The airway epithelium: more than just a structural barrier

Anthony Tam, Samuel Wadsworth, Delbert Dorscheid, S.F. Paul Man and Don D. Sin

Ther Adv Respir Dis 2011 5: 255 originally published online 3 March 2011
DOI: 10.1177/1753465810396539

The airway epithelium: more than just a structural barrier

Ther Adv Respir Dis
(2011) 5(4) 255–273
DOI: 10.1177/1753465810396539

Anthony Tam, Samuel Wadsworth, Delbert Dorscheid, S.F. Paul Man and Don D. Sin

***Abstract*:** The mammalian airway is lined by a variety of specialized epithelial cells that not only serve as a physical barrier but also respond to environment-induced damage through the release of biologically active factors and constant cellular renewal. The lung epithelium responds to environmental insults such as pathogens, cigarette smoke and pollution by secreting inflammatory mediators and antimicrobial peptides, and by recruiting immune cells to the site of infection or damage. When the epithelium is severely damaged, basal cells and Clara cells that have stem-cell-like properties are capable of self-renewal and proliferation in the affected area, to repair the damage. In order to effectively fight off infections, the epithelium requires the assistance of neutrophils recruited from the peripheral circulation through transendothelial followed by transepithelial migration events. Activated neutrophils migrate across the epithelium through a series of ligand–receptor interactions to the site of injury, where they secrete proteolytic enzymes and oxidative radicals for pathogen destruction. However, chronic activation and recruitment of neutrophils in airway diseases such as chronic obstructive pulmonary disease and asthma has been associated with tissue damage and disease severity. In this paper, we review the current understanding of the airway epithelial response to injury and its interaction with inflammatory cells, in particular the neutrophil.

***Keywords*:** airway epithelium, endothelium, gap junctions, immune barrier, neutrophils, tight junction

Introduction

The airway epithelium is a dynamic tissue that undergoes slow but constant renewal [Crystal *et al.* 2008]. Since the lung is constantly exposed to inhaled pathogens (such as bacteria and viruses) and particulate matter (including diesel exhaust and wood smoke) from the environment, the airway epithelium must play a critical role in maintaining normal airway function from the trachea to the alveoli. The airway epithelium is composed of a variety of cell types such as ciliated, mucous, basal, and Clara cells in the bronchial epithelium, and Type I and Type II cells in the alveolar epithelium (Figure 1). Pseudostratification of the airway epithelium is defined by the differing heights of the cells and nuclei within the cells, yet all of the cells are resting on the epithelial basement membrane. In addition to acting as a physical barrier, the lung epithelium regulates lung fluid balance, modulates metabolism and clearance of inhaled agents, and secretes numerous mediators, several of which recruit and activate inflammatory cells in response to injury or infection [Knight and Holgate, 2003]. Dysregulation of airway epithelial cell function related to environmental triggers may contribute to the pathogenesis of major lung diseases such as chronic obstructive pulmonary disease (COPD) and asthma. In this paper, we review the various roles of the airway epithelium as an innate immune organ, and its interaction with inflammatory cells specifically neutrophils, in response to environmental challenges.

Components of the airway epithelium

Pulmonary neuroendocrine cells

The pulmonary neuroendocrine cell (PNEC) system originated from the ancestors of air-breathing vertebrates such as the primitive phyla (amphibians, reptiles) to advanced (birds, mammals) organisms [Rogers, 1989]. During lung development, PNECs are the first cell type to form and differentiate within the airway

Correspondence to:
Don D. Sin, MD, MPH
UBC James Hogg Research Centre, St Paul's Hospital, Room 166, 1081 Burrard Street, Vancouver, BC, Canada V6Z 1Y6
don.sin@hli.ubc.ca

Anthony Tam, BSc
Samuel Wadsworth, BSc, MSc, PhD
Delbert Dorscheid, MD, PhD
S.F. Paul Man, MD, FRCPC
The UBC James Hogg Research Centre, Providence Heart and Lung Centre and Department of Medicine, University of British Columbia (UBC), Vancouver, BC, Canada

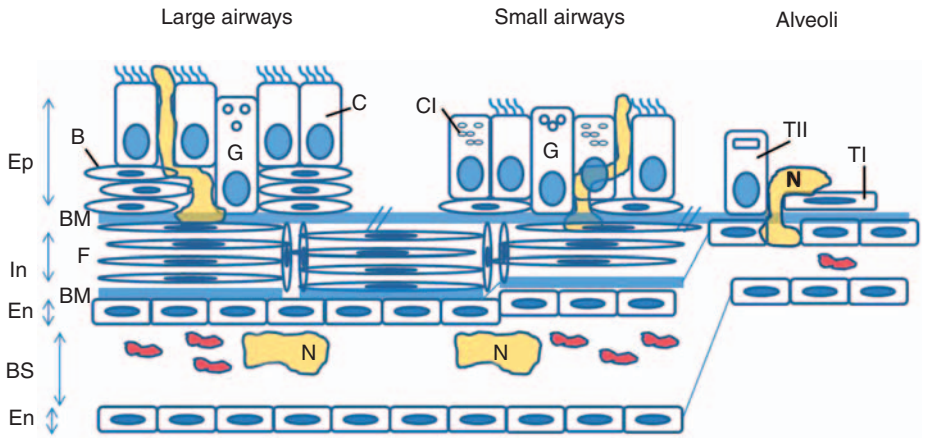

Figure 1. Major cell types of the lung epithelium. In the large airways, the major cell types are ciliated columnar (C), goblet (G) and basal (B) cells. In the small airways, the cell types are similar with relatively more Clara cell (Cl) type. The endothelium (En) separates the epithelium (Ep) from the blood stream (BS). As the small airways merge with the alveolar epithelium, Type I (TI) and Type II (TII) cells become predominant. Immune cells such as neutrophils are shown to migrate from the blood to the air space through the endothelium, endothelial basement membrane (BM), interstitial tissues (In) containing type I fibroblasts (F) (parallel to epithelium) and type II myofibroblast (perpendicular to epithelium), and finally through the epithelium via a series of ligand–receptor interactions discussed in the text. Not shown are a variety of less common cell types such as cartilage cells and neuroendocrine cells.

epithelium, increase in number at birth and peak during the neonatal period [Gustafsson *et al.* 2008]. In the postnatal phase, PNEC system represents the lung stem cell niche that is central to airway epithelial regeneration and lung carcinogenesis [Cutz *et al.* 2007]. In healthy individuals, there is approximately one PNEC per 2500 epithelial cells [Gosney *et al.* 1988]. PNECs are typically tall and pyramidal in shape, extend from the basal lamina of the epithelium and possess apical microvilli [Gustafsson *et al.* 2008]. PNECs are located within the epithelium lining the larynx, trachea, bronchi, and down to the bronchiole–alveolar junctions [Lauweryns *et al.* 1985]. The interaction of PNECs with neural elements and smooth muscles facilitate the regulatory properties of bronchomotor and vascular tone [Adriaensen *et al.* 2006].

Bronchial epithelium

Basal cells. Basal cells (BCs) are highly abundant in the large airway (50%) and in the small airways (81%) [Boers *et al.* 1998]. They are relatively undifferentiated and characteristically express transcription factor Trp-63 (p63) and cytokeratins 5 and 14 (Krt5/14) [Evans *et al.* 2001]. BCs have sparse electron-dense cytoplasm and appear to be the only cells that express hemidesmosomes, which are firmly attached to the basement membrane via integrins ($\alpha 6\beta 4$) [Evans *et al.* 1989]. In rodents, BCs are populated among ciliated and secretory cells in the trachea [Rock *et al.* 2009], whereas BCs in the human lungs are present throughout the airways including the bronchioles [Evans *et al.* 2001]. However, the cell count decreases as the airways get progressively smaller [Evans and Plopper, 1988]. There is a correlation between the thickness of the epithelium and the number of basal cells and columnar cells attached to the basement membrane [Knight and Holgate, 2003]. BCs have also been demonstrated to possess stem-cell-like properties in that they can self-renew and give rise to secretory and ciliated epithelial cells in response to epithelial injury [Hong *et al.* 2004]. In addition to their structural and progenitor roles, basal cells have been shown to produce a variety of bioactive molecules including neutral endopeptidase, 15-lipoxygenase products and cytokines [Knight and Holgate, 2003].

Columnar ciliated epithelial cells. Ciliated epithelial cells account for over 50% of all epithelial cells within the human airways [Spina, 1998]. They are believed to be terminally differentiated cells that arise from either basal or secretory cells [Ayers and Jeffery, 1988]. Typically, these cells possess up to 300 cilia per cell and a large number of mitochondria are found immediately

beneath the apical surface, which are responsible for providing energy to the cilia for mucous clearance up and out of the airways via coordinated ciliary beating [Harkema, 1991].

Mucous goblet cells. Mucous cells are defined by electron-lucent acidic-mucin granules that secrete mucous into the airway to trap foreign objects such as pathogens and dust particles [Jeffery, 1983]. In normal human trachea, it is estimated that there are up to 6800 mucous-secreting cells/mm^2 of surface epithelium [Lumsden *et al.* 1984]. The mucous layer present in the airway from the level of the trachea to the bronchioles consists of a mixture of highly glycosylated mucin proteins. In the normal airways, there is a fine equilibrium between mucous production and clearance [Evans and Koo, 2009]. However, in chronic airway inflammatory diseases such as chronic bronchitis and asthma, mucous cell hyperplasia and metaplasia occur, which lead to excessive sputum production [Lumsden *et al.* 1984]. Mucous cells are capable of self-renewal and may also transdifferentiate into ciliated epithelial cells [Evans and Plopper, 1988].

Clara cells. In humans, Clara cells contain electron-dense granules and are found in the small airways [Knight and Holgate, 2003]. The regulation of the activity of potentially harmful proteinases secreted by neutrophils during inflammation is important for the prevention of excessive tissue damages [Sallenave *et al.* 1994]. To regulate bronchiolar epithelial integrity and immunity, Clara cells have been shown to produce bronchiolar surfactants and specific antiproteases such as secretory leukocyte protease inhibitor [De Water *et al.* 1986]. In addition, Clara cells can produce p450 mono-oxygenases [De Water *et al.* 1986], which are able to metabolize xenobiotic compounds such as aromatic hydrocarbons, which are found in cigarette smoke (CS). Recent evidence has suggested that these cells may also possess important stem cell potential and may act as progenitors for both ciliated and mucous-secreting cells [Hong *et al.* 2001].

Basement membrane. The dense basement membrane of extracellular matrix (ECM) molecules beneath the airway epithelium has several important roles in maintaining epithelial integrity: (i) it acts as an anchor facilitating adhesion of epithelial cells; (ii) it establishes and maintains correct cellular polarity, (iii) it acts as a barrier between the surface epithelium and the underlying mesenchymal compartment, and (iv) it provides essential survival signals to the epithelium [Wadsworth *et al.* 2004; Boudreau *et al.* 1996; Terranova *et al.* 1980]. The upper layer of the basement membrane, the lamina densa, consists of type IV collagen and laminin (predominantly type V) secreted by epithelial cells [Knight and Holgate, 2003] (Figure 2). The lower lamina reticularis layer consists of type III and V collagen and fibronectin and is synthesized by subepithelial fibroblasts [Paulsson, 1992]. It has long been speculated that infiltrating immune cells migrate across the epithelium to the site of injury via enzymatic digestion of the extracellular matrix within the basement membrane. However, recent studies have demonstrated that the basement membrane also contains pores, with average density of 737–863/mm^2 and a mean diameter of 1.76 μm, through which immune cells can transverse without perturbing the extracellular matrix [Howat *et al.* 2002, 2001].

Alveolar epithelium

Gas exchange occurs in the alveolus. Alveolar walls comprise greater than 99% of the internal surface area of the mammalian lung [Dobbs and Johnson, 2007]. A very thin layer of liquid is found on the epithelial surface of the alveolus. The fluid lining the alveolus is composed of surfactant phospholipids and an aqueous subphase, which together has an average thickness that ranges from 0.1 to 0.9 μm [Bastacky *et al.* 1995]. Surfactants are responsible for the regulation of surface tension and gas exchange within the alveolus. Surface tension provided by the surfactant is responsible for keeping the alveolar space open and is important for elastic recoil of the lung. The volume and contents of alveolar fluid is tightly regulated by local cellular mechanisms [Dobbs and Johnson, 2007]. The regulation of alveolar ion and liquid transport has been shown *in vivo* and in isolated lungs to be dependent on alveolar epithelial cells, which are the major sites of Na^+ transport and fluid absorption [Olivera *et al.* 1994]. The alveolar epithelium facing the air-filled compartment is mostly composed of Type I (TI) and Type II (TII) cells.

TI cells. TI cells have extremely thin cytoplasmic extensions that cover more than 98% of the internal surface of the lung, thus providing a narrow anatomic barrier between the air and blood compartment [Dobbs and Johnson, 2007]. Although TI cells have been thought to be biologically

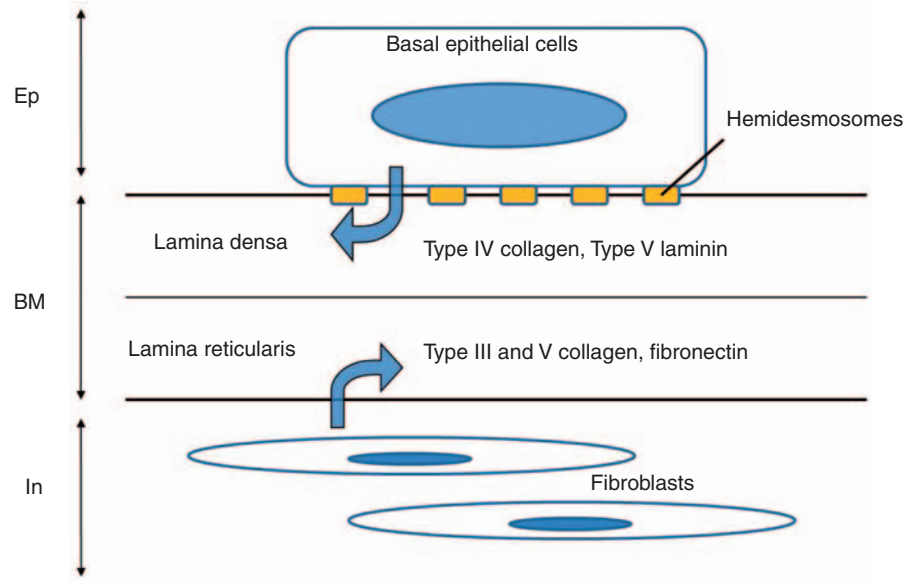

Figure 2. The basement membrane (BM) is made up of different types and forms of extracellular matrix components and is divided in two major layers: lamina densa (type IV collagen, and type V laminin) predominantly secreted by basal epithelial cells and the lamina reticularis (type III and V collagen and fibronectin) primarily synthesized by subepithelial fibroblasts in the interstitium. Basal epithelial cells adhere to the lamina densa via specialized structures, hemidesmosomes.

inert, the presence of caveolae [Gumbleton, 2001] and small intracellular vacuoles have suggested that TI cells may have endocytic function and metabolic activities [Dobbs and Johnson, 2007]. Similar to TII cells, TI cells contain microvilli, abundant mitochondria, and both rough and smooth endoplasmic reticulum, suggesting that they may also perform active biosynthetic functions [Dobbs and Johnson, 2007].

TII cells. Unlike TI cells, TII cells are smaller, cuboidal cells characterized by their distinctive secretory granules called lamellar bodies, intracellular storage organelles for pulmonary surfactants [Dobbs and Johnson, 2007]. TII cells are commonly known for the synthesis, secretion, and reuptake of pulmonary surfactants [Wright and Hawgood, 1989]. In addition, TII cells can also act as progenitor cells following injury to the alveolar epithelium [Wright, 2005].

Mucociliary functions in the airway epithelium

Two primary functions of the airway epithelium are mucous production and mucociliary transport where mucous cells and ciliated epithelial cells work in collaboration to trap and remove inhaled foreign materials from the airways [Kilburn, 1968].

As the predominant form of mucin in the human airway, MUC5AC is produced mainly in goblet cells in the surface epithelium, while MUC5B is produced mainly in mucous cells of the submucosal glands [Wickstrom *et al.* 1998; Hovenberg *et al.* 1996]. MUC5AC may represent an acute-response mucin as a result of direct contact with environmental insults, whereas MUC5B may be involved in the response to chronic infection and inflammation [Thornton *et al.* 2008]. Mucin production has been shown to be regulated by inflammatory mediators, such as lipopolysaccharide (LPS) [Smirnova *et al.* 2003], tumour necrosis factor alpha (TNF-α) [Yoon *et al.* 1999], interleukin 1 (IL-1) [Yoon *et al.* 1999], IL-17 [Chen *et al.* 2003], IL-13 [Danahay *et al.* 2002] and β neutrophil elastase [Voynow *et al.* 1999], growth factors such as epidermal growth factor (EGF), transforming growth factor α (TGF-α) [Takeyama *et al.* 1999], and environmental insults such as CS [Shao *et al.* 2004] and bacteria [Kohri *et al.* 2002]. In addition, it has been shown that female sex hormone such as estrogen can upregulate MUC5B gene expression in normal human nasal epithelial cells [Choi *et al.* 2009; Fujimoto *et al.* 2004; Helmi *et al.* 1975].

According to the mucociliary transport model proposed by Yates and colleagues in 1980

[Yates *et al.* 1980], a viscoelastic layer of mucous floats on the periciliary layer tethered to the apical cell surface by mucins 1, 4 and 16 and glycolipids [Sheehan *et al.* 2006]. Hydration of the apical epithelial surface has been shown to be regulated by the release, metabolism and retention of nucleotides on airway surfaces [Lazarowski *et al.* 2003]. The mucous layer acts as a fluid reservoir by accepting or donating liquid to maintain normal airway surface liquid (ASL) level that approximates the height of the outstretched cilia (6–7 μm) [Leopold *et al.* 2009; Tarran *et al.* 2001]. To ensure correct mucous movement in the airway, cilia beat in a highly coordinated fashion. During the effective stroke, the ciliary tips penetrate into the mucous layer, and during the recovery stroke, they withdraw from this layer [Yates *et al.* 1980]. Reverdin and colleagues have demonstrated the presence of actin in the basal body region of ciliated cells in rat trachea [Reverdin *et al.* 1975], suggesting that this contractile protein may participate in the ciliary movement and in the coordination of the ciliary beat. Cilia are anchored to the apical surface of ciliated cells via specialized structures known as basal bodies [Dalen, 1983]. The presence of claw-like structures on the cilia have been suggested to serve as a special anchorage device for pushing the entrapped particulate matter on the mucous sheet towards the pharynx [Jeffery and Reid, 1975]. Ciliary beating is an energetic process, reflected by the high numbers of mitochondria found in the apical regions of ciliated cells [Harkema, 1991].

Regulation of ASL and mucociliary clearance is critical for maintaining normal airway function. In normal airways, the cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial Na^+ channel (ENaC) (that coexist in the apical plasma membrane of airway epithelial cells with Ca^+-activated chloride channel [CaCC], outwardly rectifying Cl^- channel [ORCC], and Cl^- channel 2 [CLC2]) are fully functional [Zeitlin, 2008]. The combination of Cl^- secretion and reduced Na^+ reabsorption favours a healthy ion composition and depth of airway surface liquid, which enables effective ciliary beat for proper mucociliary clearance [Zeitlin, 2008]. In chronic airway diseases such as cystic fibrosis, CFTR is absent or dysfunctional and ENaC is no longer regulated, leading to hyperabsorption of Na^+ and an increased driving force for fluid reabsorption [Zeitlin, 2008]. The ASL depth is reduced, the mucosal glands are hypertrophied and excessive mucous is secreted [Zeitlin, 2008]. The excessive production of viscous mucous impairs mucociliary clearance, resulting in airflow obstruction and bacterial colonization of the lungs [Zeitlin, 2008].

In other rarer diseases, impaired mucociliary transport is the direct result of defective ciliary function. Patients with ciliary dyskinesia for example secrete normal amounts and types of airway mucous, however clearance from the airway is compromised due to disordered ciliary beating [Noone *et al.* 2004; Bush *et al.* 1998].

Properties and functions of junctional complexes in cell–cell communication

In the intact airway epithelium, junctional complexes at cell–cell contact sites are control points that play important roles in maintaining lung epithelial function by regulating the flow of solute across the epithelium (through tight junctions) and from one cell to another (through gap junctions). They form a tight barrier around the apical membrane of the cells resulting in close contact with each other [Farquhar and Palade, 1963]. Adhesion junctions including cadherin-containing adherens junctions and desmosomes play vital roles in maintaining the structural integrity of the epithelium by promoting direct cell–cell adhesion. For the purpose this review, we briefly address tight junctions and gap junctions on the airway epithelium. For a more extensive review on airway junctional complexes, please refer to the additional sources [Green and Jones, 1996; Schneeberger and Lynch, 1992].

Tight junctions

Several tight-junction-associated proteins have been identified, including occludin and claudins, and junctional adhesion molecules such as zonula occludens (ZO-1, ZO-2 and ZO-3), [Harhaj and Antonetti, 2004], which are important for epithelial integrity. The ZO family plays a central role in orchestrating tight junction complexes by interacting with the cytoplasmic C-terminal region of the occludin for structural stability [Harhaj and Antonetti, 2004]. ZO-1, in particular, has also been shown to regulate cell cycle and may be involved in the genesis of certain malignancies [Polette *et al.* 2007; Gonzalez-Mariscal *et al.* 2000]. ZO-1 has been used extensively as a marker of epithelial integrity [Bolton *et al.* 1998] and may be of clinical relevance in

monitoring morphologic changes and permeability of the bronchial epithelium in CS-induced respiratory diseases such as COPD [Evans *et al.* 2002]. In an *in vitro* airway epithelial model, the addition of matrix metalloproteinase 9 (MMP9) to the apical epithelium led to an increase in transepithelial conductance and a decrease in tight junction protein expression [Vermeer *et al.* 2009], suggesting that increased levels of MMP9 in asthmatics may play an important role in airway remodelling.

Gap junctions

Cells in the bronchial and alveolar epithelium are interconnected by gap junction channels that enable transport of antioxidants, cytoplasmic metabolites and signals in neighbouring cells [Koval, 2002]. TI and TII alveolar cells express at least six gap junction proteins (connexin) that are involved in intracellular signalling between homogenous (TI–TI and TII–TII cells) and heterogeneous (TI–TII cells) cultures of alveolar cells [Boitano *et al.* 2004]. Alveolar epithelial cells have the capacity to transmit signals both directly through gap junctions and indirectly through nucleotide secretion and paracrine stimulation of purinergic receptors [Boitano *et al.* 2004]. In one study, mechanical stimulation of TI cells *in situ* increased intracellular Ca^{+2} concentrations, which led to an influx of Ca^{+2} to pass through gap junctions and stimulated secretion of surfactant proteins in neighbouring TII cells [Ashino *et al.* 2000].

The airway epithelium as an immune barrier

In addition to being a physical barrier, the airway epithelium also acts an immune barrier via specific Fas (CD95/Apo-1) and Fas-ligand (FasL, CD95L, CD178) interactions [Hamann *et al.* 1998; Fine *et al.* 1997; Uhal *et al.* 1995]. The Fas receptor is a type I membrane-receptor protein belonging to the TNF family, it is ubiquitously expressed on structural and inflammatory cells [Watanabe-Fukunaga *et al.* 1992]. Fas receptor interaction with membrane-bound FasL results in the activation of a cascade of intracellular caspases leading to apoptosis of the Fas-presenting cell [Muzio *et al.* 1997; Itoh *et al.* 1991]. FasL is predominantly expressed on immune cells but has also been shown to be expressed in some structural cells including the corneal epithelium, Sertoli cells of the testes [French *et al.* 1996; Griffith *et al.* 1995] and the airway epithelium [Gochuico *et al.* 1998] at sites of 'immune privilege'.

Experimental data suggest that expression of FasL at these sites protects against tissue injury by inducing apoptosis in infiltrating immune cells during immunologic reactions and infections [French *et al.* 1996; Griffith *et al.* 1995]. For example, removal of airway epithelium in guinea pigs results in infiltration of the airway wall by eosinophils, while restitution of the epithelium is associated with eosinophilic apoptosis [Erjefalt *et al.* 1996]. During active allergen-induced airway inflammation, there is a downregulation of FasL expression in the airway epithelium [Gochuico *et al.* 1998], suggesting that FasL may be a key player in protecting the epithelial barrier from tissue injuries. Interestingly, bronchial epithelial expression of MMP7 is increased in asthmatic patients, and IL-13 stimulation of airway epithelial MMP7 *in vitro* has been shown to trigger FasL cleavage from the membrane into a soluble form [Wadsworth *et al.* 2010]. Soluble FasL released after MMP7 cleavage cannot trigger Fas trimerization and apoptosis but can function as a pro-inflammatory chemokine for neutrophils [Ottonello *et al.* 1999; Schneider *et al.* 1998; Seino *et al.* 1998], thus contributing to persistent chronic airway neutrophilic inflammation and epithelial damage in asthma.

Responses of epithelium to environmental factors

The lung epithelium is an important physical barrier, which acts as a shield against airborne toxins such as CS, airborne pollutants such as particulate matter, and foreign pathogens such as bacteria and viruses. However, the epithelium is also able to respond to injurious stimuli by releasing soluble factors important in stimulating and directing the cellular innate immune response.

CS has been shown to trigger the production of IL-1β and IL-8 from bronchial epithelial cells *in vitro*, while TNF-α and IL-6 are released by CS-treated alveolar macrophages [Mio *et al.* 1997]. IL-1β produced from the bronchial epithelium can induce the release of neutrophils from bone marrow, while IL-8 promotes neutrophil chemotaxis to the site of damage [Chung, 2001]. Elevated protein expressions of MCP-1, TGF-β1, IL-8 and their mRNA have also been observed in the bronchiolar epithelium from smokers with COPD compared with those without COPD [de Boer *et al.* 2000]. CS extract has also been shown to cause disassembly of tight junctions, which are critical in regulating the flow of solutes

through the paracellular pathway [Petecchia *et al.* 2009]. CS condensate-induced tight junction disassembly were partially blocked by the MEK inhibitor (U0126) and were completely blocked by the EGFR inhibitor, suggesting that the EGFR-MAPK ERK1/2 pathway may be one of the mechanisms by which toxic chemicals from CS may cause cellular damage [Luppi *et al.* 2007].

In addition to the secretion of inflammatory mediators and chemotactic mediators, the airway epithelium also exhibits certain features that enable protection against bacterial infections. Mechanisms that recognize pathogens by the airway are essential to mount protective responses of the innate immune system [Bals and Hiemstra, 2004]. To date, 10 Toll-like receptors (TLRs) have been well documented in human airway epithelial cells [Kaisho and Akira, 2006; Uematsu and Akira, 2006; Akira and Takeda, 2004], which have been shown to be involved in a variety of gene regulations including cytokines, chemokines and antimicrobial peptides. TLRs can be broadly divided into those that exhibit antibacterial (TLR-1, TLR-2, TLR-4, TLR-5, TLR-6 and TLR-9) and antiviral responses (TLR-3, TLR-7 and TLR-8) [Bals and Hiemstra, 2004]. Under normal resting conditions, TLR-4 is expressed minimally in the intracellular compartment of airway epithelial cells [Guillot *et al.* 2004], and are generally unresponsive to Gram-negative bacteria endotoxin (LPS) exposure [Monick *et al.* 2003]. However, respiratory syncytial virus (RSV) induced TLR-4 mRNA, proteins and membrane localization in airway epithelial cells *in vitro* [Monick *et al.* 2003]. Unlike primary airway epithelial cells, when TLR4 positive human airway epithelial cell lines are challenged with bacterial endotoxin (LPS), IL-6 and CXCL8/IL-8 proteins become upregulated, suggesting that TLR4 activation leads to the production of pro-inflammatory mediators [Guillot *et al.* 2004]. TLR-2 has been shown to respond to lipoproteins and lipoteichoic acid (LTA) from Gram-positive bacteria [Lien *et al.* 1999]. TLR-5 recognises flagellin, which is a component of the flagellae responsible for bacterial motility [Hayashi *et al.* 2001]. For a more extensive review on TLRs and their mechanistic pathways, please refer to these selected articles [Chaudhuri and Sabroe, 2008; Bals and Hiemstra, 2004].

Furthermore, epithelial cells secrete small antimicrobial peptides such as β-defensins and LL-37, which are involved in the growth inhibition of inhaled microorganisms until removal by phagocytosis and/or mucociliary clearance [Bals and Hiemstra, 2004]. Bacteria, including *Pseudomonas aeruginosa* [Li *et al.* 1997], *Staphylococcus aureus* [Lemjabbar and Basbaum, 2002], *Streptococcus pneumoniae* [Ha *et al.* 2007], nontypeable *Haemophilus influenzae* [Wang *et al.* 2002] and *Mycoplasma pneumoniae* [Kraft *et al.* 2008] can activate cell surface receptors resulting in nuclear factor (NF)-κB activation and *MUC*2 and/or *MUC*5AC transcriptional upregulation [Rose and Voynow, 2006]. Production of mucous by the airway epithelium is a natural defence mechanism against foreign pathogens such as bacteria by trapping them and facilitating their clearance through the mucociliary elevator.

Epithelial damage and repair

When the airway epithelium is damaged, tissue repair becomes critical to re-establish the integrity of the damaged tissue. However, the precise mechanisms by which the epithelium repairs itself are still controversial. In an *in vivo* study where the tracheal epithelium of guinea pig was wounded, epithelial cells including the secretory and ciliated cells near the damaged area began to dedifferentiate, flatten and migrate over the denuded area after 15 minutes (Figure 3) [Erjefalt *et al.* 1995]. The damaged area then becomes covered by a tight layer of flattened undifferentiated epithelium before epithelial redifferentiation restores the functional integrity of the tissue [Erjefalt *et al.* 1995]. These data suggest that the lung epithelium is capable of self-renewal and proliferation after injury. Increased expression of specific integrins such as αvβ1 and αvβ6 are present on the basal surface of regenerating epithelium to promote regeneration of the physical barrier [Horiba and Fukuda, 1994]. In addition, there is increased expression of fibronectin, collagen IV and laminin in bovine tracheal epithelial cell culture lesions, which promote the restoration of the basement membrane matrix and allow epithelial cell migration [Rickard *et al.* 1993]. Stem cell capacity has been shown in resident side population cells (~0.1% of total epithelial cell population) in the human airways where they had sustained colony-forming capacity, maintained telomere length over serial passage, and the ability to form a differentiated, multilayered epithelium [Hackett *et al.* 2008]. Within the population of epithelium cells, Clara cell secretory protein (CCSP)-expressing cells of proximal airways

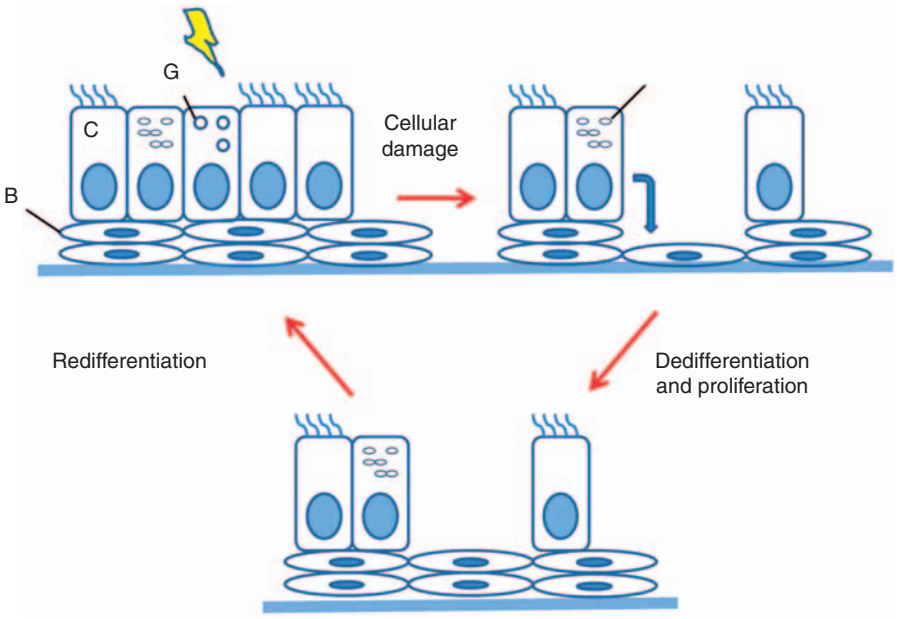

Figure 3. When the epithelium is damaged by toxic chemicals such as cigarette smoke, cells undergo cell death. Clara cells (Cl) and basal cells (B) have been shown to have stem-cell-like properties for self-renewal and re-establishment of the intact epithelium. These cells are able to undergo dedifferentiation, proliferation and redifferentiation into goblet cells (G) and ciliated epithelial cells.

behaved like classical transit-amplifying (TA) cells such as those of the intestinal epithelium, whereas CCSP-expressing cells of bronchiolar airways behaved more like the self-renewing progenitor cells present within the interfollicular epidermis [Chen *et al.* 2009].

Regulation of immune cells in the airway

Alveolar macrophages

The alveolar macrophage (AM) is the resident mononuclear phagocyte in the alveolar air spaces. It plays an important role in the defence against microorganisms and pollutants from the environment. The AM has a large internal compartment of organelles and inclusions which contain a vast repertoire of enzymes, which can be used to destroy organisms encountered from the external environment [Mehta *et al.* 2008]. When the lung is challenged by bacteria, AMs become activated and produce reactive nitric oxide (NO) [Pendino *et al.* 1993], which is regulated through an inducible form of nitric oxide synthase (iNOS) by LPS and TNF-α [Lowenstein *et al.* 1993]. AM mediates bacterial killing by releasing NO and other reactive oxygen and nitrogen species [Nathan, 1987]. CS, on the other hand, has damaging effects on AM that lead to compromised cellular functions. In an *in vitro* study by Aoshiba and colleagues, mouse, rat and human AMs cultured in CS extract experienced a decrease in the capacity to kill and clear inflammatory cells and debris from the lungs [Kirkham *et al.* 2004; Aoshiba *et al.* 2001]. Chronic CS exposure also increases the production of proteolytic enzymes such as the gelatinases (MMP2 and MMP9) and macrophage metalloelastase (MMP12) in AMs, which are involved in lung tissue destruction [Taraseviciene-Stewart and Voelkel, 2008]. Mice with knocked out genes for MMP12 do not develop increased airspace size after chronic smoke exposure. However, it should be noted that alveolar macrophages are just one potential source of MMPs in the airway, epithelial cells, neutrophils, fibroblasts and smooth muscle cells also contribute to protease activity in the lung (refs)

Neutrophils

The neutrophil plays a pivotal role in human host defence against bacterial infection [Howard *et al.* 1990]. Polymorphonuclear neutrophils (PMNs) belong to the innate immune system and constitute the main defence against invading bacteria and fungi [Zarbock and Ley, 2009]. An early response to infection is the recruitment of

neutrophils from the circulation with migration to the affected tissue. Neutrophil adhesion to the microvascular endothelium is thus an early event in the induction of an acute inflammatory response [Tonnesen *et al.* 1989]. Neutrophils respond to these stimulants by secreting granule contents and generating oxygen radicals in order to penetrate the tissue barrier to the site of infection and degrade foreign pathogens [Russo *et al.* 1981]. However, massive secretion of granule contents and oxygen radicals during infections may cause damage to adjacent cells and connective tissue structures [Fantone and Ward, 1982]. Recent *in vitro* studies have demonstrated that human neutrophils exposed to small amounts of LPS prior to stimulation with FMLP have enhanced production of granule components [Haslett *et al.* 1985] and cause increased damage to cultured vascular endothelial cells [Smedly *et al.* 1986].

Neutrophil migration

In addition to the secretion of powerful proteolytic enzymes to digest microbial pathogens, an integral function of the neutrophil is the ability to travel through the vasculature and into the site of infection [Zemans *et al.* 2009]. During its journey into the lung, neutrophils pass through the endothelium, interstitial tissues and epithelium before arriving in the airspaces. Neutrophil migration consists of three major sequential events: (1) transendothelial migration, (2) basement membrane and interstitial migration, and (3) transepithelial migration.

Neutrophil transendothelial migration

Neutrophils exit from the circulation and emigrate through the endothelium in three major phases: leukocyte adhesion, rolling and transmigration. The initial step of neutrophil adhesion is mediated by interactions between L-, E- and P-selectins that are expressed on leukocytes, and P-selectin glycoprotein ligand (PSBL1) that are expressed on the apical surface of vascular endothelium [Zemans *et al.* 2009]. Selectin-triggered signalling in neutrophils mediates slow rolling, which is followed by complete arrest of the neutrophils on the endothelial surface via β1 and β2 integrins expressed on leukocytes and adhesion molecules (intercellular adhesion molecule [ICAM] and vascular adhesion molecule [VCAM]) on the endothelium [Zemans *et al.* 2009]. The final phase of neutrophil transmigration involves spreading and crawling, which are mediated by interactions between CD11b/CD18 (αmβ2 or Mac-1) on the neutrophil and ICAM on the endothelium [Zemans *et al.* 2009]. Neutrophils then migrate across the endothelium through either a paracellular route involving platelet endothelial cell adhesion molecules (PECAM)1 and junctional adhesion molecules (JAMs), or a transcellular route involving ICAM1, PECAM1 and caveolins on the endothelium [Zemans *et al.* 2009]. *In vitro* studies have demonstrated that neutrophil adherence to cultured large vessel endothelial cells (human umbilical vein, bovine aorta and porcine aorta) is increased in the presence of a chemotactic stimulus [Hoover *et al.* 1978]. These chemotactic stimuli include chemotactic peptides, formyl-methionyl-leucyl-phenylalanine (FMLP) and C5a, and the lipid mediators, leukotriene B4 (LTB4) and platelet activating factor (PAF), which enhanced human neutrophil adherence to cultured human microvascular endothelial cells via expression of Mac-1, LFA-1, p150,95 glycoprotein family on the neutrophil surfaces [Tonnesen *et al.* 1989].

Neutrophil migration across basement membrane

Proteases

After migrating across the endothelium, neutrophils must first penetrate the subendothelial basement membrane before gaining access to the interstitial space [Burns *et al.* 2003]. Whether this process involves proteolytic degradation of the basement membrane is controversial. Observations of neutrophil migration have not provided evidence of significant disruption of the basement membrane [Shaw, 1980; Marchesi and Gowans, 1964]. Although neutrophil elastase and gelatinase B have a high capacity for ECM degradation, normal neutrophil migration at inflammatory foci has been observed in mice that are genetically deficient of elastase [Belaaouaj *et al.* 1998] or gelatinase B [Betsuyaku *et al.* 1999], suggesting that the penetration of neutrophils across the basement membrane does not require proteases. In addition, reports have shown that fibronectin associated with subendothelial basement membrane [Matzner *et al.* 1990], and type IV collagen (a major constituent of alveolar capillary basement membrane) [Amenta *et al.* 1988; Zibrak *et al.* 1985; Sage, 1982; Kefalides *et al.* 1979] act as protective substrates and inhibit neutrophil activation, resulting in reduced potential for damage

as neutrophils migrate from the blood to the airspaces.

Pores in basement membrane

Neutrophils are believed to gain access to the interstitium and subsequently the epithelium by migrating through pre-existing holes in the endothelial and epithelial basement membranes, respectively [Behzad *et al.* 1996; Walker *et al.* 1995]. In control rabbits, neutrophils were observed to pass through small holes in the endothelial basement membrane at the same loci occupied by fibroblasts [Walker *et al.* 1995], suggesting that basement membrane holes occupied by fibroblasts are sites through which neutrophils migrate. However, before a neutrophil is allowed to pass through the hole, the fibroblast extensions must be withdrawn or displaced [Burns *et al.* 2003]. This mechanism has been demonstrated in dermal explants where mechanical stress leads to fibroblast retraction and causes the long cytoplasmic extrusions of myofibroblasts to become spherical and lose contact with the ECM [Burns *et al.* 2003]. Evidence from electron microscopy suggests that the holes must be at least 1 μm in diameter for neutrophil migration [Walker *et al.* 1995].

Neutrophil migration across interstitium: fibroblast as source of contact and stimulation

As neutrophils cross the subendothelial basement membrane of airway vessels, they emerge into the interstitium [Burns *et al.* 2003]. The interstitium is composed of two main types of fibroblast; the first is associated with fibrous connective tissues and is oriented parallel to the epithelium, whereas the second type is a myofibroblast that is stellate, perpendicular to the epithelium [Burns *et al.* 2003], and capable of contraction [Kapanci *et al.* 1992; Leslie *et al.* 1992]. Myofibroblasts within the airway wall are connected by adherens-like junctions on the cytoplasmic extrusions [Burns *et al.* 2003]. Myofibroblasts have been shown to act as a bridge from pre-existing holes in the subendothelium, through the interstitium, to the holes in the basement membrane of the subepithelium [Behzad *et al.* 1996]. The close contact between the dense fibrillar cytoplasm of the neutrophil and the fibroblast supported the idea that this proximal association is an adhesive contact [Behzad *et al.* 1996]. Unlike the myofibroblasts, ECM elements including collagen and elastin are not arranged in a manner that provide a continuous structure that bridge between holes in the endothelial and epithelial basement membrane, suggesting that fibroblasts play a more directional role in guiding neutrophil migration than the ECM [Burns *et al.* 2003]. Several *in vitro* studies have shown that activated neutrophils adhere to skin and lung fibroblasts through a CD18-dependent interaction [Giuliani *et al.* 1993; Shock and Laurent, 1991]. Furthermore, fibroblasts have also shown to respond to TNF-α by secreting IL-8 proteins, which are chemotactic for neutrophils [Burns *et al.* 1996]. Collectively, this evidence suggests that lung fibroblasts can function as an adhesive substrate and a source of stimulation for neutrophil migration.

Neutrophil transepithelial migration

Once the neutrophil has traversed the epithelial basement membrane, the subsequent migration across the lung epithelia can be categorized into three sequential stages: adhesion, migration and postmigration events [Zen and Parkos, 2003]. The initial step of neutrophil transepithelial migration is characterized by adhesion of neutrophils to the basolateral surface of the epithelium by ligation of CD11b/CD18 on the neutrophil surface to molecules on the epithelial surface including fucosylated proteoglycans and JAMs [Zen and Parkos, 2003]. Unlike transendothelial migration, neutrophils have only been shown to travel through the epithelium via the paracellular route. Neutrophils migrate between cells via sequential binding to a number of epithelial cell surface molecules such as CD47 via signal regulatory protein (SIRP-α) on the neutrophil [Zen and Parkos, 2003]. At the level of the tight junction, neutrophil JAM-L binds to epithelial coxsackie and adenovirus receptor (CAR) [Zen and Parkos, 2003]. After the neutrophils have completely migrated across the epithelial monolayer, they bind to the apical surface of the epithelium and constitute a defence barrier via important neutrophil–apical cell interactions including the binding of FcR to apical antigen, neutrophil CD97 to epithelial DAF, and CD11b/CD18 to ICAM on the epithelium [Zen and Parkos, 2003].

In vitro models of transepithelial neutrophil migration

Cell culture model

Much of our knowledge of the mechanism of neutrophil transepithelial migration has derived from cell culture models [Walker *et al.* 1995]. Earlier studies employed alveolar epithelial cells

cultured on the upper surface of porous tissue culture inserts where neutrophils were allowed to migrate in a nonphysiologic apical to basolateral direction [Lin *et al.* 1995]. More recently, epithelial cells are cultured on the underside of the insert such that neutrophils can migrate physiologically from the basolateral surface to the apical surface [Parkos *et al.* 1991]. To further improve the model of neutrophil transendothelial and transepithelial migration, some groups have developed a more sophisticated cell culture model where endothelial cells and alveolar epithelial cells are grown on opposite sides of the insert to investigate transendothelial and transepithelial neutrophil migration [Hu *et al.* 2005]. These cell culture models have been used to examine the effect of respiratory drugs on neutrophil migration since they resemble the existing blood–air barrier in the airway epithelium.

Airway epithelial cell culture in air–liquid interface models *in vivo* epithelial properties

Primary human airway epithelial cells grown in air–liquid interface cultures (Figure 4) have been shown to express similar properties to the intact airway epithelium *in vivo* [Wadsworth *et al.* 2006; Choe *et al.* 2003]. Much of our knowledge from the interactions between environmental and inflammatory stimuli, and the airway epithelium has been derived extensively from *in vitro* cell culture models. Some of these functional phenotypes of the pseudostratified epithelial layer include tightly packed cells that form a barrier to the external elements with intercellular junctions such as desmosomes, ciliagenesis and mucous secretion from goblet cells [Wadsworth *et al.* 2006; Choe *et al.* 2003]. High transepithelial resistance in fully differentiated cell cultures reflects the tight cellular packing and junctional adhesions formed in the intact airway epithelium *in vivo* [Rowe *et al.* 2004].

The airway epithelial surface is covered by a thin layer of fluid, which serves to protect the underlying epithelium [Candiano *et al.* 2007]. One group has used a proteomics approach to identify proteins secreted from the airway surface liquid of differentiated human airway epithelium and has found that pro-inflammatory mediators such as IL-4, IL-1β, TNF-α and IFN-γ modified the ion transport properties by changing the expression and activity of ion channels like the CFTR, the epithelial sodium channel and, and the Ca^{+2}-dependent Cl^{-} channel [Candiano *et al.* 2007]. Similarly in another *in vitro* study, IL-13, an inflammatory mediator associated with asthma and chronic sinusitis [Humbert *et al.* 1997; Hamilos *et al.* 1996; Huang *et al.* 1995], alters the volume and composition of epithelial lining fluid by converting the human bronchial epithelium from an absorptive to a secretory phenotype [Danahay *et al.* 2002]. In chronic lung diseases such as asthma and COPD, the airway may also be subjected to epithelial wounding. The effect of glucocorticoids on epithelial wound repair [Wadsworth *et al.* 2006] and neutrophil migration via fibroblasts [Gao and Issekutz, 1997] have been examined in cell culture models. Taken together, *in vitro* studies have shown that differentiated cell culture is an invaluable model in understanding the physiological properties of the human airway epithelium. In the next section, we specifically examine the effect of inhaled steroids on neutrophil migration in cell culture models.

Effect of drugs on neutrophil migration

Airway inflammation in COPD is associated with increased numbers of macrophages and T lymphocytes in the airway wall [Di Stefano *et al.* 1996; Keatings *et al.* 1996], and neutrophils in the airway lumen [Saetta *et al.* 2001; O'Shaughnessy *et al.* 1997]. Studies have shown that neutrophils recruited to the site of inflammation by microbial products are able to recruit additional neutrophils by secreting IL-8, TNF-α and IL-1β [van Wetering *et al.* 2002; Cassatella, 1995]. TNF-α and IL-1β have been shown to induce endothelial adhesion molecule expression [Kaiser *et al.* 1993], suggesting that neutrophil–endothelial interactions via inflammatory cytokines may potentiate inflammation, creating a cycle of damage to the airways via excessive release of free radicals and proteases in chronic airway diseases.

Corticosteroids such as dexamethasone, budesonide, prednisolone, and fluticasone are commonly used in asthma and COPD patients to reduce airway inflammation. The primary role by which glucocorticoids mediate their actions is through modulating expression of genes, involved in metabolism, immunological responses and inflammation [Munck *et al.* 1990; Schleimer, 1990]. One of the important effects of steroids is to reduce adherence of polymorphonuclear cells to endothelial cells [van Overveld *et al.* 2000; McGillen and Phair, 1979], and infiltration of inflammatory cells from the blood to the tissues [McGillen and Phair, 1979]. *In vitro*

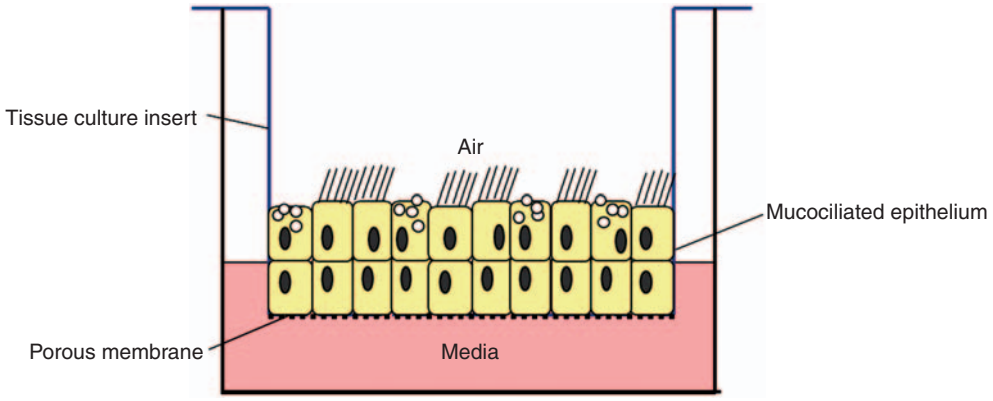

Figure 4. Airway epithelial cells are cultured on a semipermeable membrane in an air—liquid interface system where the apical cells are in contact to the air and the basolateral compartments are submerged in media. This model resembles the blood—air barrier in the airway epithelium.

studies have demonstrated that corticosteroids are able to inhibit chemotaxis of neutrophils [Lomas *et al.* 1991; Rivkin *et al.* 1976], and superoxide anion generation [Fuenfer *et al.* 1979]. Previous studies have shown that dexamethasone reduces the binding of P-selectin on endothelial cells to circulating leukocytes [Yamaki *et al.* 1998], and inhibits both LPS- and IL-1β-induced synthesis and expression of E-selectin and ICAM-1 on endothelial cells [Cronstein *et al.* 1992]. In another *in vitro* study, dexamethasone, budesonide and prednisolone individually inhibited LPS-induced leukocyte migration in a dose-dependent manner [van Overveld *et al.* 2003]. However, more studies must be conducted to elucidate the exact mechanism of these respiratory drugs.

Conclusion

The airway epithelium is not simply a physical barrier to environmental pollutants and pathogens. The airway epithelium is composed of specialized cells that have unique structures and functions. The epithelium is an active part of the innate immune system that responds to environmental challenges by secreting inflammatory mediators to trigger immune cell recruitment to sites of injury and by secreting antimicrobial agents. When the epithelium senses pathogens or pollutants via specialized receptors, neutrophils are recruited to the site of injury through transendothelial and transepithelial migration involving a series of ligand—receptor interactions. Neutrophils, in turn, unleash a series of digestive enzymes which kill their target. In a normal healthy individual, airway inflammation as a result of pathogens or pollutants will subside when the stimulants are removed. However, in chronic airway diseases, persistent neutrophilic infiltration in the lungs may lead to tissue damage from the unfocussed action of cytotoxins that they release. Therefore, cell culture models, in addition to *in vivo* models, have been used to examine the effect of drugs on cytokine and chemokine production by neutrophils and the airway epithelium, to examine neutrophil migration from the blood to the airspaces in various respiratory conditions. By exploiting the growing understanding of the epithelium and its interactions with inflammatory cells, it may be possible in the near future to develop pharmacologic compounds to treat the characteristic epithelial dysregulation which contributes to or even causes various inflammatory lung conditions.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

Anthony Tam declared no competing interests. Samuel Wadsworth declared no competing interests. Delbert Dorscheid declared no competing interests. S. F. Paul Man received educational grants from Glaxo-Smith-Kline and Astro-Zenecca to support research. Don D. Sin holds a Canada Research Chair in COPD and a senior clinical scholarship with the Michael Smith Foundation for Health Research (MSFHR)

References

Adriaensen, D., Brouns, I., Pintelon, I., De Proost, I. and Timmermans, J.P. (2006) Evidence for a role of neuroepithelial bodies as complex airway sensors: comparison with smooth muscle-associated airway receptors. *J Appl Physiol* 101: 960–970.

Akira, S. and Takeda, K. (2004) Toll-like receptor signalling. *Nat Rev Immunol* 4: 499–511.

Amenta, P.S., Gil, J. and Martinez-Hernandez, A. (1988) Connective tissue of rat lung. II: Ultrastructural localization of collagen types III, IV, and VI. *J Histochem Cytochem* 36: 1167–1173.

Aoshiba, K., Tamaoki, J. and Nagai, A. (2001) Acute cigarette smoke exposure induces apoptosis of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 281: L1392–L1401.

Ashino, Y., Ying, X., Dobbs, L.G. and Bhattacharya, J. (2000) [Ca(2+)](i) oscillations regulate type II cell exocytosis in the pulmonary alveolus. *Am J Physiol Lung Cell Mol Physiol* 279: L5–L13.

Ayers, M.M. and Jeffery, P.K. (1988) Proliferation and differentiation in mammalian airway epithelium. *Eur Respir J* 1: 58–80.

Bals, R. and Hiemstra, P.S. (2004) Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J* 23: 327–333.

Bastacky, J., Lee, C.Y., Goerke, J., Koushafar, H., Yager, D., Kenaga, L. *et al.* (1995) Alveolar lining layer is thin and continuous: low-temperature scanning electron microscopy of rat lung. *J Appl Physiol* 79: 1615–1628.

Behzad, A.R., Chu, F. and Walker, D.C. (1996) Fibroblasts are in a position to provide directional information to migrating neutrophils during pneumonia in rabbit lungs. *Microvasc Res* 51: 303–316.

Belaaouaj, A., McCarthy, R., Baumann, M., Gao, Z., Ley, T.J., Abraham, S.N. *et al.* (1998) Mice lacking neutrophil elastase reveal impaired host defense against Gram negative bacterial sepsis. *Nat Med* 4: 615–618.

Betsuyaku, T., Shipley, J.M., Liu, Z. and Senior, R.M. (1999) Neutrophil emigration in the lungs, peritoneum, and skin does not require gelatinase B. *Am J Respir Cell Mol Biol* 20: 1303–1309.

Boers, J.E., Ambergen, A.W. and Thunnissen, F.B. (1998) Number and proliferation of basal and parabasal cells in normal human airway epithelium. *Am J Respir Crit Care Med* 157: 2000–2006.

Boitano, S., Safdar, Z., Welsh, D.G., Bhattacharya, J. and Koval, M. (2004) Cell-cell interactions in regulating lung function. *Am J Physiol Lung Cell Mol Physiol* 287: L455–L459.

Bolton, S.J., Anthony, D.C. and Perry, V.H. (1998) Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. *Neuroscience* 86: 1245–1257.

Boudreau, N., Werb, Z. and Bissell, M.J. (1996) Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. *Proc Natl Acad Sci U S A* 93: 3509–3513.

Burns, A.R., Simon, S.I., Kukielka, G.L., Rowen, J.L., Lu, H., Mendoza, L.H. *et al.* (1996) Chemotactic factors stimulate CD18-dependent canine neutrophil adherence and motility on lung fibroblasts. *J Immunol* 156: 3389–3401.

Burns, A.R., Smith, C.W. and Walker, D.C. (2003) Unique structural features that influence neutrophil emigration into the lung. *Physiol Rev* 83: 309–336.

Bush, A., Cole, P., Hariri, M., Mackay, I., Phillips, G., O'Callaghan, C. *et al.* (1998) Primary ciliary dyskinesia: diagnosis and standards of care. *Eur Respir J* 12: 982–988.

Candiano, G., Bruschi, M., Pedemonte, N., Musante, L., Ravazzolo, R., Liberatori, S. *et al.* (2007) Proteomic analysis of the airway surface liquid: modulation by proinflammatory cytokines. *Am J Physiol Lung Cell Mol Physiol* 292: L185–L198.

Cassatella, M.A. (1995) The production of cytokines by polymorphonuclear neutrophils. *Immunol Today* 16: 21–26.

Chaudhuri, N. and Sabroe, I. (2008) Basic science of the innate immune system and the lung. *Paediatr Respir Rev* 9: 236–242.

Chen, H., Matsumoto, K. and Stripp, B.R. (2009) Bronchiolar progenitor cells. *Proc Am Thorac Soc* 6: 602–606.

Chen, Y., Thai, P., Zhao, Y.H., Ho, Y.S., DeSouza, M.M. and Wu, R. (2003) Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 278: 17036–17043.

Choe, M.M., Sporn, P.H. and Swartz, M.A. (2003) An in vitro airway wall model of remodeling. *Am J Physiol Lung Cell Mol Physiol* 285: L427–L433.

Choi, H.J., Chung, Y.S., Kim, H.J., Moon, U.Y., Choi, Y.H., Van Seuningen, I. *et al.* (2009) Signal pathway of 17beta-estradiol-induced MUC5B expression in human airway epithelial cells. *Am J Respir Cell Mol Biol* 40: 168–178.

Chung, K.F. (2001) Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 34: 50s–59s.

Cronstein, B.N., Kimmel, S.C., Levin, R.I., Martiniuk, F. and Weissmann, G. (1992) A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A* 89: 9991–9995.

Crystal, R.G., Randell, S.H., Engelhardt, J.F., Voynow, J. and Sunday, M.E. (2008) Airway epithelial cells: current concepts and challenges. *Proc Am Thorac Soc* 5: 772–777.

Cutz, E., Yeger, H. and Pan, J. (2007) Pulmonary neuroendocrine cell system in pediatric lung disease-recent advances. *Pediatr Dev Pathol* 10: 419–435.

Dalen, H. (1983) An ultrastructural study of the tracheal epithelium of the guinea-pig with special reference to the ciliary structure. *J Anat* 136: 47–67.

Danahay, H., Atherton, H., Jones, G., Bridges, R.J. and Poll, C.T. (2002) Interleukin-13 induces a hypersecretory ion transport phenotype in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 282: L226–L236.

Danahay *et al.* (2004).

de Boer, W.I., Sont, J.K., van Schadewijk, A., Stolk, J., van Krieken, J.H. and Hiemstra, P.S. (2000) Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. *J Pathol* 190: 619–626.

De Water, R., Willems, L.N., Van Muijen, G.N., Franken, C., Fransen, J.A., Dijkman, J.H. *et al.* (1986) Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. *Am Rev Respir Dis* 133: 882–890.

Di Stefano, A., Turato, G., Maestrelli, P., Mapp, C.E., Ruggieri, M.P., Roggeri, A. *et al.* (1996) Airflow limitation in chronic bronchitis is associated with T-lymphocyte and macrophage infiltration of the bronchial mucosa. *Am J Respir Crit Care Med* 153: 629–632.

Dobbs, L.G. and Johnson, M.D. (2007) Alveolar epithelial transport in the adult lung. *Respir Physiol Neurobiol* 159: 283–300.

Erjefalt, J.S., Erjefalt, I., Sundler, F. and Persson, C.G. (1995) In vivo restitution of airway epithelium. *Cell Tissue Res* 281: 305–316.

Erjefalt, J.S., Sundler, F. and Persson, C.G. (1996) Eosinophils, neutrophils, and venular gaps in the airway mucosa at epithelial removal-restitution. *Am J Respir Crit Care Med* 153: 1666–1674.

Evans, C.M. and Koo, J.S. (2009) Airway mucus: the good, the bad, the sticky. *Pharmacol Ther* 121: 332–348.

Evans, M.J., Cox, R.A., Shami, S.G., Wilson, B. and Plopper, C.G. (1989) The role of basal cells in attachment of columnar cells to the basal lamina of the trachea. *Am J Respir Cell Mol Biol* 1: 463–469.

Evans, M.J. and Plopper, C.G. (1988) The role of basal cells in adhesion of columnar epithelium to airway basement membrane. *Am Rev Respir Dis* 138: 481–483.

Evans, M.J., Van Winkle, L.S., Fanucchi, M.V. and Plopper, C.G. (2001) Cellular and molecular characteristics of basal cells in airway epithelium. *Exp Lung Res* 27: 401–415.

Evans, S.M., Blyth, D.I., Wong, T., Sanjar, S. and West, M.R. (2002) Decreased distribution of lung epithelial junction proteins after intratracheal antigen or lipopolysaccharide challenge: correlation with neutrophil influx and levels of BALF sE-cadherin. *Am J Respir Cell Mol Biol* 27: 446–454.

Fantone, J.C. and Ward, P.A. (1982) Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am J Pathol* 107: 395–418.

Farquhar, M.G. and Palade, G.E. (1963) Junctional complexes in various epithelia. *J Cell Biol* 17: 375–412.

Fine, A., Anderson, N.L., Rothstein, T.L., Williams, M.C. and Gochuico, B.R. (1997) Fas expression in pulmonary alveolar type II cells. *Am J Physiol* 273: L64–L71.

French, L.E., Hahne, M., Viard, I., Radlgruber, G., Zanone, R., Becker, K. *et al.* (1996) Fas and Fas ligand in embryos and adult mice: ligand expression in several immune-privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover. *J Cell Biol* 133: 335–343.

Fuenfer, M.M., Carr Jr, E.A. and Polk Jr, H.C. (1979) The effect of hydrocortisone on superoxide production by leukocytes. *J Surg Res* 27: 29–35.

Fujimoto, N., Suzuki, T., Honda, H. and Kitamura, S. (2004) Estrogen enhancement of androgen-responsive gene expression in hormone-induced hyperplasia in the ventral prostate of F344 rats. *Cancer Sci* 95: 711–715.

Gao, J.X. and Issekutz, A.C. (1997) The beta 1 integrin, very late activation antigen-4 on human neutrophils can contribute to neutrophil migration through connective tissue fibroblast barriers. *Immunology* 90: 448–454.

Giuliani, A.L., Spisani, S., Cavalletti, T., Reali, E., Melchiorri, L., Ferrari, L. *et al.* (1993) Fibroblasts increase adhesion to neutrophils after stimulation with phorbol ester and cytokines. *Cell Immunol* 149: 208–222.

Gochuico, B.R., Miranda, K.M., Hessel, E.M., De Bie, J.J., Van Oosterhout, A.J., Cruikshank, W.W. *et al.* (1998) Airway epithelial Fas ligand expression: potential role in modulating bronchial inflammation. *Am J Physiol* 274: L444–L449.

Gonzalez-Mariscal, L., Namorado, M.C., Martin, D., Luna, J., Alarcon, L., Islas, S. *et al.* (2000) Tight junction proteins ZO-1, ZO-2, and occludin along isolated renal tubules. *Kidney Int* 57: 2386–2402.

Gosney, J.R., Sissons, M.C. and Allibone, R.O. (1988) Neuroendocrine cell populations in normal human lungs: a quantitative study. *Thorax* 43: 878–882.

Green, K.J. and Jones, J.C. (1996) Desmosomes and hemidesmosomes: structure and function of molecular components. *FASEB J* 10: 871–881.

Griffith, T.S., Brunner, T., Fletcher, S.M., Green, D.R. and Ferguson, T.A. (1995) Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270: 1189–1192.

Guillot, L., Medjane, S., Le-Barillec, K., Balloy, V., Danel, C., Chignard, M. *et al.* (2004) Response of human pulmonary epithelial cells to lipopolysaccharide involves Toll-like receptor 4 (TLR4)-dependent signaling pathways: evidence for an intracellular compartmentalization of TLR4. *J Biol Chem* 279: 2712–2718.

Gumbleton, M. (2001) Caveolae as potential macromolecule trafficking compartments within alveolar epithelium. *Adv Drug Deliv Rev* 49: 281–300.

Gustafsson, B.I., Kidd, M., Chan, A., Malfertheiner, M.V. and Modlin, I.M. (2008) Bronchopulmonary neuroendocrine tumors. *Cancer* 113: 5–21.

Ha, U., Lim, J.H., Jono, H., Koga, T., Srivastava, A., Malley, R. *et al.* (2007) A novel role for IkappaB kinase (IKK) alpha and IKKbeta in ERK-dependent up-regulation of MUC5AC mucin transcription by Streptococcus pneumoniae. *J Immunol* 178: 1736–1747.

Hackett, T.L., Shaheen, F., Johnson, A., Wadsworth, S., Pechkovsky, D.V., Jacoby, D.B. *et al.* (2008) Characterization of side population cells from human airway epithelium. *Stem Cells* 26: 2576–2585.

Hamann, K.J., Dorscheid, D.R., Ko, F.D., Conforti, A.E., Sperling, A.I., Rabe, K.F. *et al.* (1998) Expression of Fas (CD95) and FasL (CD95L) in human airway epithelium. *Am J Respir Cell Mol Biol* 19: 537–542.

Hamilos, D.L., Leung, D.Y., Wood, R., Bean, D.K., Song, Y.L., Schotman, E. *et al.* (1996) Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha. *Am J Respir Cell Mol Biol* 15: 443–450.

Harhaj, N.S. and Antonetti, D.A. (2004) Regulation of tight junctions and loss of barrier function in pathophysiology. *Int J Biochem Cell Biol* 36: 1206–1237.

Harkema, J.R., Mariassy St, A., George, J., Hyde, D.M. and Plopper, C.G. (1991) Epithelial cells of the conducting airways: a species comparison. In: Farmer, S.G. and Hay, D.W.P. (eds), The Airway Epithelium: Physiology, Pathophysiology, and Pharmacology, Marcel Dekker: New York, pp. 3–39.

Haslett, C., Guthrie, L.A., Kopaniak, M.M., Johnston Jr, R.B. and Henson, P.M. (1985) Modulation of multiple neutrophil functions by preparative methods or trace concentrations of bacterial lipopolysaccharide. *Am J Pathol* 119: 101–110.

Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R. *et al.* (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410: 1099–1103.

Helmi, A.M., El Ghazzawi, I.F., Mandour, M.A. and Shehata, M.A. (1975) The effect of oestrogen on the nasal respiratory mucosa. An experimental histopathological and histochemical study. *J Laryngol Otol* 89: 1229–1241.

Hong, K.U., Reynolds, S.D., Giangreco, A., Hurley, C.M. and Stripp, B.R. (2001) Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. *Am J Respir Cell Mol Biol* 24: 671–681.

Hong, K.U., Reynolds, S.D., Watkins, S., Fuchs, E. and Stripp, B.R. (2004) In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am J Physiol Lung Cell Mol Physiol* 286: L643–L649.

Hoover, R.L., Briggs, R.T. and Karnovsky, M.J. (1978) The adhesive interaction between polymorphonuclear leukocytes and endothelial cells in vitro. *Cell* 14: 423–428.

Horiba, K. and Fukuda, Y. (1994) Synchronous appearance of fibronectin, integrin alpha 5 beta 1, vinculin and actin in epithelial cells and fibroblasts during rat tracheal wound healing. *Virchows Arch* 425: 425–434.

Hovenberg, H.W., Davies, J.R. and Carlstedt, I. (1996) Different mucins are produced by the surface epithelium and the submucosa in human trachea: identification of MUC5AC as a major mucin from the goblet cells. *Biochem J* 318: 319–324.

Howard, T.H., Wang, D. and Berkow, R.L. (1990) Lipopolysaccharide modulates chemotactic peptide-induced actin polymerization in neutrophils. *J Leukoc Biol* 47: 13–24.

Howat, W.J., Barabas, T., Holmes, J.A., Holgate, S.T. and Lackie, P.M. (2002) Distribution of basement membrane pores in bronchus revealed by microscopy following epithelial removal. *J Struct Biol* 139: 137–145.

Howat, W.J., Holmes, J.A., Holgate, S.T. and Lackie, P.M. (2001) Basement membrane pores in human bronchial epithelium: a conduit for infiltrating cells? *Am J Pathol* 158: 673–680.

Hu, M., Du, Q., Vancurova, I., Lin, X., Miller, E.J., Simms, H.H. *et al.* (2005) Proapoptotic effect of curcumin on human neutrophils: activation of the p38 mitogen-activated protein kinase pathway. *Crit Care Med* 33: 2571–2578.

Huang, S.K., Xiao, H.Q., Kleine-Tebbe, J., Paciotti, G., Marsh, D.G., Lichtenstein, L.M. *et al.* (1995) IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 155: 2688–2694.

Humbert, M., Durham, S.R., Kimmitt, P., Powell, N., Assoufi, B., Pfister, R. *et al.* (1997) Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol* 99: 657–665.

Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S., Sameshima, M. *et al.* (1991) The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66: 233–243.

Jeffery, P.K. (1983) Morphologic features of airway surface epithelial cells and glands. *Am Rev Respir Dis* 128: S14–S20.

Jeffery, P.K. and Reid, L. (1975) New observations of rat airway epithelium: a quantitative and electron microscopic study. *J Anat* 120: 295–320.

Kaiser, J., Bickel, C.A., Bochner, B.S. and Schleimer, R.P. (1993) The effects of the potent glucocorticoid budesonide on adhesion of eosinophils to human vascular endothelial cells and on endothelial expression of adhesion molecules. *J Pharmacol Exp Ther* 267: 245–249.

Kaisho, T. and Akira, S. (2006) Toll-like receptor function and signaling. *J Allergy Clin Immunol* 117: 979–987; quiz 988.

Kapanci, Y., Ribaux, C., Chaponnier, C. and Gabbiani, G. (1992) Cytoskeletal features of alveolar myofibroblasts and pericytes in normal human and rat lung. *J Histochem Cytochem* 40: 1955–1963.

Keatings, V.M., Collins, P.D., Scott, D.M. and Barnes, P.J. (1996) Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 153: 530–534.

Kefalides, N.A., Alper, R. and Clark, C.C. (1979) Biochemistry and metabolism of basement membranes. *Int Rev Cytol* 61: 167–228.

Kilburn, K.H. (1968) A hypothesis for pulmonary clearance and its implications. *Am Rev Respir Dis* 98: 449–463.

Kirkham, P.A., Spooner, G., Rahman, I. and Rossi, A.G. (2004) Macrophage phagocytosis of apoptotic neutrophils is compromised by matrix proteins modified by cigarette smoke and lipid peroxidation products. *Biochem Biophys Res Commun* 318: 32–37.

Knight, D.A. and Holgate, S.T. (2003) The airway epithelium: structural and functional properties in health and disease. *Respirology* 8: 432–446.

Kohri, K., Ueki, I.F., Shim, J.J., Burgel, P.R., Oh, Y.M., Tam, D.C. *et al.* (2002) Pseudomonas aeruginosa induces MUC5AC production via epidermal growth factor receptor. *Eur Respir J* 20: 1263–1270.

Koval, M. (2002) Sharing signals: connecting lung epithelial cells with gap junction channels. *Am J Physiol Lung Cell Mol Physiol* 283: L875–L893.

Kraft, M., Adler, K.B., Ingram, J.L., Crews, A.L., Atkinson, T.P., Cairns, C.B. *et al.* (2008) Mycoplasma pneumoniae induces airway epithelial cell expression of MUC5AC in asthma. *Eur Respir J* 31: 43–46.

Lauweryns, J.M., Van Lommel, A.T. and Dom, R.J. (1985) Innervation of rabbit intrapulmonary neuroepithelial bodies. Quantitative and qualitative ultrastructural study after vagotomy. *J Neurol Sci* 67: 81–92.

Lazarowski, E.R., Boucher, R.C. and Harden, T.K. (2003) Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. *Mol Pharmacol* 64: 785–795.

Lemjabbar, H. and Basbaum, C. (2002) Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells. *Nat Med* 8: 41–46.

Leopold, P.L., O'Mahony, M.J., Lian, X.J., Tilley, A.E., Harvey, B.G. and Crystal, R.G. (2009) Smoking is associated with shortened airway cilia. *PLoS One* 4: e8157.

Leslie, K.O., Mitchell, J. and Low, R. (1992) Lung myofibroblasts. *Cell Motil Cytoskeleton* 22: 92–98.

Li, J.D., Dohrman, A.F., Gallup, M., Miyata, S., Gum, J.R., Kim, Y.S. *et al.* (1997) Transcriptional activation of mucin by Pseudomonas aeruginosa lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proc Natl Acad Sci U S A* 94: 967–972.

Lien, E., Sellati, T.J., Yoshimura, A., Flo, T.H., Rawadi, G., Finberg, R.W. *et al.* (1999) Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 274: 33419–33425.

Lin, Y., Xia, L., Turner, J.D. and Zhao, X. (1995) Morphologic observation of neutrophil diapedesis across bovine mammary gland epithelium in vitro. *Am J Vet Res* 56: 203–207.

Lomas, D.A., Ip, M., Chamba, A. and Stockley, R.A. (1991) The effect of in vitro and in vivo dexamethasone on human neutrophil function. *Agents Actions* 33: 279–285.

Lowenstein, C.J., Alley, E.W., Raval, P., Snowman, A.M., Snyder, S.H., Russell, S.W. *et al.* (1993) Macrophage nitric oxide synthase gene: two upstream regions mediate induction by interferon gamma and lipopolysaccharide. *Proc Natl Acad Sci U S A* 90: 9730–9734.

Lumsden, A.B., McLean, A. and Lamb, D. (1984) Goblet and Clara cells of human distal airways: evidence for smoking induced changes in their numbers. *Thorax* 39: 844–849.

Luppi, F., Longo, A.M., de Boer, W.I., Rabe, K.F. and Hiemstra, P.S. (2007) Interleukin-8 stimulates cell proliferation in non-small cell lung cancer through epidermal growth factor receptor transactivation. *Lung Cancer* 56: 25–33.

Marchesi, V.T. and Gowans, J.L. (1964) The Migration of Lymphocytes through the Endothelium of Venules in Lymph Nodes: An Electron Microscope Study. *Proc R Soc Lond B Biol Sci* 159: 283–290.

Matzner, Y., Vlodavsky, I., Michaeli, R.I. and Eldor, A. (1990) Selective inhibition of neutrophil activation by the subendothelial extracellular matrix: possible role in protection of the vessel wall during diapedesis. *Exp Cell Res* 189: 233–240.

McGillen, J. and Phair, J. (1979) Polymorphonuclear leukocyte adherence to nylon: effect of oral corticosteroids. *Infect Immun* 26: 542–546.

Mehta, H., Nazzal, K. and Sadikot, R.T. (2008) Cigarette smoking and innate immunity. *Inflamm Res* 57: 497–503.

Mio, T., Romberger, D.J., Thompson, A.B., Robbins, R.A., Heires, A. and Rennard, S.I. (1997) Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* 155: 1770–1776.

Monick, M.M., Yarovinsky, T.O., Powers, L.S., Butler, N.S., Carter, A.B., Gudmundsson, G. *et al.* (2003) Respiratory syncytial virus up-regulates TLR4 and sensitizes airway epithelial cells to endotoxin. *J Biol Chem* 278: 53035–53044.

Munck, A., Mendel, D.B., Smith, L.I. and Orti, E. (1990) Glucocorticoid receptors and actions. *Am Rev Respir Dis* 141: S2–S10.

Muzio, M., Salvesen, G.S. and Dixit, V.M. (1997) FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogens. *J Biol Chem* 272: 2952–2956.

Nathan, C.F. (1987) Secretory products of macrophages. *J Clin Invest* 79: 319–326.

Noone, P.G., Leigh, M.W., Sannuti, A., Minnix, S.L., Carson, J.L., Hazucha, M. *et al.* (2004) Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 169: 459–467.

O'Shaughnessy, T.C., Ansari, T.W., Barnes, N.C. and Jeffery, P.K. (1997) Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. *Am J Respir Crit Care Med* 155: 852–857.

Olivera, W., Ridge, K., Wood, L.D. and Sznajder, J.I. (1994) Active sodium transport and alveolar epithelial Na-K-ATPase increase during subacute hyperoxia in rats. *Am J Physiol* 266: L577–L584.

Ottonello, L., Tortolina, G., Amelotti, M. and Dallegri, F. (1999) Soluble Fas ligand is chemotactic for human neutrophilic polymorphonuclear leukocytes. *J Immunol* 162: 3601–3606.

Parkos, C.A., Delp, C., Arnaout, M.A. and Madara, J.L. (1991) Neutrophil migration across a cultured intestinal epithelium. Dependence on a CD11b/CD18-mediated event and enhanced efficiency in physiological direction. *J Clin Invest* 88: 1605–1612.

Paulsson, M. (1992) Basement membrane proteins: structure, assembly, and cellular interactions. *Crit Rev Biochem Mol Biol* 27: 93–127.

Pendino, K.J., Laskin, J.D., Shuler, R.L., Punjabi, C.J. and Laskin, D.L. (1993) Enhanced production of nitric oxide by rat alveolar macrophages after inhalation of a pulmonary irritant is associated with increased expression of nitric oxide synthase. *J Immunol* 151: 7196–7205.

Petecchia, L., Sabatini, F., Varesio, L., Camoirano, A., Usai, C., Pezzolo, A. *et al.* (2009) Bronchial airway epithelial cell damage following exposure to cigarette smoke includes disassembly of tight junction components mediated by the extracellular signal-regulated kinase 1/2 pathway. *Chest* 135: 1502–1512.

Polette, M., Mestdagt, M., Bindels, S., Nawrocki-Raby, B., Hunziker, W., Foidart, J.M. *et al.* (2007) Beta-catenin and ZO-1: shuttle molecules involved in tumor invasion-associated epithelial-mesenchymal transition processes. *Cells Tissues Organs* 185: 61–65.

Reverdin, N., Gabbiani, G. and Kapanci, Y. (1975) Actin in tracheo-bronchial ciliated epithelial cells. *Experientia* 31: 1348–1350.

Rickard, K.A., Taylor, J., Rennard, S.I. and Spurzem, J.R. (1993) Migration of bovine bronchial epithelial cells to extracellular matrix components. *Am J Respir Cell Mol Biol* 8: 63–68.

Rivkin, I., Foschi, G.V. and Rosen, C.H. (1976) Inhibition of in vitro neutrophil chemotaxis and spontaneous motility by anti-inflammatory agents. *Proc Soc Exp Biol Med* 153: 236–240.

Rock, J.R., Onaitis, M.W., Rawlins, E.L., Lu, Y., Clark, C.P., Xue, Y. *et al.* (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci U S A* 106: 12771–12775.

Rogers, D.C. (1989) Phylogenetic aspects of the neuroepithelial bodies. *Arch Histol Cytol* 52(Suppl): 397–402.

Rose, M.C. and Voynow, J.A. (2006) Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 86: 245–278.

Rowe, R.K., Brody, S.L. and Pekosz, A. (2004) Differentiated cultures of primary hamster tracheal airway epithelial cells. *In Vitro Cell Dev Biol Anim* 40: 303–311.

Russo, R.G., Liotta, L.A., Thorgeirsson, U., Brundage, R. and Schiffmann, E. (1981) Polymorphonuclear leukocyte migration through human amnion membrane. *J Cell Biol* 91: 459–467.

Saetta, M., Turato, G., Maestrelli, P., Mapp, C.E. and Fabbri, L.M. (2001) Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 163: 1304–1309.

Sage, H. (1982) Collagens of basement membranes. *J Invest Dermatol* 79(Suppl 1): 51s–59s.

Sallenave, J.M., Shulmann, J., Crossley, J., Jordana, M. and Gauldie, J. (1994) Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. *Am J Respir Cell Mol Biol* 11: 733–741.

Schleimer, R.P. (1990) Effects of glucocorticosteroids on inflammatory cells relevant to their therapeutic applications in asthma. *Am Rev Respir Dis* 141: S59–S69.

Schneeberger, E.E. and Lynch, R.D. (1992) Structure, function, and regulation of cellular tight junctions. *Am J Physiol* 262: L647–L661.

Schneider, P., Holler, N., Bodmer, J.L., Hahne, M., Frei, K., Fontana, A. *et al.* (1998) Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. *J Exp Med* 187: 1205–1213.

Seino, K., Iwabuchi, K., Kayagaki, N., Miyata, R., Nagaoka, I., Matsuzawa, A. *et al.* (1998) Chemotactic activity of soluble Fas ligand against phagocytes. *J Immunol* 161: 4484–4488.

Shao, M.X., Nakanaga, T. and Nadel, J.A. (2004) Cigarette smoke induces MUC5AC mucin overproduction via tumor necrosis factor-alpha-converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol Lung Cell Mol Physiol* 287: L420–L427.

Shaw, J.O. (1980) Leukocytes in chemotactic-fragment-induced lung inflammation. Vascular emigration and alveolar surface migration. *Am J Pathol* 101: 283–302.

Sheehan, J.K., Kesimer, M. and Pickles, R. (2006) Innate immunity and mucus structure and function. *Novartis Found Symp* 279: 155–166; discussion 167–159, 216–159.

Shock, A. and Laurent, G.J. (1991) Adhesive interactions between fibroblasts and polymorphonuclear neutrophils in vitro. *Eur J Cell Biol* 54: 211–216.

Smedly, L.A., Tonnesen, M.G., Sandhaus, R.A., Haslett, C., Guthrie, L.A., Johnston Jr, R.B. *et al.* (1986) Neutrophil-mediated injury to endothelial cells. Enhancement by endotoxin and essential role of neutrophil elastase. *J Clin Invest* 77: 1233–1243.

Smirnova, M.G., Guo, L., Birchall, J.P. and Pearson, J.P. (2003) LPS up-regulates mucin and cytokine mRNA expression and stimulates mucin and cytokine secretion in goblet cells. *Cell Immunol* 221: 42–49.

Spina, D. (1998) Epithelium smooth muscle regulation and interactions. *Am J Respir Crit Care Med* 158: S141–S145.

Takeyama, K., Dabbagh, K., Lee, H.M., Agusti, C., Lausier, J.A., Ueki, I.F. *et al.* (1999) Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci U S A* 96: 3081–3086.

Taraseviciene-Stewart, L. and Voelkel, N.F. (2008) Molecular pathogenesis of emphysema. *J Clin Invest* 118: 394–402.

Tarran, R., Grubb, B.R., Gatzy, J.T., Davis, C.W. and Boucher, R.C. (2001) The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J Gen Physiol* 118: 223–236.

Terranova, V.P., Rohrbach, D.H. and Martin, G.R. (1980) Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. *Cell* 22: 719–726.

Thornton, D.J., Rousseau, K. and McGuckin, M.A. (2008) Structure and function of the polymeric mucins in airways mucus. *Annu Rev Physiol* 70: 459–486.

Tonnesen, M.G., Anderson, D.C., Springer, T.A., Knedler, A., Avdi, N. and Henson, P.M. (1989) Adherence of neutrophils to cultured human microvascular endothelial cells. Stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. *J Clin Invest* 83: 637–646.

Uematsu, S. and Akira, S. (2006) Toll-like receptors and innate immunity. *J Mol Med* 84: 712–725.

Uhal, B.D., Joshi, I., True, A.L., Mundle, S., Raza, A., Pardo, A. *et al.* (1995) Fibroblasts isolated after fibrotic lung injury induce apoptosis of alveolar epithelial cells in vitro. *Am J Physiol* 269: L819–L828.

van Overveld, F.J., Demkow, U.A., Gorecka, D., Zielinski, J. and De Backer, W.A. (2003) Inhibitory capacity of different steroids on neutrophil migration across a bilayer of endothelial and bronchial epithelial cells. *Eur J Pharmacol* 477: 261–267.

van Overveld, F.J., Vermeire, P.A. and De Backer, W.A. (2000) Induced sputum of patients with chronic obstructive pulmonary disease (COPD) contains adhesion-promoting, therapy-sensitive factors. *Inflamm Res* 49: 8–13.

van Wetering, S., Mannesse-Lazeroms, S.P., van Sterkenburg, M.A. and Hiemstra, P.S. (2002) Neutrophil defensins stimulate the release of cytokines by airway epithelial cells: modulation by dexamethasone. *Inflamm Res* 51: 8–15.

Vermeer, P.D., Denker, J., Estin, M., Moninger, T.O., Keshavjee, S., Karp, P. *et al.* (2009) MMP9 modulates tight junction integrity and cell viability in human airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 296: L751–L762.

Voynow, J.A., Young, L.R., Wang, Y., Horger, T., Rose, M.C. and Fischer, B.M. (1999) Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. *Am J Physiol* 276: L835–L843.

Wadsworth, S.J., Atsuta, R., McIntyre, J.O., Hackett, T.L., Singhera, G.K. and Dorscheid, D.R. (2010) IL-13 and T(H)2 cytokine exposure triggers matrix metalloproteinase 7-mediated Fas ligand cleavage from

bronchial epithelial cells. *J Allergy Clin Immunol*, 126(2): 366–374, 374 e1–8.

Wadsworth, S.J., Freyer, A.M., Corteling, R.L. and Hall, I.P. (2004) Biosynthesized matrix provides a key role for survival signaling in bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 286: L596–L603.

Wadsworth, S.J., Nijmeh, H.S. and Hall, I.P. (2006) Glucocorticoids increase repair potential in a novel in vitro human airway epithelial wounding model. *J Clin Immunol* 26: 376–387.

Walker, D.C., Behzad, A.R. and Chu, F. (1995) Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 50: 397–416.

Wang, B., Lim, D.J., Han, J., Kim, Y.S., Basbaum, C.B. and Li, J.D. (2002) Novel cytoplasmic proteins of nontypeable Haemophilus influenzae up-regulate human MUC5AC mucin transcription via a positive p38 mitogen-activated protein kinase pathway and a negative phosphoinositide 3-kinase-Akt pathway. *J Biol Chem* 277: 949–957.

Watanabe-Fukunaga, R., Brannan, C.I., Itoh, N., Yonehara, S., Copeland, N.G., Jenkins, N.A. *et al.* (1992) The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J Immunol* 148: 1274–1279.

Wickstrom, C., Davies, J.R., Eriksen, G.V., Veerman, E.C. and Carlstedt, I. (1998) MUC5B is a major gel-forming, oligomeric mucin from human salivary gland, respiratory tract and endocervix: identification of glycoforms and C-terminal cleavage. *Biochem J* 334: 685–693.

Wright, J.R. (2005) Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 5: 58–68.

Wright, J.R. and Hawgood, S. (1989) Pulmonary surfactant metabolism. *Clin Chest Med* 10: 83–93.

Yamaki, K., Thorlacius, H., Xie, X., Lindbom, L., Hedqvist, P. and Raud, J. (1998) Characteristics of histamine-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. *Br J Pharmacol* 123: 390–399.

Yates, G.T., Wu, T.Y., Johnson, R.E., Cheung, A.T. and Frand, C.L. (1980) A theoretical and experimental study on tracheal muco-ciliary transport. *Biorheology* 17: 151–162.

Yoon, J.H., Kim, K.S., Kim, H.U., Linton, J.A. and Lee, J.G. (1999) Effects of TNF-alpha and IL-1 beta on mucin, lysozyme, IL-6 and IL-8 in passage-2 normal human nasal epithelial cells. *Acta Otolaryngol* 119: 905–910.

Zarbock, A. and Ley, K. (2009) Neutrophil adhesion and activation under flow. *Microcirculation* 16: 31–42.

Zeitlin, P.L. (2008) Cystic fibrosis and estrogens: a perfect storm. *J Clin Invest* 118: 3841–3844.

Zemans, R.L., Colgan, S.P. and Downey, G.P. (2009) Transepithelial migration of neutrophils: mechanisms and implications for acute lung injury. *Am J Respir Cell Mol Biol* 40: 519–535.

Zen, K. and Parkos, C.A. (2003) Leukocyte-epithelial interactions. *Curr Opin Cell Biol* 15: 557–564.

Zibrak, J.D., Faris, B., Vaccaro, C.A. and Brody, J.S. (1985) Isolation and characterization of a rat lung fraction enriched in alveolar wall basement membranes. *Exp Lung Res* 8: 15–28.