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Perspective

Airway Epithelial Cell Function and Respiratory Host Defense in Chronic Obstructive Pulmonary Disease

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Introduction

Our lungs have a vital role in mediating the exchange of oxygen and carbon dioxide between the air we breathe and the body. This function is under constant pressure as inhaled air contains numerous particles, gasses, and microorganisms that may cause injury and infection to the lungs. Removal and neutralization of potential harmful substances from inhaled air is a main function of the airway epithelium. This pseudo-stratified layer of cells covers the surface of the conducting airways and plays an important role in protecting the alveoli, where gas exchange takes place, from injury. The airway epithelium has a range of properties that contribute to lung defense, including constitutive host defense mechanisms and regulation of airway innate immunity. Moreover, epithelial cells display wound-healing properties, which allow rapid recovery of airway tissues upon injury. Airway epithelial host defense functions are important to maintain proper gas exchange and lung homeostasis. Epithelial exposures to inhaled noxious particles and gasses may have detrimental outcomes on this host defense function of the airway epithelium.

This is seen in chronic obstructive pulmonary disease (COPD), a disease in which an impaired epithelial function and epithelial remodeling caused by smoking and exposure to other inhaled toxicants contributes to an accelerated decline in lung function. COPD is a severe inflammatory lung disease, regarded as one of the most prevalent burdens in global health[1] and is predicted as the 3rd cause of death and the leading lung disease worldwide by 2030.[2] Airflow limitation in COPD is accompanied by persistent inflammation, airway remodeling, and destruction of lung tissue, resulting in clinical symptoms such as dyspnea, chronic cough, and fatigue. Exposure to biomass, occupational dusts, and chemicals are examples of cytotoxic insults that are associated with COPD development and progression.[3-5] However, smoking is regarded as the main risk factor that is associated with the disease in industrialized countries. In addition to a progressive decline in lung function in stable COPD, exacerbations – defined as an acute worsening of symptoms – contribute markedly to morbidity and mortality in COPD. Respiratory infections are considered as an important trigger for COPD exacerbations, and patients with frequent exacerbations are more susceptible to recurrent exacerbations.[6] This increased colonization and infections with opportunistic respiratory pathogens have been attributed in part to impaired epithelial host defense functions. However, the molecular and cellular mechanisms that are affected in the airway epithelium by smoking and that may lead to COPD are largely unclear.

Microbial Colonization and Respiratory Infections in Chronic Obstructive Pulmonary Disease

Microbial colonization and infections are an important pathophysiological aspect in certain COPD patients. While many studies focused on the detection of respiratory tract bacterial and viral infections during disease exacerbations, colonization during the stable phase of the disease has now been extensively studied. Based on traditional culture-based

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techniques, it was shown that clinically stable COPD patients were colonized with opportunistic respiratory pathogens, most notably nontypeable *Haemophilus influenzae*.[7-10] Colonization with respiratory pathogens was furthermore associated with elevated levels of inflammatory markers in upper- and lower airway fluid samples.[8,9,11,12] This suggests a role of microbial colonization in airway inflammation in COPD patients. Recent understanding of the presence of complex lung microbial communities (the lung microbiome) has further supported a role for microbial colonization in COPD pathogenesis. Compared to healthy individuals, it has been shown in various studies[13,14] that COPD patients have altered microbiomes in the upper and lower airways, which are characterized by a less diverse microbial composition. In line with culture-based studies, respiratory pathogens such as *Haemophilus* spp., were observed more frequently in the airway microbiome of COPD patients.[15,16] In addition, the COPD airway microbiome is characterized by the absence of microbes that are common in healthy individuals.[15,17] These promising findings suggest that an imbalance in the microbiome or dysbiosis is a hallmark of COPD. Besides colonization in stable COPD, acute bacterial or viral infections are associated with approximately 50% of disease exacerbations. In particular, acquisition of new bacterial strains is assumed to cause acute worsening of patient symptoms.[18] Furthermore, recent studies suggest alterations in the airway microbiome during COPD exacerbations, which are characterized by an increase in airway pathogens.[19] Overall, these observational studies highlight the importance of a better understanding of the role of microbial colonization and infections in COPD pathogenesis and its interaction with epithelial host defenses.

The underlying mechanism linking smoking with microbial colonization and infections in COPD can be explained by the vicious circle hypothesis.[13] According to this hypothesis, smoking stimulates the development and progression of COPD by initiating a vicious circle of airway injury, microbial colonization/infections, and inflammation [Figure 1].[13]

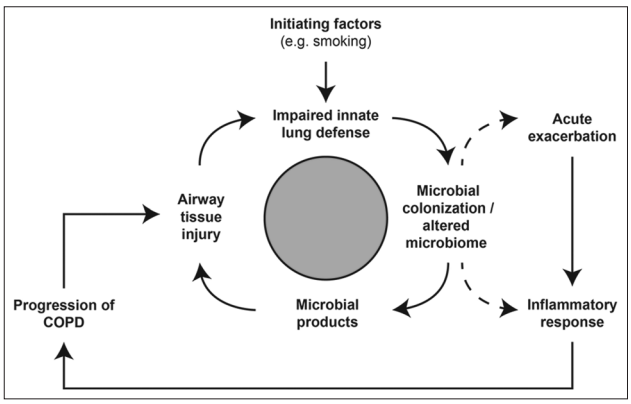

Figure 1: Vicious circle hypothesis of COPD. A model that explains the development and progression of COPD, focusing on a persistent cycle of microbial colonization and infections, inflammation, and airway tissue injury. Adapted from: Mammen and Sethi, 2016. COPD: Chronic obstructive pulmonary disease.

Cigarette smoke exposure of airway tissues induces damage, which promotes local inflammatory responses and impairs host defense. Microbes further amplify airway inflammatory responses, whereas chronic inflammation contributes to tissue damage and degenerative repair. The persistence of this vicious circle due to repetitive smoking eventually modulates tissue repair and leads to remodeling of the airways, thereby causing progressive airflow obstruction. In line with this, endogenous lung tissue repair may be impaired in COPD as demonstrated by decreased nuclear β-catenin staining in emphysematous lung tissue.[20]

Airway Epithelium

The airway epithelium is the first target of inhaled cigarette smoke. Furthermore, epithelial cells are the first defense lining of the respiratory tract that prevents microbial colonization and infections.[21,22] Since the airway epithelium is also the first tissue to be exposed to inhaled toxicants such as those present in cigarette smoke, the airway epithelium has a central role in the vicious circle hypothesis, and alterations in host defense and epithelial remodeling may contribute to COPD development and progression.

The airway epithelium is a continuous layer that covers the surface of the respiratory tract and consists of cells that are connected by adhesion and tight junctions.[23,24] Two morphological and functional distinct types of epithelium are located in, respectively, the conductive airways and respiratory units in the lung peripheral tissue [Figure 2a]. The conductive airways start at the nasal cavity and end at the small bronchioles in the lower airways. In these regions, the epithelium facilitates the moistening and warming of inhaled air before reaching the alveoli in the respiratory units where gas exchange takes place. The airway epithelium of the conductive airways furthermore has an active role in protecting the lungs against inhaled microorganisms, and this role is closely linked to the morphology and composition of the epithelium.

In contrast to the simple columnar and cuboidal lining of the bronchioles and alveoli, the epithelium of the large conducting airways is characterized by a pseudostratified morphology.[23,25] Based on this morphology, epithelial cells can be divided into luminal cells (LCs), which are in direct contact with the environment, and basal cells (BCs) that are superimposed by LCs and located above the basement membrane [Figure 2b]. The main cell types that make up the LC population are the ciliated cells and the secretory cells which include the club cells and the mucus-producing goblet cells, which are discussed in the next paragraph. LCs and BCs have distinct functions in airway host defense, which depend on the degree of microbial threat and also whether the epithelial layer is intact or damaged. Based on this, airway epithelial host defenses can be categorized into (1) constitutive host defense mechanisms by LCs, (2) inducible innate immunity, and (3) injury-induced wound repair and defense by airway BCs [Figure 2c]. In addition, the airway epithelium plays a central role in instructing

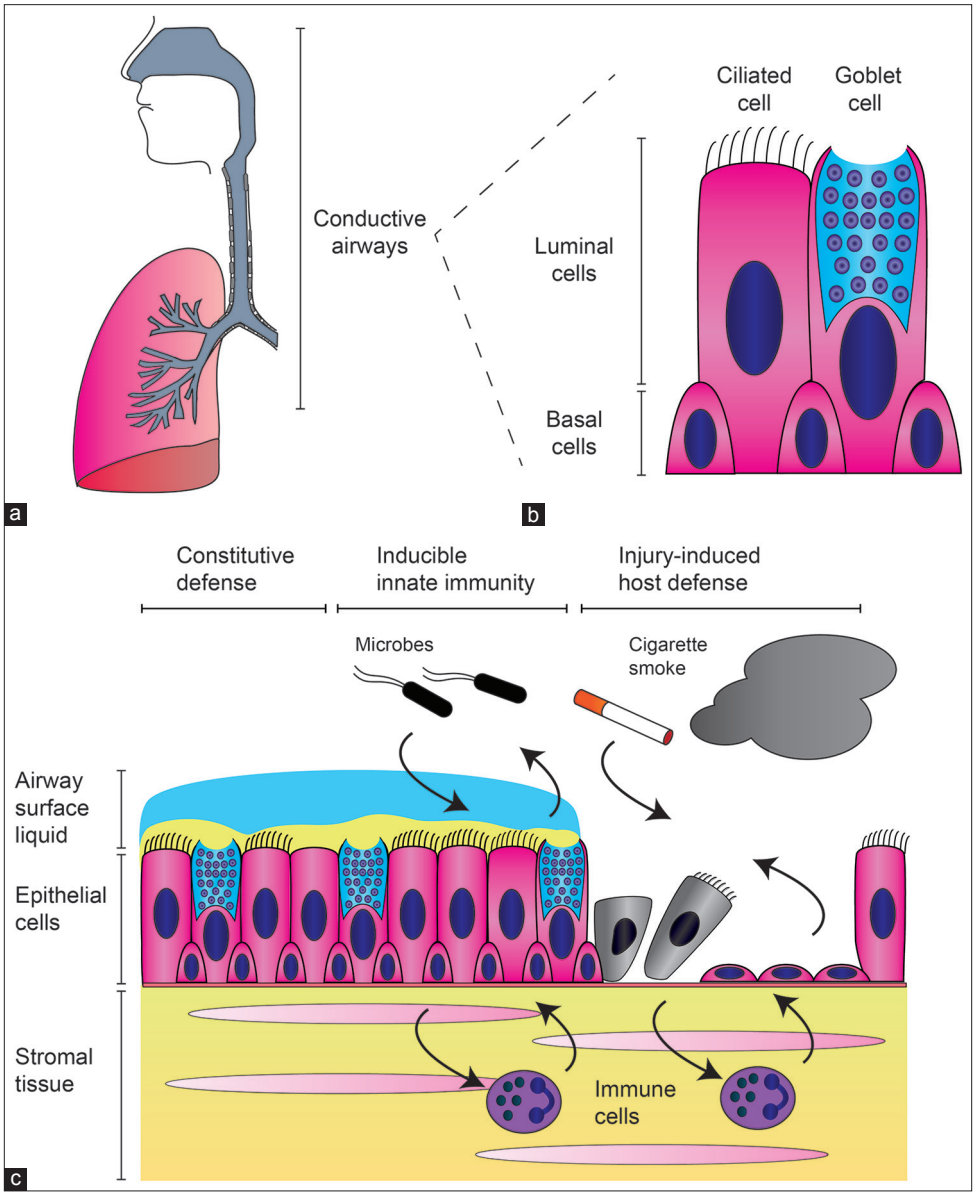

Figure 2: Schematic presentation of the airway epithelium. (a) The human respiratory tract, with the conductive airways highlighted in gray. (b) Composition of the pseudostratified airway epithelium, consisting of ciliated, secretory cells (i.e., goblet and club cells located in the upper and lower airways, respectively) and basal cells. (c) Airway epithelial host defense mechanisms include constitutive host defense mechanisms, inducible innate immunity, activated for instance by microbes, and injury-induced host defense mechanisms, activated for instance by cigarette smoking. Both inducible innate immunity and injury-induced host defense mechanisms contribute to the chemoattraction and interaction with immune cells.

adaptive immunity by interacting with dendritic cells and innate lymphoid cells, but this topic is beyond the scope of the present review.

Constitutive Luminal Cell Host Defenses

Constitutive epithelial host defense mechanisms are defined as those functions mediated by intact airway epithelium at baseline, homeostatic conditions [Figure 3a]. This includes the physical barrier functions of connected epithelial cells, but also active mechanisms mediated by LCs that are directly exposed to environmental insults. LCs comprise mature high columnar cells with specialized functions. Ciliated cells are an abundant LC type and are characterized by their multiciliated structures at the apical surface.[23,25] Moreover, the luminal epithelium includes specialized secretory cells, i.e., goblet and club cells, which are distinctively located in, respectively, the large and small airways.[26] The constitutive defense of LCs depends on the interaction between ciliated and secretory cells in regulating the fluid lining located at the epithelial surface. This airway surface liquid (ASL) consists of a mixture of host defense proteins and peptides that are secreted by the airway epithelium and immune cells.[27-29] This mixture provides a chemical shield against microorganisms and is responsible for the relatively low levels of microbes in the respiratory tract of healthy individuals. Antimicrobial proteins and peptides (AMPs) present in this ASL prevent

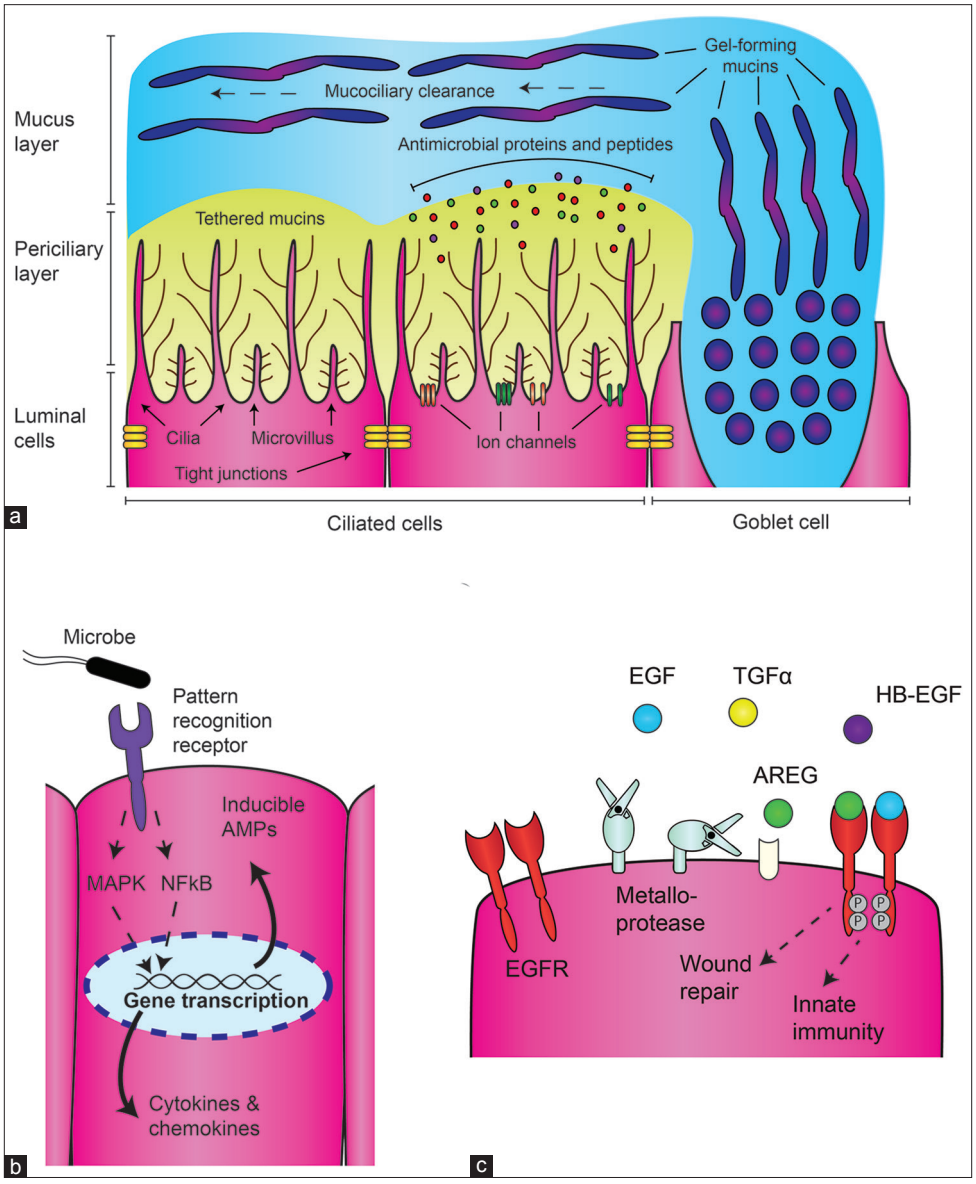

Figure 3: Airway epithelial host defense. (a) Constitutive host defense mechanisms of the luminal airway epithelium including barrier function, a cellular and tethered mucin barrier, defense through mucociliary clearance and secreted antimicrobial proteins and peptides, and regulation of airway surface liquid physiological properties through ion transport channels. (b) Inducible innate immunity can be activated upon recognition of microbes by epithelial pattern recognition receptors, which activate signaling pathways, i.e., MAPK and NF-κB, which promote the expression of inducible AMPs and pro-inflammatory mediators. (c) Epithelial injury results in the activation of EGFR located on basal cells, through various EGFR-ligands (i.e., EGF, TGF-α, HB-EGF, and AREG) produced in an autocrine manner or by luminal cells, stromal cells, or immune cells. The release of EGF-ligands is in part mediated through shedding by matrix-metalloproteases. EGFR activation subsequently promotes wound repair and innate immune responses. EGFR: Epidermal growth factor receptor; EGF: Epidermal growth factor; TGF-α: Transforming growth factor-alpha; HB-EGF: Heparin-binding-epidermal growth factor; AREG: Amphiregulin.

microbial colonization and infections by displaying direct microbial killing activity or by reducing the availability of important micronutrients.[30,31] Another host defense mechanism is mediated by secreted gel-forming mucins, present in the ASL as discontinuing floating strands or rafts.[32,33] These mucins can entrap microorganisms and large particles and are subsequently removed through mucociliary clearance. During this process, mucus is propelled from the airways toward the throat by the continuous ciliary beating of ciliated cells.[34] MUC5B and MUC5AC, the main mucins of the mucus gel, are mainly produced by the goblet cells of the surface epithelium and by the submucosal glands.[33] Moreover, it has been reported that club cells can produce MUC5B in the lower airways.[35] A second constitutive defense lining, the periciliary layer, separates the luminal airway epithelium and mucin gel. The fluidity and height of this layer is important to allow the cilia to move the mucus layer with entrapped particles. Host defense mucins that are tethered to the surface of the epithelium and present in complexes with the glycosaminoglycan keratin sulfate also contribute to host defense.[36] These complexes are mainly located at epithelial cilia and are assumed to shape

a periciliary brush, which creates an additional barrier that prevents penetration of particles and microorganisms.[37] LCs furthermore regulate the physiological conditions of the ASL. This is mediated by active ion transport, for instance by the cystic fibrosis transmembrane conductance regulator protein or calcium-activated chloride channels such as anoctamin-1 (ANO-1/TMEM16A).[38,39] Chloride secretion and reabsorption of sodium by the epithelial sodium channel have been shown to regulate ASL volume.[40] This has important consequences for mucociliary clearance as it determines the hydration state of the mucus gel, as well as the height of the periciliary layer which is an important determinant of ciliary movement.[41] Moreover, transport of bicarbonate regulates the pH of the ASL,[42] which may affect the activity of pH-sensitive AMPs and mucus viscosity.[43,44]

Inducible Innate Immunity

Constitutive host defense mechanisms provided by LCs give baseline protection during relatively low microbial exposures. Evasion from host defense mechanisms or adaptation to the host microenvironment may allow microbial outgrowth, thereby overwhelming constitutive airway epithelial defense.[45] Therefore, secondary host defense mechanisms are activated upon sensing of increased levels of microbes [Figure 3b].[46] This depends on recognition of microbes by host cell receptors, which is highly conserved between species. It was first observed in Drosophila that microbial recognition of the receptor toll resulted in the expression of AMPs.[47] Similar to Drosophila, human toll-like receptors are present at the surface of airway epithelial cells or located in membrane-enclosed compartments.[48] Moreover, other patter recognition receptors (PRRs), such as NOD-like receptors, MDA5, and RIG-1, are located in the cell cytosol.[49] Ligation of PRRs leads to activation of cellular signaling transduction pathways such as MAPK and NF-κB.[50] This subsequently leads to expression of AMPs that are not produced at baseline conditions or only at very low levels. These "inducible" AMPs increase the antimicrobial activity of the ASL, counteracting the increased levels of microbes.[51,52] In addition to increasing the expression of AMPs, activation of downstream signaling pathways also leads to epithelial expression of pro-inflammatory cytokines and chemokines.[53] These factors increase the attraction of immune cells to the site where increased microbial exposure is detected. Initially, innate immune cells, such as dendritic cells, macrophages, and neutrophils, are directed to the epithelium, but in later stages also, adaptive immune cells such as T- and B-lymphocytes are attracted. In addition to activation of the inducible innate immune system by microbes, airway epithelial cells are furthermore activated by the attracted innate and adaptive immune cells, which produce cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha.[54] Moreover, airway epithelial cells display an autocrine mechanism, in which expression of the pro-inflammatory cytokine IL-17C leads to maintained innate immune defense mechanisms.[55,56] Finally, the micronutrient Vitamin D can also induce antibacterial responses, in part through the expression of the antimicrobial peptide LL-37.[57] Taken together, inducible secondary host defense mechanisms are increased in the epithelium upon microbial exposure, during inflammation, during repair processes (discussed in the next paragraph), and upon exposure to Vitamin D, thereby providing protection upon outgrowth of microbes in the airways.

Injury-Induced Innate Defense by Airway Basal Cells

The importance of maintaining an intact airway epithelium is emphasized by the low epithelial turnover at steady-state levels.[58,59] However, exposure to cytotoxic particles and microorganisms may cause epithelial injury, leading to shedding and cell death of LCs.[25] Shedding of LCs provides defense by removal of infected cells.[60] Moreover, epithelial death induced by injury or infection leads to the release of components that contain the so-called damage-associated molecular patterns, which – like the microbial pathogen-associated molecular patterns – serve as danger signals and activate the innate immune system.[61] Nevertheless, elimination of LCs compromises epithelial host defense. In this case, airway epithelial BCs play a role in providing airway protection [Figure 3c]. BCs serve as progenitor cells and comprise approximately 30% of the airway epithelium in the large conductive airways, whereas their numbers are lower at distal regions of the conductive airways.[58] The cells are largely quiescent in intact epithelium. However, upon epithelial injury, BCs contribute to epithelial host defense by mediating recovery of the epithelial lining.[62] Initially, BCs spread and migrate on denuded basement membranes, followed by proliferation and differentiation toward mature LCs. A central role in the activation of epithelial repair involves activation of the epidermal growth factor receptor (EGFR).[63] This Erb family member is restricted to BCs and is activated by various ligands, including epidermal growth factor, amphiregulin, and transforming growth factor-alpha.[64-66] These ligands are produced and secreted by stromal cells or immune cells; however, EGFR is also activated in an autocrine manner. This occurs for instance not only through the release of EGF located at the surface of damaged luminal airway epithelial cells, but also through shedding of membrane-bound EGFR-ligands by matrix metalloproteinases.[63] In all cases, activation of EGFR leads to initiation of wound repair, particularly controlled by MAPK signaling transduction and downstream AP-1 family transcription factors. In addition, BCs contribute to airway innate immunity upon activation of PRRs.[67] Moreover, EGFR activates innate immune responses by promoting the expression of antimicrobial peptides and pro-inflammatory factors that lead to chemoattraction of immune cells to the site of injury as well as epithelial expression of AMPs. High expression of integrins and the cell type-restricted expression

of ICAM-1 allow homing of immune cells to BCs, which may provide protection against microbes at the site of injury.[68,69] Moreover, innate immune mediators produced by immune cells may increase wound repair or direct LC differentiation.[70,71]

Effect of Cigarette Smoke and Chronic Obstructive Pulmonary Disease Status on Airway Epithelial Host Defense

Basic research using cell culture models has markedly contributed to our understanding of airway epithelial cell biology. These models also serve as an important tool to understand epithelial cell responses to stimuli related to chronic inflammatory airway diseases or examine and compare cell cultures from diseased patients and controls.[72] In COPD research, a large number of studies have analyzed the effect of cigarette smoke on airway epithelial cell cultures. In particular, aqueous solutions of cigarette smoke particles, i.e., extract or condensate, have been used to study this.[73,74] However, this approach primarily takes the effects of the soluble particulate phase of cigarette smoke into account and underestimates the effect of the vapor phase and especially that of short-lived oxidants.[75] Therefore, instead of the conventional method of using an aqueous extract of cigarette smoke, we have set up a whole cigarette smoke exposure model.[76,77] In this model, epithelial cells are directly exposed to the particulate and vapor phase by leading smoke derived from a burning cigarette directly to the cells that are grown at the air-liquid interface. This allows the exposure of cells to airborne substances in a physiologically realistic fashion. Previous studies using a comparable exposure model have shown that cigarette smoke inhibits the antimicrobial activity of airway epithelial cells.[78] These results suggest that further application of the whole cigarette smoke exposure model will give insight into how other airway epithelial cell host defense functions are affected by smoking.

Although smoking is regarded as the primary risk factor of COPD, not all smokers develop the disease.[79] Therefore, it can be speculated that epithelial cells from COPD patients and non-COPD smokers display differences in host defense properties that may explain disease development. Recent studies have suggested that differences in airway epithelial activities persist in cell culture, such as an impaired airway epithelial barrier integrity, reduced wound repair, and alterations in cell differentiation.[80-83] Based on this, we hypothesized that persistent differences are present in other airway epithelial host defense properties of COPD patients and non-COPD controls. Indeed, in a recent study, we demonstrated impaired antimicrobial defenses in air–liquid interface cultures of primary airway epithelial cells from patients with COPD compared to (ex)-smoking controls.[84] This was accompanied by a lower expression of selected antimicrobial peptides, and expression of a range of such inducible AMPs was found to be further decreased by exposure to cigarette smoke. In this study, we observed that acute smoke increases epithelial inflammation and decreases host defense by differentially affecting MAP kinase and NF-κB pathways. Recently, we also reported that chronic exposure to cigarette smoke of differentiating cultured airway epithelial cells causes airway remodeling characterized by impaired LC formation and aberrant expression of Notch-signaling target genes, resulting in impaired constitutive host defense mechanisms.[85] The involvement of the Notch pathway in these *in vitro* events is in line with reports on the putative role of this pathway in aberrant epithelial differentiation in COPD. We furthermore observed that cessation of chronic CS exposure during differentiation results in the re-appearance of differentiated AEC, except for club cells. This is in line with the observation that club cells are very sensitive to smoke exposure and may help to explain aberrant epithelial repair in COPD in view of the capacity of club cells to self-renew and differentiate into ciliated and goblet cells. Interestingly, in another study, we showed that exposure of human airway epithelial cell cultures to diesel exhaust displayed similar effects on airway epithelial cell innate immune function as acute exposure to cigarette smoke.[86] These findings highlight the risk of air pollution for the development of respiratory diseases. Collectively, these studies show that cigarette smoke alters the innate immune function of the airway epithelium, resulting in decreased host defense and increased inflammation. The observation that such responses are also altered in cultured cells outside the COPD lung environment indicates that some of the aspects of altered epithelial cell function in COPD persist, which may in part be explained by epigenetic changes in the airway epithelium.

Conclusions

The use of basic and translational science approaches to study airway epithelial cell function in COPD has provided us with novel insights into dysregulated innate immunity in COPD. Impaired host defense in smokers with and without COPD can thus be explained by a range of mechanisms, including disrupted mucociliary clearance, decreased barrier function, and decreased antimicrobial activity of the airway epithelium. These are in part explained by acute effects of exposure to inhaled toxicants such as cigarette smoke, but also result from remodeling of the airway epithelium. The observation that some of these dysregulations in airway epithelial cell function persist in cultured cells can possibly be explained by epigenetic mechanisms. Such epigenetic mechanisms may also contribute to the chronicity of impaired epithelial host defense and inflammation in COPD. Further studying the abnormal function of the airway epithelial cell progenitors such as the BCs in COPD may provide important clues for understanding COPD pathogenesis and development of disease-modifying treatments.

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Conflicts of interest

There are no conflicts of interest.

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