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The innate immune function of airway epithelial cells in inflammatory lung disease

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Abstract

The airway epithelium is now considered central to the orchestration of pulmonary inflammatory and immune responses, and is also key to tissue remodelling. It acts as a first barrier in the defence against a wide range of inhaled challenges, and is critically involved in the regulation of both innate and adaptive immune responses to these challenges. Recent progress in our understanding of the developmental regulation of this tissue, the differentiation pathways, recognition of pathogens and antimicrobial responses is now exploited to help understand how epithelial cell function and dysfunction contributes to the pathogenesis of a variety of inflammatory lung diseases. In the review, advances in our knowledge of the biology of airway epithelium, as well as its role and (dys)function in asthma, COPD and cystic fibrosis, are discussed.

Introduction

Airway epithelial cells (AECs) are located at the interface between the external and internal milieu, and are exposed daily to an array of inhaled gases and particles. The classic view of the airway epithelium is that of a structural barrier that regulates water and ion transport, and contributes to the clearance of inhaled substances through mucociliary clearance. However, recent research reveals that the airway epithelium is highly dynamic and displays a broad spectrum of activities related to inflammation, immunity, host defence and tissue remodelling. This review focuses on the role of the airway epithelium in innate immunity and host defence against bacterial and viral infections in health, as well as in asthma, COPD and cystic fibrosis.

Development and structure of the airway epithelium

AECs line the airways and serve as a means of transportation of gases to and from the alveoli. They are the most abundant cell type in the lung to first encounter inhaled substances, and are therefore important in the regulation of host defence. Inhaled air

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contains numerous substances, including toxic compounds and micro-organisms and microbial products, that may cause pulmonary injury. The airway epithelium contributes to defence by a variety of mechanisms that are discussed in more detail in the other sections of this contribution. Briefly, these mechanisms include the barrier function of the epithelium and mucociliary clearance, as well as the production of antimicrobial peptides and proteins, reactive oxygen and nitrogen species, and a range of cytokines, chemokines and growth factors (1;2). This allows the airway epithelium to contribute directly to host defence, and augment the response via the recruitment of a range of leukocytes and communication with mesenchymal cells in the airway wall. This intense communication network involves not only soluble mediators but also cell-cell contacts, and allows AECs to transfer a signal resulting from exposure to inhaled substances to other cells, and thus instruct adaptive immunity.

In recent years, significant progress has been made in our understanding of lung development, including the airway epithelium. This is important for understanding the innate immune function of the airway epithelium, because it is increasingly recognized that early life events during development contribute to adult lung disease. Furthermore, lung development research has resulted in new tools to study epithelial cell function in inflammatory lung disease. Indeed, recently, the ability to generate AECs from human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) (3–5) has contributed insights into the development of the airway epithelium, and will likely continue to do so. Excellent recent reviews on lung development and lung stem/progenitor cells are available for the interested reader (6–8).

The pseudostratified airway epithelium of the newborn and adult is composed of a variety cell types, including basal cells, ciliated cells, mucus-producing goblet cells, club cells (formerly called Clara cells) and neuroendocrine cells. Submucosal glands are mainly located in the large airways and are composed of terminal serous and mucous cells and the collecting duct cells. The balance of these various cell types differs between the trachea, bronchi and the more distal airways where e.g. goblet cells are normally nearly absent. However, the composition of the epithelium is clearly altered in asthma, COPD and cystic fibrosis, with e.g. an increase in goblet cells and obstruction by mucus in especially the small airways. The epithelial layer forms a tight barrier separating the lumen of the airways with its inhaled content from the underlying tissue. An essential component of this barrier is the luminal junctional complex that is composed of the most apical tight junctions (TJ) and adherens junctions (AJ). This complex is disturbed by inhaled substances such as pollutants, micro-organisms and inhaled allergens, and is dysfunctional in a variety of airway diseases, including asthma and COPD. This impaired epithelial barrier function also impacts susceptibility to respiratory infections.

Basal cells are progenitors capable of self-renewal and generation of all surface epithelium cell types, and are characterized in the human by expression of p63 and KRT14 (9). Differentiated AEC can also dedifferentiate into stem cells and thus contribute to regeneration of the differentiated airway epithelium (10). Epithelial cell development and differentiation is tightly regulated by epigenetic mechanisms, including histone modifications and microRNAs (miRNAs) (7). The discovery of miRNAs as regulators of

post-transcriptional gene expression was one of the most exciting advances in biology during the late 20th century. MiRNAs mediate changes in gene expression post-transcriptionally through translational repression or mRNA degradation (11). Many lines of evidence now show that changes in miRNA actions can influence epithelial cell function in asthma, COPD and cystic fibrosis. Injury and repair, cell adhesion, cell proliferation, cell migration, and differentiation are finely regulated by miRNAs. Several studies have identified roles for miRNAs in lung development (7;12;13) and disease states including COPD (11) and asthma (14). Their role in cystic fibrosis is more extensively discussed in the section on cystic fibrosis. Therefore, miRNAs and other epigenetic mechanisms have a major effect on epithelial cell development and function.

Airway epithelial cell culture models

Advances in basic and translational studies require suitable model systems to bridge the gap between heterologous cell populations and human studies. Investigations of airway epithelial cell biology have benefited greatly from the development and continued refinement of primary cultures derived of surface epithelia from the trachea, bronchi, and alveoli. An air-liquid interface (ALI) culture method of cells grown on a permeable filter with air above and cell culture media below is widely employed (15). The validity of this model is supported by studies of the ultrastructure, but also the function of the epithelium, including mucus secretion, ASL regulation, mucociliary transport, as well as inflammatory and antimicrobial mediator production. This is supported by gene expression profiling experiments that have revealed remarkable similarities between the mRNA expression profiles of ALI cultured epithelia and bronchoscopically obtained tracheal and bronchial brushings from the human airways (16;17). These findings support the utility of this culture model and strengthen the rationale for its use as an experimental platform to complement *in vivo* studies. In addition, the ALI model is also used to generate primary epithelial cell cultures from genetically modified animals and thus helps to define the function of individual genes in epithelial cell function (18). Developments in imaging and culture techniques including super resolution (STED) microscopy techniques, epithelial cell and lung organoids, microfluidic lung-on-a-chip system and use of lung tissue slices *in vitro* are also of interest. A significant recent advance is the application of a Rho kinase (ROCK) inhibitor and/or fibroblast feeder cells or conditioned media as a strategy to greatly expand primary cell populations by inhibiting cellular senescence, making cultures derived from small samples from patients such as nasal or bronchial brushings even more feasible (19;20).

Recent progress in the production of induced pluripotent stem cells (iPSCs) from human and animal sources provides an opportunity for banking and expanding disease and patient specific cells. Several groups are making progress towards the goal of differentiating iPSCs into AECs (4;5). Further advances in this space will greatly facilitate studies of cell therapies, tissue engineering, pharmacogenomics, and personalized medicine. New gene targeting and gene repair technologies including zinc finger nucleases, TALENs, and CRISPR-cas9 (21) provide further opportunities to generate isogenic models for investigations.

Although differentiated cultures derived from primary airway epithelial cells and iPSCs do show remarkable similarities with airway epithelial cells in tissue with regard to morphology, cell specific markers and gene expression profiles, it needs to be noted that the cells in these model systems may differ from native cells in tissue. In addition, it is important that results obtained using these model system are confirmed using cells from multiple donors. This way full advantage can be taken from the superiority of these models compared to those based on immortalized or tumour cell lines.

The microbiome of the lung

A number of recent studies have shown that micro-organisms are present in the lungs. AECs are continuously exposed to this distinct collection of bacteria, viruses, fungi, and their products. The term “microbiome” is defined as the “ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share a specific site of the body” (22). Currently, real time PCR, pyrosequencing, restriction fragment length polymorphism analysis, and direct sequencing are applied to analyse the microbiome. These approaches showed that the lung microbiome is complex in healthy individuals and altered in diseases. In asthma and COPD the microbiome of the lung is different from healthy individuals and is likely mechanistically linked to disease processes (23;24). This is confirmed by studies in mouse models of allergic airways inflammation showing a link between the development of the lung microbiome and immunologic tolerance to allergens (25). In cystic fibrosis lung disease, analysis of the lung microbiome revealed a complex composition far beyond the current knowledge (26;27). Furthermore, the lung microbiome is dynamic and for example changes significantly after rhinovirus infection in COPD (28).

Analogous to the gut, the interaction between epithelial cells and the microbiome is likely an important factor in maintaining stable homeostasis (29). Also in the lung, AEC are in continuous contact with the microbiome and this interaction is critical to maintain a sufficient barrier. Hypothetically, inhaled or aspirated microorganisms are quickly inactivated and killed, and their products interact with immune cells and AEC, and thus help shape immune responses (30).

Sensing microbial presence

The continuous threat posed by microbial exposure requires sensitive detection mechanisms, as well as scaling of responses to avoid unwanted inflammation and tissue injury. In 1989, Janeway proposed in the pattern recognition theory that the host innate immune system senses microbial presence through the detection of so-called pathogen-associated molecular patterns (PAMPs), invariant molecular structures that are present in pathogens but absent in the host (31). The identification of the Toll-like receptor (TLR) family of transmembrane proteins was the first step in delineating the pattern recognition receptors (PRR) that bind and respond to PAMP exposures, and subsequent studies also showed the presence of cytosolic receptors for sensing microbial presence. These and other mechanisms that contribute to microbial detection by AECs are discussed below (Figure 1).

Pattern recognition receptors: Toll-like receptors, lectin-type receptors and intracellular receptors

PRRs are germline encoded receptors with currently four families: the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), the cytoplasmic proteins retinoic acid-inducible gene (RIG)-I-like (RLRs) and the NOD-like receptors (NLRs) (32). Orthologous receptors have been identified in multiple species including drosophila, mouse and human (33). The functions of Toll receptors in Drosophila comprise developmental regulation and host defence (34), while in mammals TLRs have a restricted role as PRR in innate immunity by recognizing microbial and endogenous ligands. The 10 TLRs identified in humans and the 12 TLRs in mice are structurally characterized by N-terminal leucine-rich repeats (LRRs), a transmembrane region, and a cytoplasmic Toll/IL-1R homology (TIR) domain that mediates signalling. Their cellular localization and ligands are summarized in Table 1. TLR-mediated signalling is complex and involves MyD88 and TRIF (35). Whereas AECs express most TLRs (36), they often seem to be hyporesponsive to microbial stimulation and alveolar macrophages are necessary to induce a full epithelial host defence reaction (37).

CLRs comprise a large number of proteins highly conserved in vertebrates that were originally characterized by a C-type lectin domain (mannose binding), but now encompass more structurally divergent molecules (38). Dectin-1 is a CLR expressed in AEC and involved in detection of mycobacteria and Aspergillus (39;40). RLRs are complex cytoplasmic receptors that recognize the presence of genomic RNA of dsRNA viruses or dsRNA intermediates from ssRNA viruses. Melanoma differentiation-associated gene 5 (MDA5) is expressed in AECs and involved in the detection of rhinovirus and other viruses (41). Finally NLRs are intracellular PRRs that are characterized by a central nucleotide-binding and oligomerization domain (NOD or NACHT) and C-terminal leucine-rich repeats (LRRs) (42); limited information is available about NOD function in AECs.

Integrated stress response and endoplasmic reticulum stress

The integrated stress response (ISR) can be activated by one of four stress-sensing kinases, that are triggered by a variety of signals including those generated during infection (43). These kinases cause phosphorylation of the α-subunit of the eukaryotic translation initiation factor 2 (eIF2α), resulting in the inhibition of protein synthesis which may be cytoprotective e.g. by inhibiting viral replication. eIF2α phosphorylation also causes translation of selected mRNAs, including the transcription factor ATF4 that results in enhanced expression of genes involved in adaptation to cellular stress. The kinase PKR is an example of a stress-sensing kinase that causes phosphorylation of eIF2α during infection, since it is triggered by double-stranded RNA present during viral infection. The kinase PERK senses protein (mis)folding in the endoplasmic reticulum (ER), and is activated during ER stress which may result from the increased demand on the ER during infection as a result of production of e.g. antimicrobial peptides and pro-inflammatory mediators. Thereby, PERK and the downstream signalling that is shared with three other kinases of the ISR comprises one of the three arms of the unfolded protein response (UPR) to ER stress (44). The crosstalk between immune activation by e.g. TLRs and the responses to cellular stress by the UPR and ISR provides evidence for the numerous possibilities for fine-tuning the epithelial response to infection (45). Whereas acute activation of the ISR and UPR may contribute to cell

survival and host defence against infection, chronic activation as observed in COPD and CF (43) may be detrimental.

Integration of signals: scaling of danger—The availability of this variety of receptors and mechanisms to detect microbial presence and the ability to integrate this information allows the innate immune system to scale responses (46). Recent studies showing that production of antimicrobial peptides by upper airway AECs is triggered by bitter receptors (T2R), a response that is blocked by sweet taste receptors (T1R2/3), provides additional information on the regulation of innate immunity at the epithelial surface (47). This is based on the hypothesis that glucose consumption at sites of infection by micro-organisms may reverse this inhibition by T1R2/3 receptors, which is in line with the hypothesis that detection of microbial viability is an important means to scale the response.

Epithelial effector mechanisms in host defence against infection

A variety of antimicrobial mechanisms contribute to clearance of micro-organisms that are a potential threat to the host (Figure 1). The vast redundancy in the system prevents microbial resistance, although many mechanisms are most effective against bacteria in their planktonic phase, and less active against bacteria in biofilms.

Mucins

Mucus is an important component of mucociliary clearance and is an extracellular gel comprised of water, mucins, and numerous associated molecules (48). Mucins are large glycoproteins released by several secretory cells, including goblet cells, club cells, and the serous and mucous cells of the glands (49). Mucin proteins are polydispersed in size (2–50 MDa) and display a random coil formation in solution, stabilized by disulphide-linked subunits. Large carbohydrate chains are attached to the protein backbone. Mucins are encoded by 17 MUC genes: 10 code for cell-tethered mucins (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC16, MC16, MUC17, and MUC20), 7 code for secreted mucins (MUC2, MUC5A/C, MUC5B, MUC6, and MUC19), and 2 code for non-polymeric glycoproteins (MUC7 and MUC8) (49). The mucin content of airway mucus is mainly characterized by glycoproteins encoded by MUC5AC, MUC5B, and MUC2. Amongst these airway mucins, recently an important role of Muc5b in mucociliary clearance and host defence in the murine airways was described (50). Mucin gene expression is regulated by a variety of factors including inhaled toxins or micro-organisms, inflammatory cytokines, and ErbB-receptor ligands. After synthesis, mucins are stored in condensed granules and their release is tightly controlled by calcium, ATP (via apical $P2Y_2$ receptors) and other mechanisms (51).

A number of smaller molecules are associated with mucus, mediated by charge interactions between these molecules and the negatively charged mucins. Many of these proteins display antimicrobial activity, including secretory IgA (sIgA), antimicrobial peptides, lysozyme, and collectins.

The current view is that a discontinuous mucus layer is located above the periciliary space with the tips of the cilia reaching the mucus. It is separated from the epithelial surface by the

periciliary layer (PCL) or sol layer, in which macromolecules (cell-tethered mucins, glycosaminoglycans) are attached to the cilia and form a protected area that cannot be penetrated by mucus or other molecules from above (52). The mucus layer and PCL together comprise the airway surface liquid (ASL), and its structure and properties are controlled by different mechanisms including the transepithelial ion- and water transport. Ion channels such as the CFTR and ENaC regulate the volume and composition of airway secretions (53).

Mucociliary transport

The coordinated beating of cilia interacting with mucus provides an important mechanism for clearance of inhaled or aspirated particulates or microbes via mucociliary transport (MCT). Decreased clearance of pathogens and inflammatory mediators results in inflammation, infection and tissue destruction. In cystic fibrosis, loss of CFTR function reduces ASL pH, impairs liquid secretion, and causes mucus strands to remain tethered to submucosal gland ducts resulting in reduced mucociliary transport and altered host defence (54;55).

Antimicrobial peptides

Antimicrobial peptides (AMPs) are small peptides (~10–50 amino acids) that have antimicrobial activity against bacteria, viruses, and fungi. In addition, many of them likely act as modulators of inflammation, repair, regeneration and other processes. In a narrow view, AMPs are gene encoded; in a broader view, AMPs also arise from proteolytic fragments of larger proteins. AECs of mammals produce AMPs of the defensin and cathelicidin families.

The structural hallmark of defensins is the presence of 6 cysteines that form three intramolecular disulphide bonds. Based on their structure, defensins can be subdivided into α-, β- or θ-defensins. While α-defensins are expressed in myeloid cells and θ-defensins are not functionally expressed in humans, AECs produce β-defensins. Human β-defensin 1, 2, 3, and 4 are mainly expressed in AECs, synthesized as precursors and secreted after the cleavage from the propiece. While hBD-1 appears to be secreted constitutively, the production of other β-defensins is induced by various signalling pathways such as the TLR or NF-kB pathways or the presence of proinflammatory cytokines. Interestingly, the number of defensin genes varies between individuals from 2 to 12 (copy number variants). The number of present defensin gene clusters modulates disease outcomes for psoriasis, but no correlation has been confirmed for COPD or asthma (56). The antimicrobial activity of defensins is likely based on interactions with microbial lipids and covers gram-positive, -negative bacteria, viruses, and fungi. In addition to their antimicrobial function, β-defensins were shown to chemoattract immune cells and to activate dendritic cells. The role of β-defensins in the lung is not entirely clear. Murine knockout models did not provide a clear answer due to the redundancy of these AMPs and indicated a host defence function (57;58). In human disease, acute inflammation seems to increase defensin expression, while chronic inflammation may suppress expression of these AMPs (59).

The cathelicidins are a large group of AMPs characterized by the homologous propiece, called “cathelin”, which acts as inhibitor of cathepsin L. The structures of the C-terminal

active peptide differ amongst species. In human and the mouse, only one cathelicidin gene is present and encodes the peptides hCAP-18 / LL-37 (gene name: *CAMP*) or CRAMP (gene name: *Camp*), respectively. Both peptides are linear molecules that have an α-helical structure in physiological solution. These peptides undergo posttranslational modification and have broad activity against various micro-organisms including viruses. In the lung, AECs, neutrophils, macrophages and other cells produce LL-37 (60). The biological role in pulmonary host defence has been shown using the CRAMP knockout model (61). In addition, cathelicidins appear to have a complex and broad role in the modulation of diverse biological processes mediated through specific receptors or through non-specific interactions with biomembranes (62). Cathelicidins have a role in angiogenesis, wound healing of epithelia, lung cancer growth and regulation of immune cells. A major source of LL-37 in the lung is the neutrophil, while vitamin D induces the peptide's expression in macrophages and AECs (63).

In addition to defensins and cathelicidins, numerous other proteins or peptides with antibacterial activity are secreted from AECs, including lysozyme, lactoferrin and secretory leukocyte proteinase inhibitor (SLPI). Most of these host defence molecules are positively changed and thus closely interact with negatively charged macromolecules. As outlined above, mucus (64) and neutrophil extracellular traps (NETs; suggested as host defense factors) (65) contain high concentrations of cationic host defence peptides. Interactions between AMPs and components released during inflammation and tissue injury (e.g. F-actin and DNA) likely inhibit host defence activities.

Miscellaneous mechanisms

Reactive oxygen and nitrogen species—AEC produce substantial amounts of reactive oxygen species, mainly arising from the NADPH oxidases DUOX1 and DUOX2. DUOX-derived ROS have been shown to contribute to the antimicrobial activity that is generated by AECs in the ASL. This results from the formation of antimicrobial $OSCN^-$ from DUOX-derived H_2O_2 catalysed by lactoperoxidase (LPO) (66), a system that may be defective in the airways of cystic fibrosis (CF) patients because of a deficient secretion of SCN^- by the CF airway epithelium (67). In addition to ROS, AECs also produce reactive nitrogen species (RNS) through the action of NOS enzymes, and the resultant NO displays a variety of functions in immune regulation and host defence against infection (68). NO production in the airways is derived from the constitutive NOS-1 and NOS-3 enzymes, and the inducible NOS-2.

Antiviral interferons—A variety of the aforementioned mechanisms contribute to antiviral defences of the airway epithelium, including mucins, antimicrobial peptides and RNS/NO. Detection of viral infection by the aforementioned membrane-bound and intracellular recognition mechanisms also triggers the production of type I interferons (IFN-α and IFN-β) and type III interferons (IFN-λ) (69). Interferons induce the expression of a range of genes encoding proteins that interfere with viral replication and protein synthesis and trafficking. In diseases including COPD, asthma, and CF, interferon-mediated host defences may be blunted (70). Based on these findings, novel drugs are being developed that are not directly antiviral, but cause activation of IFN signalling pathways and thus enhance

antiviral defences (71). Furthermore, in CF impaired IFN-mediated STAT1 signalling and impaired induction of antiviral iNOS2 and OAS1 may also contribute to increased epithelial susceptibility to infection by respiratory viruses (72).

Autophagy—Autophagy is a homeostatic mechanism that delivers unwanted cellular components to lysosomes for degradation. It plays a role in cell stress, differentiation and development, as well as the clearance of toxic components and (intracellular) pathogens (73). In contrast, excessive and uncontrolled autophagy is detrimental to the host and contributes to the pathogenesis of e.g. COPD (74).

Epithelial cell (dys)function in inflammatory lung diseases

Asthma

Asthma is characterized by airways inflammation, structural alterations in lung tissue, variable airflow limitation and airway hyperresponsiveness. In the most common form of asthma, atopic allergen-induced asthma, eosinophils and T-helper 2 (Th2) lymphocytes play central roles. Genetic studies have provided insights into the role of gene-environment interactions, and it is interesting to note that a substantial number of the genes associated with asthma, including PCHD1, IL-33 and ORMDL3, are expressed in the airway epithelium (75). Asthma is accompanied by extensive alterations in the airway epithelium, including increased fragility, decreased barrier function, impaired anti-oxidant activity, increased goblet cells, and impaired antiviral innate responses (76). Furthermore, the important function of the airway epithelium in regulating fluid and ion transport is altered in asthma. It was found that Th2 cytokines increase epithelial expression of chloride channels such as TMEM16A and SLC26A9, and that chloride secretion through these channels helps preventing mucus obstruction of the airways (77;78). In line with this, a SNP in SLC26A9 that is associated with reduced protein expression of the chloride channel is associated with asthma (78).

Infections play an important role in asthma, and especially rhinovirus infections are associated with asthma exacerbations. Studies on the human airway microbiome have also confirmed the involvement of bacterial colonization and/or infection in the pathogenesis of asthma (79). Increased susceptibility to infection can be explained by the decreased barrier activity of the airway epithelium in asthma, decreased mucociliary clearance resulting from excessive mucus production, but also decreased epithelial innate immune responses to respiratory viruses (80) and the inhibitory effect of Th2 cytokines on the production of antimicrobial peptides (81). These respiratory infections contribute to inflammation, and have been suggested to also induce a “memory pool” of basal progenitor cells in the epithelium that e.g. produce excessive amounts of IL-33 (82).

It has long been thought that inflammation is a main driver of airway remodelling in asthma. However, also inhalation of a bronchoconstrictor in patients with mild atopic asthma resulted in an increase in measures of airway remodelling and an increase in epithelial TGF-β1 (83). This suggests that mechanical forces exerted during bronchoconstriction cause stress in the airway epithelium resulting in airway remodelling. However this increased epithelial expression of TGF-β1 may also affect host defence. TGF-β can inhibit production

of the antimicrobial serine protease inhibitor secretory leukocyte proteinase inhibitor (SLPI) by AEC (84) and promotes rhinovirus replication in AEC (85). On the other hand, TGF-β may also have beneficial effects by counteracting the cigarette-smoke induced disruption of epithelial barrier function (86) and suppressing epithelial mucin production induced by non-typeable *Haemophilus influenzae* (via suppression of p38 MAPK; (87)) or IL-13 (an isoform-specific effect of TGF-β2; (88)).

COPD

Asthma and COPD share an increased susceptibility to respiratory infections. In COPD and smokers without COPD, a variety of studies have shown that cigarette smoke exposure impairs host defence by decreasing epithelial barrier function, ciliary function, antimicrobial peptide production and antiviral responses, while increasing mucus production (59;76;89). Cigarette smoke is the main risk factor for development of COPD, and many of these epithelial features of COPD can be explained by a direct or indirect result of cigarette smoke exposure. Exposure of cultured AEC to cigarette smoke increases inflammatory mediator release and decreases barrier function (90) and expression of antimicrobial peptides (59). Furthermore, smoke exposure also increases citrullination of the antimicrobial peptide LL-37, resulting in impaired antimicrobial and increased pro-inflammatory activity of this peptide (91). These observations show that cigarette smoke increases inflammation, while decreasing host defence against infections which is compatible with what is seen in COPD patients. Cigarette smoke may affect epithelial cell functions through a variety of mechanisms, including direct oxidant activity, TLR signalling, but also e.g. may induce ER stress and activate the ISR (43;92). These latter findings are supported by studies in lung tissue from COPD patients (93). Another feature of COPD that is recently observed in lung tissue and culture, is the impaired epithelial CFTR function and expression that was found to be associated with COPD and smoking (94). Interestingly, as also observed in asthma and CF, epithelial features may persist after culturing AEC. This is shown by various studies, including that of Schulz *et al* who demonstrated that AECs from COPD patients release more IL-8 than cells from smoking control subjects (95). AECs from patients with α1-antitrypsin deficiency, the main genetic risk factor for COPD, also display a proinflammatory phenotype in culture (96). Holtzman *et al* provided new insights into how infections may promote chronic inflammation in the airways. They showed that viral infection may activate basal cells, leading to a population of long-lived basal cells with increased expression of IL-33. IL-33 expression in this population was associated with increased IL-13 and mucin expression in mouse models of viral infection and in severe COPD (82).

Cystic fibrosis

The early pathogenic steps in cystic fibrosis lung disease have been difficult to elucidate because they occur in infants and pre-school aged children. Mouse models of loss of CFTR function have not resulted in spontaneous lung disease with similarities to humans. To overcome this limitation, groups have developed new animal models by disrupting the CFTR gene in pigs (97;98), ferrets (99), and rats (100). Both CF pigs and ferrets spontaneously develop lung disease with several similarities to children with CF including a

mucosal host defence defect, ineffective eradication of bacteria, bacterial colonization, increased mucus production, and airway remodelling (101–103).

Experimental findings in these animal models are stimulating bench to bedside and back studies that are providing new insights into early steps in lung disease onset. For example, as a consequence of reduced bicarbonate transport due to loss of CFTR, the ASL of newborn CF pigs has a lower pH than littermate controls (54). The reduction in ASL pH impairs the function of resident host defence peptides and proteins and reduces the antimicrobial activity of airway secretions. The ASL pH differences between non-CF and CF subjects may vary depending on age and disease state. McShane and coworkers found no differences in ASL pH between people with CF and non-CF controls aged 3 years or older (104). In contrast, Abou Alaiwa and co-workers recently reported that CF babies had a lower nasal ASL pH compared to non-CF neonates, while nasal pH values in older CF children and adults were similar to values obtained in non-CF subjects (105). Together, these findings raise the possibility that interventions aimed at increasing ASL pH in the neonatal airways might improve host defence (106).

Impaired host defence against respiratory infections may result not only from the reduced activity of ASL antimicrobials, but also from a decreased mechanical clearance of inhaled pathogens. It is also widely believed that loss of CFTR function leads to amiloride-sensitive Na^+ hyperabsorption via ENaC, depletion of the periciliary liquid layer, dehydration of the airways, and impaired mucociliary clearance (107;108). However, recent findings in new CF animal models call into question whether sodium hyperabsorption is a primary early event in CF that contributes to decreased MCT at the time of disease onset. In line with findings in adult CF patients, newborn CF pigs exhibited impaired MCT under conditions of cholinergic stimulation (55). However, this reduced MCT was not associated with sodium hyperabsorption or periciliary liquid depletion. Remarkably, the mucus released from CF submucosal glands was anchored to gland ducts. These findings were present at birth, in the absence of infection or inflammation, and indicate that impaired MCT is a primary defect in CF. Interestingly, airway epithelia in newborn CF pigs do not hyperabsorb sodium (109). Additionally, experiments in neonatal CF ferrets (103;110) and CF rats (100), and some results in human CF airway epithelia (111) similarly find no evidence of sodium hyperabsorption. These results suggest that loss of CFTR function is not associated with increased ENaC activity at the time of disease onset.

It remains possible that over time secondary disease-associated changes in the airways lead to sodium hyperabsorption. Sodium hyperabsorption can alter airway function as shown in transgenic mice overexpressing beta-ENaC. They develop a lung disease characterized by ASL volume depletion, mucus obstruction, chronic inflammation, and structural lung damage (112). The concept that impaired MCT in people with established CF lung disease might result from ASL volume depletion secondary to impaired or absent CFTR function and resulting in mucin hyperconcentration is supported by a recent study demonstrating higher mucin concentrations in secretions of adults with CF (113). The investigators posit that the predicted increased osmotic pressures of CF mucus results in osmotic compression of the periciliary liquid layer in CF airways.

The post-translational regulation of CFTR expression and function by miRNAs is now established. Oglesby and colleagues reported an increase in miR-494 levels in bronchial epithelial brushings from human CF airways (114). Repression of *CFTR* by miR-494 has also been reported (115;116). The reasons for dysregulated microRNA expression in CF are unknown. Cellular responses to inflammation, infection, cytokines, other inflammatory mediators, or other changes in the ASL environment likely contribute. Additionally, Ramachandran *et al* demonstrated that human airway epithelia transfected with the mimics of miR-509-3p or -494 exhibited reduced CFTR expression and their respective anti-miRs had the opposite effect (117). Interestingly, both miRNAs acted cooperatively in regulating CFTR expression and function. Additionally, miR-138, through interactions with the transcriptional repressor *SIN3A* mRNA and other targets, helps regulate both CFTR transcription and post-translational processing (118). Changes in CFTR abundance and function regulated by miRNAs might help dynamically regulate ASL volume and composition, host defence, and mucociliary clearance. The concept of therapeutic modification of miRNAs is now established (119). As this field advances, there may be opportunities for the development of new therapies aimed at enhancing or reducing the function of specific miRNAs and their targets (120).

Conclusions

The airway epithelium is more than a physical barrier and the central player in mucociliary clearance of the lung. The innate immune functions of the epithelium include not only the secretion of a variety of antimicrobial substances, but also cytokines and growth factors that mediate leukocyte recruitment, modulation of adaptive immunity, and tissue repair and remodelling. An increasing number of studies demonstrate that several of these functions are altered or decreased in asthma, COPD and cystic fibrosis (Figure 2). Novel mechanisms that contribute to the dysfunctional airway epithelium are being discovered, and include miRNAs, endoplasmic reticulum stress and the integrated stress response. Furthermore, various features of a dysfunctional innate immune function of AEC appear to persist in culture, indicating that not only acute exposures to inhaled substances or local inflammation affects the epithelium, but that genetic and epigenetic mechanisms also contribute. Thus the development of advanced epithelial cell culture systems and patient-derived epithelial cell cultures are becoming a valuable tool to study pathogenetic mechanisms and novel diagnostics. In addition, these facilitate target identification for drug discovery purposes, as well as evaluation of therapeutic approaches. This is important, because although the anti-inflammatory effects of inhaled steroids may partly restore epithelial cell function in airway diseases, this is by far not optimal. Therefore, other approaches are needed to restore the dysfunctional airway epithelium in these diseases. Such approaches include using vitamin D to boost epithelial defences, CFTR correctors and potentiators, miRNA modification, small molecules to increase production of antiviral interferons, as well as blocking IL-4 and IL-13.

In summary, our increased understanding of the innate immune functions of the airway epithelium in the healthy lung and in asthma, COPD and cystic fibrosis, will contribute to better diagnostics and treatment of patients with these chronic inflammatory diseases. Therefore current and future basic and translational studies on airway epithelial cell function

will likely continue to contribute to meeting the unmet medical need in the treatment of these diseases with marked morbidity and mortality.

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Reference List

1. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. Eur Respir J. 2004 Feb; 23(2):327–33. [PubMed: 14979512]
2. Parker D, Prince A. Innate Immunity in the Respiratory Epithelium. Am J Respir Cell Mol Biol. 2011 Aug 1; 45(2):189–201. [PubMed: 21330463]
3. Li Y, Eggermont K, Vanslembrouck V, Verfaillie CM. NKX2–1 activation by SMAD2 signaling after definitive endoderm differentiation in human embryonic stem cell. Stem Cells Dev. 2013 May 1; 22(9):1433–42. [PubMed: 23259454]
4. Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. Nat Biotechnol. 2012 Sep; 30(9):876–82. [PubMed: 22922672]
5. Firth AL, Dargitz CT, Qualls SJ, Menon T, Wright R, Singer O, et al. Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2014 Apr 29; 111(17):E1723–E1730. [PubMed: 24706852]
6. Rock JR, Hogan BLM. Epithelial Progenitor Cells in Lung Development, Maintenance, Repair, and Disease. Annu Rev Cell Dev Biol. 2011 Oct 10; 27(1):493–512. [PubMed: 21639799]
7. Herriges M, Morrisey EE. Lung development: orchestrating the generation and regeneration of a complex organ. Development. 2014 Feb 1; 141(3):502–13. [PubMed: 24449833]
8. Wansleeben C, Barkauskas CE, Rock JR, Hogan BLM. Stem cells of the adult lung: their development and role in homeostasis, regeneration, and disease. WIREs Dev Biol. 2013 Jan 1; 2(1): 131–48.
9. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc Natl Acad Sci U S A. 2009 Aug 4; 106(31): 12771–5. [PubMed: 19625615]
10. Tata PR, Mou H, Pardo-Saganta A, Zhao R, Prabhu M, Law BM, et al. Dedifferentiation of committed epithelial cells into stem cells in vivo. Nature. 2013 Nov 14; 503(7475):218–23. [PubMed: 24196716]
11. Rupani H, Sanchez-Elsner T, Howarth P. MicroRNAs and respiratory diseases. Eur Respir J. 2013 Mar; 41(3):695–705. [PubMed: 22790917]
12. Khoshgoo N, Kholdebarin R, Iwasiow BM, Keijzer R. MicroRNAs and lung development. Pediatr Pulmonol. 2013 Apr; 48(4):317–23. [PubMed: 23281163]
13. Harris KS, Zhang Z, McManus MT, Harfe BD, Sun X. Dicer function is essential for lung epithelium morphogenesis. Proc Natl Acad Sci U S A. 2006 Feb 14; 103(7):2208–13. [PubMed: 16452165]
14. Williams AE, Larner-Svensson H, Perry MM, Campbell GA, Herrick SE, Adcock IM, et al. MicroRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy. PLoS One. 2009 Jun 12.4(6):e5889. [PubMed: 19521514]
15. Fulcher ML, Randell SH. Human nasal and tracheo-bronchial respiratory epithelial cell culture. Methods Mol Biol. 2013; 945:109–21. [PubMed: 23097104]
16. Pezzulo AA, Starner TD, Scheetz TE, Traver GL, Tilley AE, Harvey BG, et al. The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of in vivo airway epithelia. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2011 Jan 1; 300(1):L25–L31. [PubMed: 20971803]

17. Dvorak A, Tilley AE, Shaykhiev R, Wang R, Crystal RG. Do airway epithelium air-liquid cultures represent the in vivo airway epithelium transcriptome? Am J Respir Cell Mol Biol. 2011 Apr; 44(4):465–73. [PubMed: 20525805]
18. Horani, A.; Dickinson, J.; Brody, S. Applications of Mouse Airway Epithelial Cell Culture for Asthma Research. In: Allen, IC., editor. Mouse Models of Allergic Disease. 1032. Humana Press; 2013. p. 91-107.
19. Suprynowicz FA, Upadhyay G, Krawczyk E, Kramer SC, Hebert JD, Liu X, et al. Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. Proc Natl Acad Sci U S A. 2012 Dec 4; 109(49):20035–40. [PubMed: 23169653]
20. Horani A, Nath A, Wasserman MG, Huang T, Brody SL. Rho-Associated Protein Kinase Inhibition Enhances Airway Epithelial Basal-Cell Proliferation and Lentivirus Transduction. Am J Respir Cell Mol Biol. 2013 May 28; 49(3):341–7. [PubMed: 23713995]
21. Gaj T, Gersbach CA, Barbas CF III. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013 Jul; 31(7):397–405. [PubMed: 23664777]
22. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH Human Microbiome Project. Genome Res. 2009 Dec; 19(12):2317–23. [PubMed: 19819907]
23. Hansel TT, Johnston SL, Openshaw PJ. Microbes and mucosal immune responses in asthma. Lancet. 2013 Mar 9; 381(9869):861–73. [PubMed: 23428115]
24. Dickson RP, Erb-Downward JR, Huffnagle GB. The role of the bacterial microbiome in lung disease. Expert Review of Respiratory Medicine. 2013 Jun 1; 7(3):245–57. [PubMed: 23734647]
25. Gollwitzer ES, Saglani S, Trompette A, Yadava K, Sherburn R, McCoy KD, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. Nat Med. 2014 May.11:10.
26. Sibley CD, Grinwis ME, Field TR, Eshaghurshan CS, Faria MM, Dowd SE, et al. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. PLoS One. 2011; 6(7):e22702. [PubMed: 21829484]
27. Zemanick ET, Sagel SD, Harris JK. The airway microbiome in cystic fibrosis and implications for treatment. Curr Opin Pediatr. 2011 Jun; 23(3):319–24. [PubMed: 21494150]
28. Molyneaux PL, Mallia P, Cox MJ, Footitt J, Willis-Owen SA, Homola D, et al. Outgrowth of the bacterial airway microbiome after rhinovirus exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2013 Nov 15; 188(10):1224–31. [PubMed: 23992479]
29. Song X, Gao H, Lin Y, Yao Y, Zhu S, Wang J, et al. Alterations in the microbiota drive interleukin-17C production from intestinal epithelial cells to promote tumorigenesis. Immunity. 2014 Jan 16; 40(1):140–52. [PubMed: 24412611]
30. Pezzulo AA, Kelly PH, Nassar BS, Rutland CJ, Gansemer ND, Dohrn CL, et al. Abundant DNase I-sensitive bacterial DNA in healthy porcine lungs and its implications for the lung microbiome. Appl Environ Microbiol. 2013 Oct; 79(19):5936–41. [PubMed: 23872563]
31. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989; 54(Pt 1):1–13. [PubMed: 2700931]
32. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010 Mar 19; 140(6): 805–20. [PubMed: 20303872]
33. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. Nat Rev Immunol. 2013 Jun; 13(6):453–60. [PubMed: 23681101]
34. Anderson KV, Jurgens G, Nusslein-Volhard C. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. Cell. 1985 Oct; 42(3): 779–89. [PubMed: 3931918]
35. Greene CM, McElvaney NG. Toll-like receptor expression and function in airway epithelial cells. Arch Immunol Ther Exp (Warsz). 2005 Sep; 53(5):418–27. [PubMed: 16314825]
36. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. Am J Respir Cell Mol Biol. 2004 Sep; 31(3):358–64. [PubMed: 15191912]
37. Hess C, Herr C, Beisswenger C, Zakharkina T, Schmid RM, Bals R. Myeloid RelA regulates pulmonary host defense networks. Eur Respir J. 2010 Feb; 35(2):343–52. [PubMed: 19679599]
38. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, Reis e Sousa. Myeloid C-type lectins in innate immunity. Nat Immunol. 2006 Dec; 7(12):1258–65. [PubMed: 17110942]

39. Lee HM, Yuk JM, Shin DM, Jo EK. Dectin-1 is inducible and plays an essential role for mycobacteria-induced innate immune responses in airway epithelial cells. J Clin Immunol. 2009 Nov; 29(6):795–805. [PubMed: 19633936]
40. Sun WK, Lu X, Li X, Sun QY, Su X, Song Y, et al. Dectin-1 is inducible and plays a crucial role in Aspergillus-induced innate immune responses in human bronchial epithelial cells. Eur J Clin Microbiol Infect Dis. 2012 Oct; 31(10):2755–64. [PubMed: 22562430]
41. Wang Q, Nagarkar DR, Bowman ER, Schneider D, Gosangi B, Lei J, et al. Role of double-stranded RNA pattern recognition receptors in rhinovirus-induced airway epithelial cell responses. J Immunol. 2009 Dec 1; 183(11):6989–97. [PubMed: 19890046]
42. Lipinski S, Rosenstiel P. Debug Your Bugs - How NLRs Shape Intestinal Host-Microbe Interactions. Front Immunol. 2013 Dec 27.4:479. eCollection;%2013.:479. [PubMed: 24409180]
43. van 't Wout EF, Hiemstra PS, Marciniak SJ. The Integrated Stress Response in Lung Disease. Am J Respir Cell Mol Biol. 2014 Mar 7; 50(6):1005–9. [PubMed: 24605820]
44. Marcinak SJ, Ron D. The unfolded protein response in lung disease. Proc Am Thorac Soc. 2010 Nov; 7(6):356–62. [PubMed: 21030513]
45. Claudio N, Dalet A, Gatti E, Pierre P. Mapping the crossroads of immune activation and cellular stress response pathways. EMBO J. 2013 May 2; 32(9):1214–24. [PubMed: 23584529]
46. Blander JM, Sander LE. Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. Nat Rev Immunol. 2012 Mar; 12(3):215–25. [PubMed: 22362354]
47. Lee RJ, Kofonow JM, Rosen PL, Siebert AP, Chen B, Doghramji L, et al. Bitter and sweet taste receptors regulate human upper respiratory innate immunity. J Clin Invest. 2014 Mar 3; 124(3): 1393–405. [PubMed: 24531552]
48. Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med. 2010 Dec 2; 363(23):2233–47. [PubMed: 21121836]
49. Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. Annu Rev Physiol. 2008 Mar 6; 70(1):459–86. [PubMed: 17850213]
50. Roy MG, Livraghi-Butrico A, Fletcher AA, McElwee MM, Evans SE, Boerner RM, et al. Muc5b is required for airway defence. Nature. 2014 Jan 16; 505(7483):412–6. [PubMed: 24317696]
51. Adler KB, Tuvim MJ, Dickey BF. Regulated Mucin Secretion from Airway Epithelial Cells. Frontiers in Endocrinology. 2013:4. [PubMed: 23420531]
52. Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK, et al. A Periciliary Brush Promotes the Lung Health by Separating the Mucus Layer from Airway Epithelia. Science. 2012 Aug 24; 337(6097):937–41. [PubMed: 22923574]
53. Mall MA, Button B, Johannesson B, Zhou Z, Livraghi A, Caldwell RA, et al. Airway surface liquid volume regulation determines different airway phenotypes in liddle compared with betaENaC-overexpressing mice. J Biol Chem. 2010 Aug 27; 285(35):26945–55. [PubMed: 20566636]
54. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature. 2012 Jul 5; 487(7405):109–13. [PubMed: 22763554]
55. Hoegger MJ, Fischer AJ, McMenimen JD, Ostedgaard LS, Tucker AJ, Awadalla MA, et al. Cystic fibrosis. Impaired mucus detachment disrupts mucociliary transport in a piglet model of cystic fibrosis. Science. 2014 Aug 15; 345(6198):818–22. [PubMed: 25124441]
56. Wain LV, Odenthal-Hesse L, Abujaber R, Sayers I, Beardsmore C, Gaillard EA, et al. Copy number variation of the beta-defensin genes in europeans: no supporting evidence for association with lung function, chronic obstructive pulmonary disease or asthma. PLoS One. 2014 Jan 3.9(1):e84192. [PubMed: 24404154]
57. Moser C, Weiner DJ, Lysenko E, Bals R, Weiser JN, Wilson JM. beta-Defensin 1 contributes to pulmonary innate immunity in mice. Infect Immun. 2002 Jun; 70(6):3068–72. [PubMed: 12010999]
58. Morrison G, Kilanowski F, Davidson D, Dorin J. Characterization of the mouse beta defensin 1, Defb1, mutant mouse model. Infect Immun. 2002 Jun; 70(6):3053–60. [PubMed: 12010997]
59. Herr C, Beisswenger C, Hess C, Kandler K, Suttorp N, Welte T, et al. Suppression of pulmonary innate host defence in smokers. Thorax. 2009 Feb; 64(2):144–9. [PubMed: 18852155]

60. Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci U S A. 1998 Aug 4; 95(16):9541–6. [PubMed: 9689116]
61. Kovach MA, Ballinger MN, Newstead MW, Zeng X, Bhan U, Yu FS, et al. Cathelicidin-related antimicrobial peptide is required for effective lung mucosal immunity in Gram-negative bacterial pneumonia. J Immunol. 2012 Jul 1; 189(1):304–11. [PubMed: 22634613]
62. Kahlenberg JM, Kaplan MJ. Little Peptide, Big Effects: The Role of LL-37 in Inflammation and Autoimmune Disease. The Journal of Immunology. 2013 Nov 15; 191(10):4895–901. [PubMed: 24185823]
63. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006 Mar 24; 311(5768):1770–3. [PubMed: 16497887]
64. Felgentreff K, Beisswenger C, Griese M, Gulder T, Bringmann G, Bals R. The antimicrobial peptide cathelicidin interacts with airway mucus. Peptides. 2006 Dec; 27(12):3100–6. [PubMed: 16963160]
65. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5; 303(5663):1532–5. [PubMed: 15001782]
66. Conner GE, Salathe M, Forteza R. Lactoperoxidase and hydrogen peroxide metabolism in the airway. Am J Respir Crit Care Med. 2002 Dec 15; 166(12 Pt 2):S57–S61. [PubMed: 12471090]
67. Moskwa P, Lorentzen D, Excoffon KJDA, Zabner J, McCray PB Jr, Nauseef WM, et al. A novel host defense system of airways is defective in cystic fibrosis. Am J Respir Crit Care Med. 2007 Jan 15; 175(2):174–83. [PubMed: 17082494]
68. Wink DA, Hines HB, Cheng RY, Switzer CH, Flores-Santana W, Vitek MP, et al. Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol. 2011 Jun; 89(6):873–91. [PubMed: 21233414]
69. Vareille M, Kieninger E, Edwards MR, Regamey N. The airway epithelium: soldier in the fight against respiratory viruses. Clin Microbiol Rev. 2011 Jan; 24(1):210–29. [PubMed: 21233513]
70. Holtzman M, Patel D, Kim HJ, You Y, Zhang Y. Hypersusceptibility to respiratory viruses as a shared mechanism for asthma, chronic obstructive pulmonary disease, and cystic fibrosis. Am J Respir Cell Mol Biol. 2011 Jun; 44(6):739–42. [PubMed: 21653905]
71. Patel DA, Patel AC, Nolan WC, Zhang Y, Holtzman MJ. High throughput screening for small molecule enhancers of the interferon signaling pathway to drive next-generation antiviral drug discovery. PLoS ONE. 2012; 7(5):e36594. [PubMed: 22574190]
72. Zheng S, De BP, Choudhary S, Comhair SAA, Goggans T, Slee R, et al. Impaired Innate Host Defense Causes Susceptibility to Respiratory Virus Infections in Cystic Fibrosis. Immunity. 2003 May; 18(5):619–30. [PubMed: 12753739]
73. Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. Nat Cell Biol. 2010 Sep; 12(9): 814–22. [PubMed: 20811353]
74. Cloonan SM, Lam HC, Ryter SW, Choi AM. “Ciliophagy”: The consumption of cilia components by autophagy. Autophagy. 2014 Mar; 10(3):532–4. [PubMed: 24401596]
75. Heijink IH, Nawijn MC, Hackett TL. Airway epithelial barrier function regulates the pathogenesis of allergic asthma. Clin Exp Allergy. 2014 May 1; 44(5):620–30. [PubMed: 24612268]
76. Grainge CL, Davies DE. Epithelial injury and repair in airways diseases. Chest. 2013 Dec 1; 144(6):1906–12. [PubMed: 24297122]
77. Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, et al. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science. 2008 Oct 24; 322(5901):590–4. [PubMed: 18772398]
78. Anagnostopoulou P, Riederer B, Duerr J, Michel S, Binia A, Agrawal R, et al. SLC26A9-mediated chloride secretion prevents mucus obstruction in airway inflammation. J Clin Invest. 2012 Oct 1; 122(10):3629–34. [PubMed: 22945630]
79. Huang YJ, Boushey HA. The microbiome and asthma. Ann Am Thorac Soc. 2014 Jan; 11(Suppl 1):S48–51.10.1513/AnnalsATS.201306-187MG.:S48-S51 [PubMed: 24437406]

80. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. JEM. 2005 Mar 21; 201(6):937–47.
81. Beisswenger C, Kandler K, Hess C, Garn H, Felgentreff K, Wegmann M, et al. Allergic airway inflammation inhibits pulmonary antibacterial host defense. J Immunol. 2006 Aug 1; 177(3):1833–7. [PubMed: 16849494]
82. Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. J Clin Invest. 2013 Sep 3; 123(9):3967–82. [PubMed: 23945235]
83. Grainge CL, Lau LCK, Ward JA, Dulay V, Lahiff G, Wilson S, et al. Effect of Bronchoconstriction on Airway Remodeling in Asthma. N Engl J Med. 2011 May 25; 364(21): 2006–15. [PubMed: 21612469]
84. Jaumann F, Elssner A, Mazur G, Dobmann S, Vogelmeier C. Transforming growth factor-beta1 is a potent inhibitor of secretory leukoprotease inhibitor expression in a bronchial epithelial cell line. Eur Respir J. 2000 Jun; 15(6):1052–7. [PubMed: 10885424]
85. Bedke N, Sammut D, Green B, Kehagia V, Dennison P, Jenkins G, et al. Transforming growth factor-beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response. PLoS ONE. 2012; 7(9):e44580. [PubMed: 22970254]
86. Schamberger AC, Mise N, Jia J, Genoyer E, Yildirim AO, Meiners S, et al. Cigarette Smoke-Induced Disruption of Bronchial Epithelial Tight Junctions is Prevented by Transforming Growth Factor-Beta. Am J Respir Cell Mol Biol. 2013 Dec 20.
87. Jono H, Xu H, Kai H, Lim DJ, Kim YS, Feng XH, et al. Transforming growth factor-beta-Smad signaling pathway negatively regulates nontypeable Haemophilus influenzae-induced MUC5AC mucin transcription via mitogen-activated protein kinase (MAPK) phosphatase-1-dependent inhibition of p38 MAPK. J Biol Chem. 2003 Jul 25; 278(30):27811–9. [PubMed: 12734193]
88. Harrop CA, Gore RB, Evans CM, Thornton DJ, Herrick SE. TGF-beta(2) decreases baseline and IL-13-stimulated mucin production by primary human bronchial epithelial cells. Exp Lung Res. 2013 Feb; 39(1):39–47. [PubMed: 23249391]
89. Proud D, Hudy MH, Wiehler S, Zaheer RS, Amin MA, Pelikan JB, et al. Cigarette smoke modulates expression of human rhinovirus-induced airway epithelial host defense genes. PLoS ONE. 2012; 7(7):e40762. [PubMed: 22808255]
90. Rusznak C, Mills PR, Devalia JL, Sapsford RJ, Davies RJ, Lozewicz S. Effect of Cigarette Smoke on the Permeability and IL-1beta and sICAM-1 Release from Cultured Human Bronchial Epithelial Cells of Never-Smokers, Smokers, and Patients with Chronic Obstructive Pulmonary Disease. Am J Respir Cell Mol Biol. 2000 Oct 1; 23(4):530–6. [PubMed: 11017919]
91. Kilsgard O, Andersson P, Malmsten M, Nordin SL, Linge HM, Eliasson M, et al. Peptidylarginine deiminases present in the airways during tobacco smoking and inflammation can citrullinate the host defense peptide LL-37, resulting in altered activities. Am J Respir Cell Mol Biol. 2012 Feb; 46(2):240–8. [PubMed: 21960546]
92. Nyunoya T, Mebratu Y, Contreras A, Delgado M, Chand HS, Tesfaigzi Y. Molecular Processes that Drive Cigarette Smoke-Induced Epithelial Cell Fate of the Lung. Am J Respir Cell Mol Biol. 2013 Oct 10; 50(3):471–82. [PubMed: 24111585]
93. Steiling K, van den Berge M, Hijazi K, Florido R, Campbell J, Liu G, et al. A dynamic bronchial airway gene expression signature of chronic obstructive pulmonary disease and lung function impairment. Am J Respir Crit Care Med. 2013 May 1; 187(9):933–42. [PubMed: 23471465]
94. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. Eur Respir J. 2014 Oct; 44(4):1042–54. [PubMed: 24925916]
95. Schulz C, Wolf K, Harth M, Kratzel K, Kunz-Schughart L, Pfeifer M. Expression and release of interleukin-8 by human bronchial epithelial cells from patients with chronic obstructive pulmonary disease, smokers, and never-smokers. Respiration. 2003 May; 70(3):254–61. [PubMed: 12915744]
96. van't Wout EFA, Dickens JA, van Schadewijk A, Haq I, Kwok HF, Ordonez A, et al. Increased ERK signalling promotes inflammatory signalling in primary airway epithelial cells expressing Z alpha1-antitrypsin. Human Molecular Genetics. 2014 Feb 15; 23(4):929–41. [PubMed: 24097797]

97. Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science. 2008 Sep 26; 321(5897): 1837–41. [PubMed: 18818360]

98. Ostedgaard LS, Meyerholz DK, Chen JH, Pezzulo AA, Karp PH, Rokhlina T, et al. The DeltaF508 mutation causes CFTR misprocessing and cystic fibrosis-like disease in pigs. Sci Transl Med. 2011 Mar 16.3(74):74ra24.

99. Sun X, Yan Z, Yi Y, Li Z, Lei D, Rogers CS, et al. Adeno-associated virus-targeted disruption of the CFTR gene in cloned ferrets. J Clin Invest. 2008 Apr; 118(4):1578–83. [PubMed: 18324338]

100. Tuggle KL, Birket SE, Cui X, Hong J, Warren J, Reid L, et al. Characterization of defects in ion transport and tissue development in cystic fibrosis transmembrane conductance regulator (CFTR)-knockout rats. PLoS One. 2014 Mar 7.9(3):e91253. [PubMed: 24608905]

101. Stoltz DA, Meyerholz DK, Pezzulo AA, Ramachandran S, Rogan MP, Davis GJ, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. Sci Transl Med. 2010 Apr 28.2(29):29ra31.

102. Sun X, Sui H, Fisher JT, Yan Z, Liu X, Cho HJ, et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. J Clin Invest. 2010 Sep; 120(9):3149–60. [PubMed: 20739752]

103. Sun X, Olivier AK, Liang B, Yi Y, Sui H, Evans TI, et al. Lung phenotype of juvenile and adult cystic fibrosis transmembrane conductance regulator-knockout ferrets. Am J Respir Cell Mol Biol. 2014 Mar; 50(3):502–12. [PubMed: 24074402]

104. McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EW. Airway surface pH in subjects with cystic fibrosis. Eur Respir J. 2003 Jan; 21(1):37–42. [PubMed: 12570106]

105. Abou Alaiwa MH, Beer AM, Pezzulo AA, Launspach JL, Horan RA, Stoltz DA, et al. Neonates with cystic fibrosis have a reduced nasal liquid pH; A small pilot study. J Cyst Fibros. 2014 Jan 11; 13(4):373–7. [PubMed: 24418186]

106. Berkebile AR, McCray PB Jr. Effects of airway surface liquid pH on host defense in cystic fibrosis. Int J Biochem Cell Biol. 2014 Feb 19.

107. Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med. 2007; 58:157–70. [PubMed: 17217330]

108. Hobbs CA, Da TC, Tarran R. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? J Physiol. 2013 Sep 15; 591(Pt 18):4377–87. [PubMed: 23878362]

109. Chen JH, Stoltz DA, Karp PH, Ernst SE, Pezzulo AA, Moninger TO, et al. Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell. 2010 Dec 10; 143(6):911–23. [PubMed: 21145458]

110. Fisher JT, Tyler SR, Zhang Y, Lee BJ, Liu X, Sun X, et al. Bioelectric Characterization of Epithelia from Neonatal CFTR Knockout Ferrets. American Journal of Respiratory Cell and Molecular Biology. 2013 Jun 19; 49(5):837–44. [PubMed: 23782101]

111. Itani OA, Chen JH, Karp PH, Ernst S, Keshavjee S, Parekh K, et al. Human cystic fibrosis airway epithelia have reduced Cl-conductance but not increased Na+ conductance. Proceedings of the National Academy of Sciences. 2011 Jun 21; 108(25):10260–5.

112. Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med. 2004 May; 10(5):487–93. [PubMed: 15077107]

113. Henderson AG, Ehre C, Button B, Abdullah LH, Cai LH, Leigh MW, et al. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. J Clin Invest. 2014 Jul 1; 124(7):3047–60. [PubMed: 24892808]

114. Oglesby IK, Bray IM, Chotirmall SH, Stallings RL, O'neill SJ, McElvaney NG, et al. miR-126 is downregulated in cystic fibrosis airway epithelial cells and regulates TOM1 expression. J Immunol. 2010 Feb 15; 184(4):1702–9. [PubMed: 20083669]

115. Gillen AE, Gosalia N, Leir SH, Harris A. MicroRNA regulation of expression of the cystic fibrosis transmembrane conductance regulator gene. Biochem J. 2011 Aug 15; 438(1):25–32. [PubMed: 21689072]

116. Megiorni F, Cialfi S, Dominici C, Quattrucci S, Pizzuti A. Synergistic post-transcriptional regulation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) by miR-101 and miR-494 specific binding. PLoS One. 2011; 6(10):e26601. [PubMed: 22028919]

117. Ramachandran S, Karp PH, Osterhaus SR, Jiang P, Wohlford-Lenane C, Lennox KA, et al. Post-transcriptional regulation of cystic fibrosis transmembrane conductance regulator expression and function by microRNAs. Am J Respir Cell Mol Biol. 2013 Oct; 49(4):544–51. [PubMed: 23646886]

118. Ramachandran S, Karp PH, Jiang P, Ostedgaard LS, Walz AE, Fisher JT, et al. A microRNA network regulates expression and biosynthesis of wild-type and DeltaF508 mutant cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A. 2012 Aug 14; 109(33):13362–7. [PubMed: 22853952]

119. Greene CM, Gaughan KP. microRNAs in asthma: potential therapeutic targets. Curr Opin Pulm Med. 2013 Jan; 19(1):66–72. [PubMed: 23095468]

120. Jackson AL, Levin AA. Developing microRNA therapeutics: approaching the unique complexities. Nucleic Acid Ther. 2012 Aug; 22(4):213–25. [PubMed: 22913594]

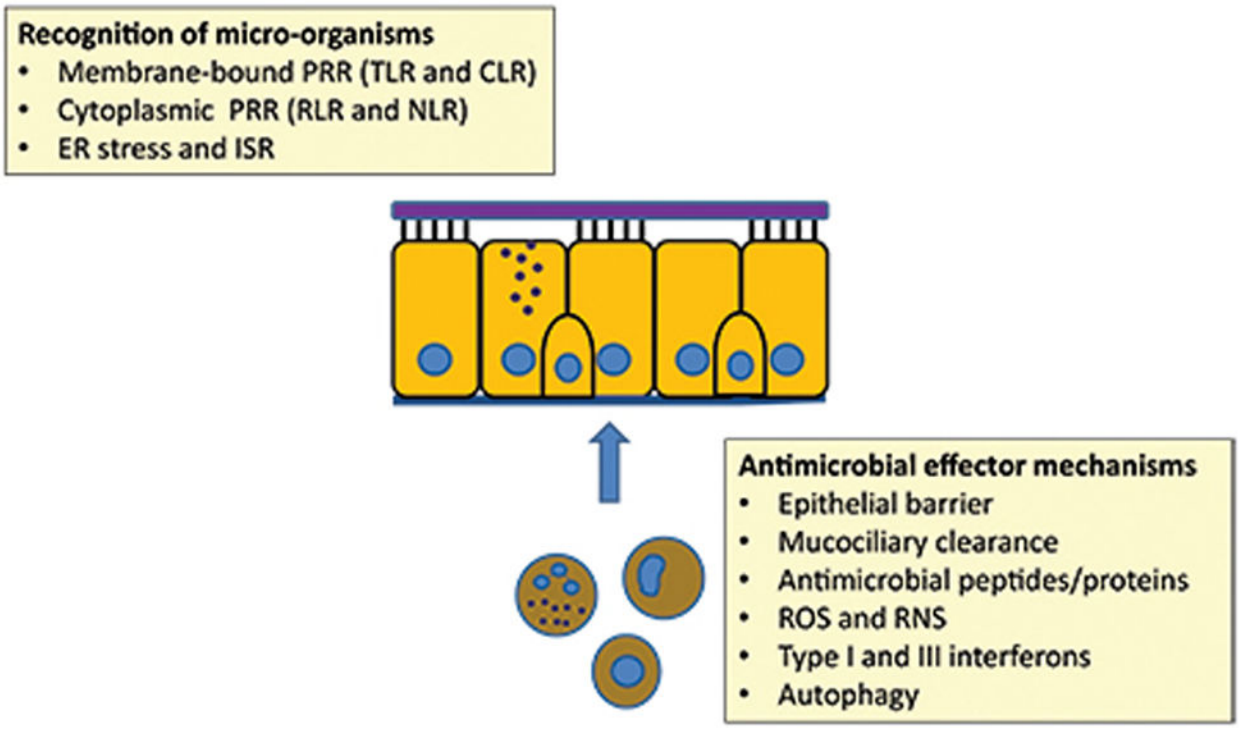

Figure 1. The innate immune function of the airway epithelium: sensing microbial presence and antimicrobial effector mechanisms

Various cell types are involved in the innate immune function of the airway epithelium, including the basal cells, goblet cells, ciliated cells and club cells. Microbial presence is detected by pattern recognition receptors (PRR): membrane-bound Toll-like receptors (TLR) and C-type lectin receptors (CLR), and cytoplasmic RIG-I-like receptors (RLR) and NOD-like receptors (NLR). Also other mechanisms are involved in this recognition process, including endoplasmic reticulum (ER) stress and the integrated stress response (ISR). Antimicrobial effector mechanisms include the barrier function of the epithelium, mucociliairy clearance and the antimicrobial activity of mucus, antimicrobial peptides, reactive oxygen species (ROS) and reactive nitrogen species (RNS), antiviral interferons (type I and III interferons) and autophagy. In addition, production of cytokines, chemokines and other mediators results in the recruitment of cells of the adaptive and innate immune system that may contribute to host defence.

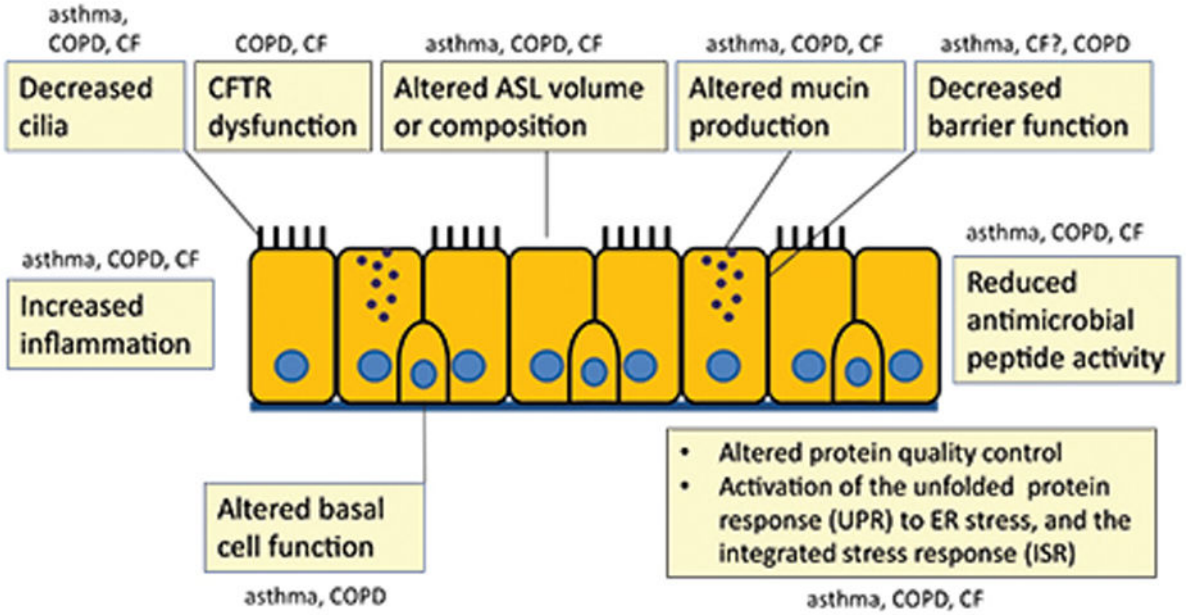

Figure 2. Airway epithelial dysfunction in asthma, COPD and cystic fibrosis

Several mechanisms related to the innate immune function of AEC are altered or dysfunctional in asthma, COPD and cystic fibrosis.

Table 1

Toll like receptors (TLRs) of humans and mice

TLR	Cellular localization	Microbial ligand
TLR1	Plasma membrane	Lipoproteins
TLR2	Plasma membrane	Lipoproteins
TLR3	Endosome	Double stranded (ds)RNA
TLR4	Plasma membrane	LPS
TLR5	Plasma membrane	Flagellin
TLR6	Plasma membrane	Diacyl lipoprotein
TLR7	Endosome	Single stranded (ss)RNA
TLR8	Endosome	Small synthetic compounds; single-stranded RNA
TLR9	Endosome	CpG-oligonucleotides
TLR10	Endosome	Putatively influenza related ligand
TLR11, 12, 13 (mouse, not humans)	Endosome	Profilin-like molecule (parasites)