IL-2)-dependent proliferation of T-cells [205]. TGF-β also inhibits cytokine production by macrophages [206]. Several cell types in the lung have been shown to produce TGF-β, including airway epithelial cells [207–209], and macrophages [210].

Other mediators with anti-inflammatory properties produced by airway epithelial cells include prostaglandin E_2 (PGE_2) and interleukin-6 (IL-6). PGE_2 has a number of anti-inflammatory effects, including reduction in the production of neutrophil chemoattractants by macrophages [211]. IL-6 is capable of reducing inflammation in several models of inflammation, including an *in vivo* model of pulmonary inflammation [212]. In the context that IL-6 also has well-documented proinflammatory effects, such cytokines may be "bifunctional", with differing activities depending on the progression of the inflammatory process.

The alveolar epithelium has also been demonstrated to have immunosuppresive activities. Using the Type II cell-like cell line, A549, a protein with T-cell suppressive activities has been identified [213]. With a molecular weight of about 70 kDa, the protein was found to arrest T-cell proliferation between G_1 and S phase. Whilst not fully characterized, the presence of such a protein in the alveolar space would provide the alveolar epithelium with an important defence against unchecked immune responses to inhaled antigens, thereby protecting the close proximation of airspace and blood needed for optimal gas exchange.

Surfactant can modulate inflammatory cell activity, downregulating lymphocyte functions and enhancing macrophage functions. Studies with whole surfactant have demonstrated inhibition of lymphocyte activities, including natural killer activity, proliferation, and immunoglobulin synthesis [214–218]. The lymphocyte

suppressor activities of surfactant have been isolated to the lipid fraction of surfactant [215], but the mechanisms responsible for the modulation of lymphocyte functions have not been established. In contrast, SP-A is probably responsible for the macrophage enhancing effects of surfactant [219]. SP-A enhances macrophage migration [220], opsonophagocytosis [219, 221, 222], intracellular killing of *Staphylococcus aureus* [221, 223], and killing of tumour cells [224]. SP-A may alter macrophage activities *via* a specific receptor [225].

Interactions with parenchymal cells

Adaptive responses to injury and the repair of epithelium surfaces following injury are essential components of normal immune responses. Experimental evidence suggests that epithelial cells, in addition to interacting with inflammatory cells, interact with pulmonary parenchymal cells. These interactions with smooth muscle cells, endothelial cells, other epithelial cells, and fibroblasts influence airway functions, such as airway tone, vascular permeability, and repair of damaged epithelium.

Smooth muscle cells

Airway epithelial cells are capable of modulating airway smooth muscle cells and, therefore, smooth muscle tone *via* several mechanisms including both direct effects and by modulation of known bronchoconstricting and bronchodilating agents. Cultured airway epithelial cells release substances with direct bronchoconstricting properties, such as endothelin [226], and arachidonic acid-derived lipoxygenase products [227]. Similarly, bronchial epithelial cells also release substances with direct bronchodilatory effect, including PGE_2 [228]. As noted above, the nonprostanoid endothelial-derived relaxing factor [229] has been shown to be primarily NO. Bronchial airway epithelial cells appear to have NOS activity [230]. However, NO does not appear to be responsible for epithelium-derived relaxing factor activity [231].

Airway epithelial cells also influence smooth muscle tone indirectly, by metabolizing agents such as neuropeptides. The membrane of bronchial epithelial cells contain the enzyme neutral endopeptidase (NEP), which degrades the bronchoconstricting neuropeptide substance P [232]. Loss of epithelial cells as a source of NEP in viral infection results in increased substance P-induced bronchoconstriction [233]. Similarly, epithelial cell-associated NEP also modulates the ability of endothelin to induce bronchoconstriction [234].

Thus, airway epithelial cells both release and metabolize substances which modulate airway smooth muscle tone. These activities are regulated by a variety of agents and injury, making the underlying airway responsiveness a balance of multiple interactions. Indirect effects of airway epithelial cells may also have long-term effects on airway smooth muscle. For example, TGF-β, a product of bronchial epithelial cells, can alter smooth muscle β-adrenergic receptors [235], and, thereby, alter the response of smooth muscle to β-agonists.

Endothelial cells

Epithelial cells have the capability of altering endothelial cell function, contributing to vascular permeability and oedema seen in inflammatory pulmonary diseases. As already noted, bronchial epithelial cells express enzymes, such as neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE), and Type I epithelial cells contain carboxypeptidase M, all enzymes capable of degrading neuropeptides released by sensory nerves in the airway [236, 237]. Thus, pulmonary epithelial cells potentially contribute to the containment of neuropeptides. The balance of active peptides and peptidases in the airway may play a role in the pathogenesis of airway inflammation, in that conditions such as cigarette smoke can both stimulate release of neuropeptides and reduce neutral endopeptidase activity [238]. In addition, agents such as corticosteroids can alter epithelial cell NEP expression [239].

Airway epithelial cells contain a variety of other enzymes, which may contribute to the metabolism of inflammatory mediators involved in airway oedema. In particular, epithelial cells are a source of histamine-degrading enzymes, such as histamine N-methyltransferase, and are, thus, capable of modulating histamine effects on vascular permeability [240]. Arachidonate 15-lipoxygenase is located in tracheal epithelial cells, and may influence the generation of metabolites affecting permeability [241]. NO production by bronchial epithelial cells may also contribute to the modulation of endothelial cell function [242–244]. Thus, epithelial cells, which are often exposed at an early stage to a variety of insults and inflammatory mediators, may play important roles in metabolizing those mediators, and subsequently influencing endothelial cell function.

Epithelial cells

Interactions between epithelial cells may be of primary importance in directing repair of injury. After airway epithelial damage, epithelial cells at the edge of the injury appear to flatten out and migrate across the provisional matrix of the wound to close the defect [245]. This ability to migrate is probably influenced by several factors, including the constituents of the extracellular matrix [246]. Soon after injury occurs, increased expression of fibronectin (Fn) and tenascin has been demonstrated in wound matrix, whilst changes in laminin and type IV collagen expression appear later [245]. Fn, in particular, is thought to have a significant role in the modulation of epithelial cell migration. Bronchial epithelial cells *in vitro* demonstrate chemotaxis to Fn using a blindwell chamber technique [247]. Furthermore, bronchial epithelial cells in culture produce chemotactic factor for epithelial cells, which is predominantly Fn [248]. This epithelial cell-derived Fn has been shown to be more potent as a chemotactic factor for airway epithelial cells than plasma-derived Fn [248]. Plasma-derived Fn appears early in a wound [249], but cellular Fn has unique properties that enhance repair [250]. The exact mechanisms by which

bronchial epithelial cell Fn modulates migration are not yet defined.

Airway epithelial cell Fn may differ from plasma Fn as a consequence of mRNA splicing or post-translational modification of the glycoprotein. Two major regions of the Fn gene are typically involved in mRNA splicing [251]. These include the extra domain regions (EIIIA and EIIIB), which are either totally included or excluded, and the variable or IIICS region, which has several splice donor and acceptor sites within the exon. The expression of splice variants has been shown to be cell and tissue specific [252]. Importantly, WANG *et al.* [253] have shown that guinea-pig tracheal epithelial cells preferentially express EIIIA containing Fn from the apical surface of cultures. The unique function of EIIIA containing Fn or other splice variants in airway repair has not been elucidated. However, the potential importance of Fn splice variants in disease is supported by the recent observation that the IIICS Fn variant is involved with the synovial inflammatory response of rheumatoid arthritis [254].

The production of matrix molecules, and Fn in particular, can be influenced by inflammatory mediators present in wounds, such as transforming growth factor-beta (TGF-β) [255, 256]. Importantly, TGF-β has been shown to modulate Fn production from airway epithelial cells *in vitro* [253, 257]. There are multiple sources of TGF-β in the airways, but bronchial epithelial cells themselves produce TGF-β which can function in an autocrine/paracrine fashion to influence epithelial cell phenotypes, including Fn production and cell morphology [258]. TGF-β is also capable of enhancing the expression of the EIIIA Fn splice variant of cultured airway epithelial cells [253]. Additionally, other mediators present in inflamed airways, such as TNF-α and histamine can modulate bronchial epithelial cell Fn production [259, 260]. Thus, the matrix production by epithelial cells is probably influenced by mediators released from epithelial cells themselves, as well as the "traditional" inflammatory cells of the airway.

The migration of epithelial cells to cover an airway wound is probably a series of complex interactions of epithelial cells with the matrix. In epithelial cell sheet migration assays using video microscopy, both cell spreading at the edge of the wound and migration of epithelial sheets have been observed as early features of repair [261]. Sheet migration is enhanced by Fn and epidermal growth factor (EGF) [262], and impaired by TGF-β [263]. TGF-β may slow sheet migration, in part, by increasing adhesion of cells to the matrix [263]. Adhesion of epithelial cells is mediated, in part, by receptor interactions with the matrix. Bronchial epithelial cells are known to express both integrin and non-integrin receptors, which can influence attachment [264–266]. TGF-β enhancement of bronchial epithelial cell attachment is mediated, in part, by integrin receptors for Fn and vitronectin [263]. The relative importance of the many aspects of migration at various stages of repair remains an active area of research.

Proliferation and differentiation of epithelial cells are also necessary in the repair of injured epithelium [267]. Inflammatory mediators released by a variety of cells in the airway, including epithelial cells themselves, are known to influence epithelial cell proliferation and differentiation [268, 269]. TGF-β inhibits epithelial cell growth and induces squamous differentiation [270, 271]. TGF-β from bronchial epithelial cells has been shown to function in an autocrine/paracrine fashion to alter cell morphology [258], and to exert growth regulation [269]. Airway epithelial cell EGF and TGF-β are also thought to regulate growth and differentiation of epithelial cells [269, 272].

At the alveolar level, the Type I cell is the most easily injured cell. Type I cells, owing to their morphology, are the most exposed cells in the alveolus, and lack the capacity for cellular repair and mitosis. Type I cells can be injured by a wide variety of insults, including inhaled environmental agents, *e.g.* ozone [273], NO_2 [274], and volcanic ash [275], infectious agents, *e.g. Pneumocystis carinii* [276], and xenobiotics, *e.g.* cyclophosphamide [277], bleomycin [278], and butylated hydroxytoluene [279]. Type II cells are much more resistant to injury. The normal response to injury of any source is destruction and exfoliation of Type I cells, followed by proliferation of Type II cells [280], and differentiation into the Type I phenotype [280]. However, under conditions of chronic inflammation, Type II hyperplasia results. Hyperplastic Type II cells form a cuboidal epithelium and are hallmarks of such diverse clinical syndromes as idiopathic pulmonary fibrosis and adult respiratory distress syndrome [281].

The control of the proliferative response of Type II cells presumably requires several factors, including loss of contact inhibition by adjacent Type I cells, stimulation with growth factors, and the presence of the appropriate extracellular matrix [282]. A number of growth factors have been identified for cultured Type II cells. These include growth factors such as EGF and TGF-α, which have been identified as airway epithelial cell products, raising the possibility that epithelial cell interactions contribute to repair of alveolar injury.

Migration, proliferation, and differentiation of epithelial cells are integral features of the repair process accompanying airway inflammation. Epithelial cell production of a variety of mediators and matrix constituents contribute to the interaction of epithelial cells with one another and the surrounding matrix to accomplish restoration of the epithelium.

Fibroblasts

Many components of epithelial cell interactions similarly influence surrounding fibroblasts (fig. 2). These epithelial cell-fibroblast interactions also play a significant role in inflammatory repair mechanisms. *In vitro*, bronchial epithelial cells release factors which influence fibroblast migration [283], growth [284], matrix production [285], and retraction. Fibroblast chemotaxis is known to be influenced by bronchial epithelial cell Fn [286]. TGF-β modulates fibroblast chemotaxis [287], and, thus, airway epithelial cell TGF-β may also be involved in fibroblast migration. TGF-β is one of the epithelial cell

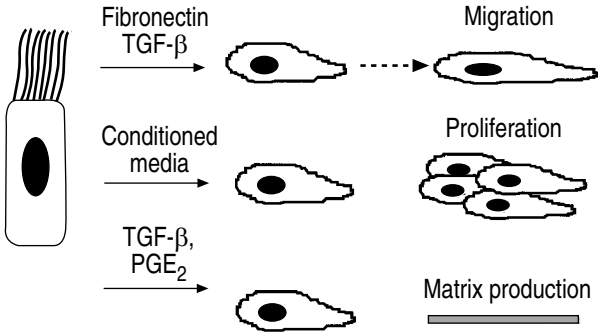

Fig. 2. – Epithelial cells interactions with fibroblasts which may be of import for the repair of injury. Epithelial cells secrete fibronectin and TGF-β which may direct fibroblast migration, uncharacterized substances in conditioned media which stimulate fibroblast proliferation, and TGF-β and PGE_2 which can modulate fibroblast secretion of matrix proteins.

products that appears to increase fibroblast Fn and collagen production [285]. In contrast, PGE_2 is released by bronchial epithelial cells [288], and functions to inhibit fibroblast matrix production [285]. A variety of other mediators known to influence matrix production are also expressed by epithelial cells, such as insulin-like growth factor-1 (IGF-1), IL-1, and IL-6, and probably are involved in epithelial cell-fibroblast interactions in airway repair [289].

Supernatants from bronchial epithelial cell cultures have been shown to contain factors which both stimulate and inhibit fibroblast proliferation [290]. The growth stimulatory factor(s) have not been elucidated, and the inhibitory factor(s) are known to include PGE_2. In addition, exposure of bronchial epithelial cells *in vitro* to endotoxin causes an increase in release of PGE_2. However, in the presence of indomethacin, endotoxin exposure caused a stimulation of fibroblast proliferation [291]. Thus, epithelial cells may modulate fibroblast growth and mediators of inflammation may influence this epithelial-derived growth activity.

Further understanding of repair mechanisms undoubtedly involves better delineation of the interactions of epithelial cell mediators with fibroblasts *in vivo.* The complexity of these interactions may be increased by the observation that fibroblasts in various locations in the airway may respond differently to inflammatory stimuli [292]. Thus, continued investigations are needed to define epithelial cell-fibroblast interactions in airway injury and repair.

Role in disease

The pulmonary epithelium is undoubtedly involved in most pulmonary disease processes. Damage can occur to epithelial cells both in the alveoli and in the airways. Damage of Type I alveolar cells is a regular feature of many acute and chronic interstitial lung diseases [293]. Similarly, damage of the airway epithelium is a regular feature in infectious processes, following toxic exposures, and as a result of immune-mediated injury, and is a regular feature of chronic diseases, such as asthma and chronic bronchitis [293].

The precise roles of individual cellular functions in the pathogenesis of specific diseases are incompletely defined. Nevertheless, loss of barrier and transport functions consequent to alveolar epithelial cell damage may contribute significantly to alveolar flooding with protein rich exudates, potentially setting the stage for secondary infections [294]. It is also possible that loss of host defence capabilities may also lead to the increased incidence of infections to which these patients are thought to be susceptible. Similarly, alterations in epithelial cell function could predispose to secondary infection by decreasing clearance, by altering the composition of airway secretions, and by making the airway surface more susceptible to bacterial colonization [1].

Damage of epithelial cells is undoubtedly linked with the initiation of repair processes. Whether started as part of an inflammatory response or as a response to injury, these processes can lead to stimulation of the mesenchymal cells of the lung, and result in permanent alteration of tissue architecture and function. Deposition of increased amounts of collagen, for example, is a regular feature of both acute and chronic interstitial lung diseases, and can result in severe restrictive ventilatory defects [293, 295, 296]. Similarly, accumulation of collagen within the lumen of the airway, for example in bronchiolitis obliterans, or within the airway wall, for example in chronic obstructive pulmonary disease (COPD), can lead to airflow obstruction [293, 297]. Thus, epithelial cells, by virtue of being linked to the responses of other parenchymal cells in the lung, can contribute to the long-term physiological consequences in many conditions. Finally, the stimulation of proliferation of epithelial cells that takes place following many stimuli may play an important role in the development of malignant neoplasms in the lung [298].

Therapeutic perspectives

There are, at present, no direct therapeutic applications of pulmonary epithelial cell biology which have reached clinical practice. A number of important therapeutic opportunities are, however, on the horizon.

It is likely that current therapies may be targeting the pulmonary epithelium. Cells of the airway epithelium, for example, are importantly involved in the production of mediators in inflammatory diseases, such as asthma and bronchitis. In this regard, airway epithelial cells have receptors for a variety of agents, including beta-agonists, acetylcholine, histamine and neuropeptides [1]. It thus seems likely that many well-established therapies may, in part, be due to effects on the airway epithelial surface. The importance of the airway epithelium as a target for widely-used medications is probably only partially explored. Erythromycin, for example, can directly affect epithelial mucin production [299], and such an action may account for the widely-recognized benefits of erythromycin during exacerbations of airways disease.

The ability to deliver therapeutic agents directly to the epithelial surface of the airways offers a number of important opportunities. Gene therapy, for example, can

be delivered directly to the airway surface using a variety of vectors, including viruses which are "predesigned" to target the airway epithelium [300, 301]. With such approaches, it is possible to introduce novel genetic material into the airway epithelium. Such strategies offer a myriad of therapeutic opportunities. Several protocols for gene therapy for cystic fibrosis are currently in progress. It has proved possible to introduce the CFTR gene into airway epithelial cells. This has been accomplished both in animal models [302, 303], and in human patients [304]. In patients with cystic fibrosis, introduction of the normal gene has been associated with normalization of airway potential differences. It seems likely that with such strategies it will be possible not only to correct underlying genetic defects, but also to modulate epithelial behaviour in acquired diseases.

Airway epithelial cells are also responsible for the production of a number of agents which determine lung function. The use of glucocorticoids to stimulate foetal lung production of surfactant by epithelial cells is a well-established therapy aimed indirectly at Type II alveolar epithelial cells [305]. These cells are also importantly involved in other parenchymal lung diseases, including both acute lung injury and chronic interstitial fibrotic disorders [293]. It seems likely that current studies defining the specific mediators released by cells of the alveolar epithelial surface which help to both drive and control these pathophysiological processes will open new therapeutic opportunities.

Finally, cancer frequently originates in the epithelial cells of the airway [298]. How carcinogens, for example from cigarette smoke, interact with cells of the airway and lead to the development of the neoplastic process is an area which has been under investigation for many years. Enzyme systems for xenobiotic metabolism which may activate or inactivate carcinogens have been related to cancer susceptibility. In addition, the genetic basis for the regulatory control of cell proliferation, including both oncogenes and anti-oncogenes, has been intensively studied. Whilst such studies have not yet yielded direct therapeutic information, it seems likely that information gained from such studies will contribute to therapies designed to either limit or treat neoplastic processes.

In conclusion, the pulmonary epithelium is composed of well structured, but heterogenous populations of cells. The cells which reside in the pulmonary epithelium play important roles in the maintenance of normal lung function. The airway epithelium provides a mechanical barrier to the surface of the airway lumen, contributes to host defence through mucociliary clearance and secretion, maintains airway tone by modulating airway smooth muscle and vascular responses, modulates pulmonary inflammatory responses, and directs repair of injury to the airway. The cells which line the alveoli serve predominantly to maintain optimal conditions for gas exchange. However, *via* the newly recognized immunomodulatory activities of SP-A, lymphocyte inhibitory factor, and expression of ICAM-1, these cells are becoming recognized as participants in local immune responses.

Morphological and functional disordering of the pulmonary epithelium cause hypersecretion, airway obstruction, abnormal fibrotic healing, and, in turn, ventilation/perfusion abnormalities. Hopefully, continuing insight into the cell biology of pulmonary epithelial function will to lead to new, rational therapies directed towards the pathogenesis of lung diseases, thereby preventing the physiological consequences of lung diseases upon their unfortunate victims.

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