

Role of Epithelial Cells in Chronic Inflammatory Lung Disease

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Introduction

Airborne pathogens entering the lungs first encounter the mucus layer overlaying epithelial cells as a first line of host defense [1, 2]. In addition to serving as the physical barrier to these toxic agents, intact epithelia also are major sources of various macromolecules including antimicrobial agents, antioxidants and antiproteases [3, 4] as well as proinflammatory cytokines and chemokines that initiate and amplify host defensive responses to these toxic agents [5]. Airway epithelial cells can be categorized as either ciliated or secretory [6]. Secretory cells, such as goblet cells and Clara cells, are responsible for the production and secretion of mucus along the apical epithelial surface and, in conjunction with ciliated cells, for the regulation of airway surface liquid viscosity. In addition, submucosal mucus glands connect to the airway lumen through a ciliated duct that propels mucins outward. These glands are present in the larger airways between bands of smooth muscle and cartilage. See Fig. 1.

Initially, inhaled toxic agents encounter a mucus layer overlying the respiratory epithelium, become trapped, and are subsequently neutralized by macromolecules. Elimination of these toxic agents depends on mucociliary clearance and cough.

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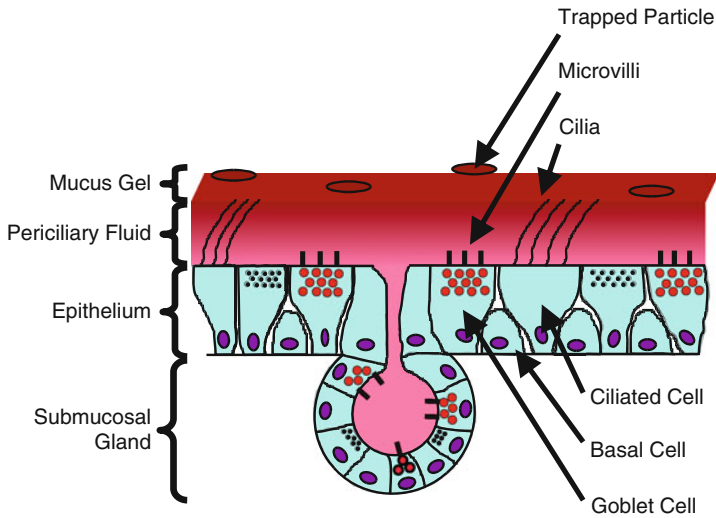


Fig. 1 The airway epithelium and mucus gel

Continuous ciliary movement propels secretions proximally at about 1 mm per minute [7], and this mucosal velocity is modified by hydration of the mucus layer [7, 8] and adrenergic and cholinergic stimuli [7, 9–11]. Efficacy of cough in the elimination of mucus depends on inspiratory muscle strength and expiratory flow velocity, which must detach the mucus from the airway surface and expel the secretions proximally.

A second layer of defense is provided by cell surface receptors (e.g. Toll-like receptors, TLRs) on epithelial cells and resident leukocytes. They bind to various components of the harmful agents and stimulate the production of proinflammatory cytokines (e.g. tumor necrosis factor- α , TNF- α) and chemokines (e.g. interleukin-8). Finally, a third layer of protection is mediated by leukocytes recruited to the lumen of the airways by chemotactic molecules that attack pathogens by direct phagocytosis, as well as through the release anti-microbial proteases (e.g. neutrophil elastase) and oxygen radicals. Details of the role of airway epithelium in the host immune responses are described in a recent review [12]. This review is focused on the role of airway mucus, particularly MUC1 mucin, in the context of chronic inflammatory lung diseases.

Clinical Consequences and Airway Pathology

Mucus is overproduced in several inflammatory lung diseases, a process detrimental to the normal lung defense against environmental toxins. To make matters worse, difficulty in secretion clearance secondary to ineffective cough or poor mucociliary transport leads to mucostasis and paradoxically predisposes to bacterial colonization and infection [13, 14]. Dyspnea, cough and sputum production result from the

Table 1 Airway structural changes in airway diseases^a

Variable	Asthma	COPD	Bronchiectasis
Mucus gland hyperplasia	++	++++	+++
Subepithelial collagen deposition	+++	+	+
Angiogenesis	+++	+	+
Increased smooth muscle	+++	+	+
Increased proteoglycan depositin	+++	+	+
Increased elastin	++	?	+
Epithelial damage	+++	++	+

^aOverall estimate of the significane of these changes in the conditions mentioned. Scores are as follows: +=mild, ++=moderate, +++=significant, ++++=marked, ?=uncertain (Adapted with permission from the American College of Chest Physicians. Bergeron C, Boulet LP. Structural Changes in Airway Diseases: Characteristics, Mechanisms, Consequences, and Pharmacologic Modulation. *Chest* 2006; 129: 1068–1087)

physical obstruction of the airways by mucus and stimulation of intrapulmonary vagal afferent nerves [15, 16]. Lung diseases such as COPD, bronchiectasis, and cystic fibrosis are characterized by mucus hypersecretion, chronic bacterial colonization, and repeated lower respiratory tract infections [17–21].

Airway disease is a crucial pathologic component of multiple inflammatory lung diseases (See Table 1). Pathologic changes in airway epithelium of COPD patients include squamous metaplasia, inflammatory cell infiltration, goblet cell hyperplasia, and mucus metaplasia, a process in which mucus is overproduced in response to inflammatory stimuli (Figs. 2 and 3) [22]. These abnormalities are seen in both the larger central airways as well as smaller respiratory bronchioles [23–26]. Airway inflammation from smoking begins early in the course of the disease, and leads to persistent and progressive airway remodeling, even after smoking cessation [27]. Niewoehner et al. discovered inflammatory changes in the peripheral airways of young smokers who died suddenly, suggesting that airway disease developed before the diagnosis of COPD could be established [28]. As further evidence of this concept, epithelial layer thickness and mucous metaplasia increase incrementally with disease severity [26, 29]. These alterations in the epithelium increase airflow obstruction by several mechanisms: (1) excess mucus occludes the airway lumen [30]; (2) epithelial layer thickening encroaches on the airway lumen, thereby reducing inner diameter [31]; and (3) increased mucus alters surface tension of the airway, predisposing it to collapse during expiration [32]. Hogg et al. found inverse relationships between inflammatory cell infiltration and luminal occlusion of the small airways and lung function [29], strongly supporting the notion that small airway pathology is responsible for severity of illness. Airway disease also has prognostic significance in COPD. Mucus metaplasia in COPD has been associated with worse physiologic response to lung volume reduction surgery [33] as well as greater mortality [34].

Although it is established that quantity of emphysema correlates well with clinical disease staging in COPD, the relationship between airway pathology, physiology and symptom severity is weak at best. Chronic bronchitis exists in 26–45 % of all smokers, but COPD develops in only 15–20 % [35, 36]. Large airway mucous metaplasia correlates poorly with the degree of airflow obstruction[37] and amount

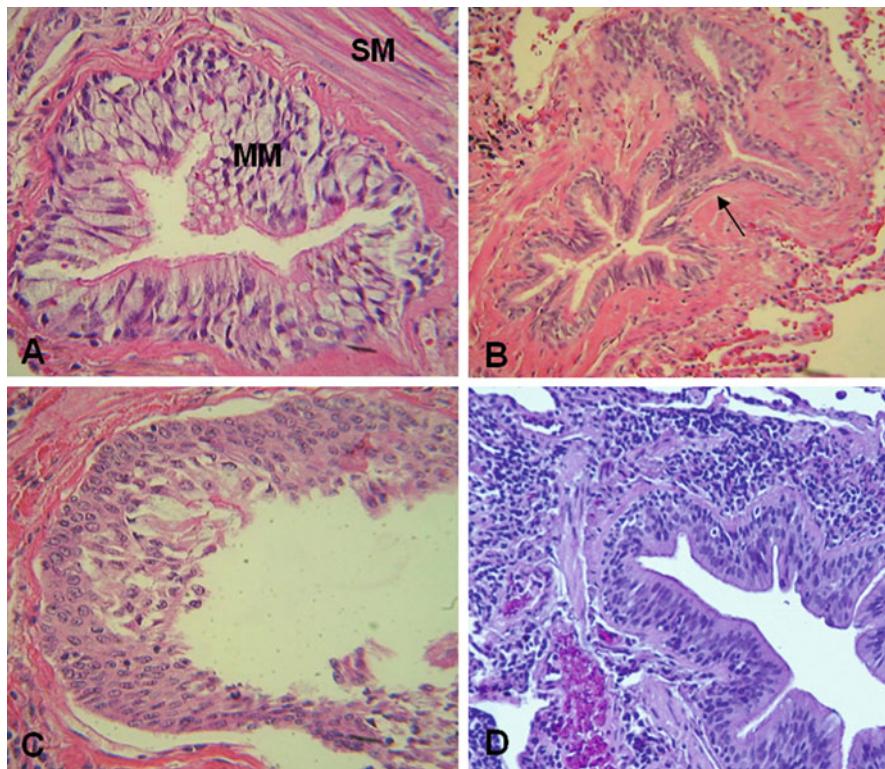


Fig. 2 Examples of airway remodeling in COPD. *A* represents mucous metaplasia (*MM*) of the epithelium and smooth muscle hypertrophy (*SM*). *B* represents peribronchial fibrosis (*black arrow*). *C* shows squamous metaplasia. *D* shows an inflammatory infiltrate of lymphocytes in the adventitia of a bronchiole (Reprinted with permission from the American Thoracic Society. Copyright© American Thoracic Society. Kim V, Rogers TJ, Criner GJ. New concepts in the pathobiology of chronic obstructive pulmonary disease. *Proc Am Thor Soc* 2008; 5: 478–485. Official Journal of the American Thoracic Society)

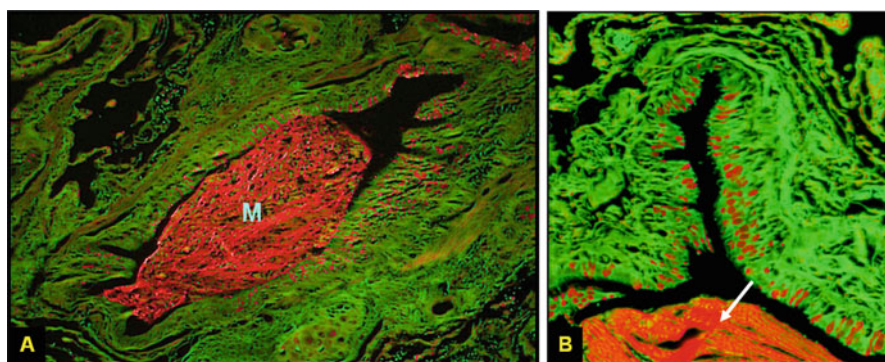


Fig. 3 Periodic Acid Fluorescent Schiff stain of a small airway from a patient with advanced emphysema. The entire airway is seen in *A* and a quadrant of the airway in *B*. Mucin granules are shown in red along the apical border of the epithelium. Note the large intraluminal mucin plug (*M*) in *A*, also noted in *B* (*white arrow*) (Reprinted with permission from the American Thoracic Society. Copyright© American Thoracic Society. Kim V, Rogers TJ, Criner GJ. New concepts in the pathobiology of chronic obstructive pulmonary disease. *Proc Am Thor Soc* 2008; 5: 478–485. Official Journal of the American Thoracic Society)

of mucus expectoration [38]. Small airway disease has been found in surgical lung specimens from those with advanced emphysema, with no clinical or radiographic evidence to suggest its presence preoperatively [29, 33, 39]. More importantly, the degree of small airway mucous metaplasia is difficult to detect clinically by burden of cough or sputum [40].

Despite the disconnect between symptoms and airway pathology, chronic cough and sputum production in COPD have multiple consequences, including an accelerated decline in lung function, [41, 42] increased exacerbation frequency [43–47], greater respiratory symptoms [43, 48], worse health related quality of life [43], and higher mortality [35, 49]. These phenomena are without a doubt a result of increased airway inflammation and worsened airflow obstruction, in addition to the aforementioned mechanisms. In a long term study of over 9,000 adults, an excess yearly rate of FEV₁ decline of 12.6–22.8 mL was attributed to chronic mucus hypersecretion [21]. We have found chronic cough and sputum production in patients with severe COPD were associated with higher dyspnea scores and more upper airway symptoms [43, 48]. In multiple studies, patients with chronic bronchitis and COPD were found to be at a 1.20–1.92-fold increased risk for COPD exacerbation compared to those without chronic bronchitis [43–47]. The cause of the observed increase in all-cause mortality, however, is still a matter of debate. It is hypothesized that the increased lung inflammation associated with chronic bronchitis causes greater systemic inflammation, resulting in numerous downstream consequences, including coronary artery disease, dyslipidemia, osteoporosis, and skeletal muscle weakness [50]. In the Tucson Epidemiological Survey of Airway Obstructive Disease, chronic bronchitis was associated with a 2.2-fold greater risk of all-cause mortality in those under the age of 50, and was also associated with higher serum levels of IL-8 and C-reactive protein at enrollment [49].

In asthma, chronic inflammation and thickening of the small airway epithelium, submucosal space, and smooth muscle has been noted in several pathologic studies [51–53]. In addition, shedding of the epithelial layer has been noted in postmortem studies, bronchoalveolar lavage fluid, and sputum samples [54], most likely as a result of weakened attachment of epithelial cells to the basement membrane. Large airway goblet cell hyperplasia and smooth muscle hypertrophy are prominent pathologic features of asthma. Goblet cell hyperplasia is more consistently seen in asthma compared to COPD, where clinical and pathologic phenotype is a highly variable combination of airway disease and emphysema. In asthma, mucus hypersecretion leads to obstruction of the majority of distal airways and ultimately respiratory failure during fatal asthma exacerbations [55]. Diffuse occlusion of the small and medium sized airways by mucus and cellular debris has been demonstrated in multiple autopsy studies of patients who died from asthma [56, 57]. Goblet cell hyperplasia is also seen in less severe cases as well; *Ordonez et al.* found a greater number of goblet cells and secreted mucins in subjects with mild to moderate asthma compared to control subjects [58].

Similar to asthma, mucus hypersecretion in cystic fibrosis leads to airflow obstruction and small airway occlusion [59]. However, cystic fibrosis is caused by dysfunction of an epithelial chloride channel, which results in sodium and water influx to the epithelial layer and therefore depletion of the airway surface liquid [19].

The increased viscosity and tenacity of secretions makes detachment from the epithelium and propulsion outward during cough exceedingly difficult. Excess mucus production combined with airway surface liquid dessication results in mucostasis, causing colonization by pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* [18–20].

Airway Mucus and Mucins

Mucus, or the airway surface liquid, is a complex mixture of ions, salts, peptides, proteins, glycoconjugates and water. Strict regulation of mucus production is indispensable for normal lung function. The protective function of mucus depends on its proper composition of constituent components, particularly mucin glycoproteins. Mucins are high molecular weight proteins with O-glycosidic linkages between the first GalNAc residue of the oligosaccharide side chains and serine and threonine amino acids of the polypeptide backbone. Over 20 mucin (MUC in human, Muc in animals) genes have been cloned, 12 of which are expressed in the lung [2, 60]. The airway mucin include secreted gene products (MUC2, 5AC, 5B, 7, 8, and 19) and membrane-tethered mucins (MUC1, 4, 11, 13, 16, and 20). MUC5AC and MUC5B are the two major secretory mucins in the respiratory tract. The levels of these mucins in mucus have been shown to significantly increase, and to directly correlate with, the number of goblet cells under the pathological conditions of goblet cell metaplasia or goblet cell hyperplasia. Although the exact roles of MUC5AC and MUC5B in the airways remain to be fully elucidated, it has been suggested that MUC5AC expression is inducible during airway inflammation, whereas MUC5B expression is constitutive [61]. A recent report supports this notion by demonstrating that MUC5AC levels correlated with the degree of airway obstruction in COPD patients [62]. Cystic fibrosis, in contrast, is characterized by greater MUC5B levels compared to MUC5AC [63, 64], suggesting that impaired mucociliary clearance is the principal mechanism responsible for the overwhelming burden of mucus in these patients.

Epithelial TLRs as Mediators of Airway Inflammation

TLRs, and related molecules, on airway epithelial cells comprise a second line of defense against inhaled microbial pathogens [65]. These pattern recognition receptors (PRRs) constitute an evolutionary conserved family of gene products that interact with pathogen-associated molecular patterns (PAMPs) to initiate downstream signal transduction and innate inflammatory responses. In general, all TLRs possess a leucine-rich repeat region in their ectodomains and an intracellular Toll/interleukin-1 receptor (TIR) domain. TLR signaling is activated by agonist-induced receptor homodimerization, recruitment of cytoplasmic adaptor proteins (MyD88, TIRAP, TRIF) to the TIR domain, and activation of protein kinases (IRAKs,

TRAF6) [66]. Although all of the 10 known human TLRs are expressed by airway epithelial cells, TLR2 and TLR5 are the predominant respiratory PRRs [67, 68]. TLR5 engages flagellin, the major protein component of the bacterial flagellum, while TLR2 recognizes a diverse array of components from Gram-positive and Gram-negative bacteria, including lipoproteins and peptidoglycan. It remains unclear how a single receptor (TLR2) can recognize such a broad diversity of stimuli, but a possible explanation is the ability of TLR2 to form heterodimers with TLR1 and TLR6. For example, bacterial peptidoglycan interacted with the TLR2/6 co-receptor complex on airway epithelial cells to activate NF- κ B and stimulate production of TNF- α [69]. The magnitude of the response generated by the TLR2/6 heterodimer was greater than that produced by TLR2 alone. While TLR5 homodimers are clearly capable of binding to flagellin, Mizel et al. [70] reported that nitric oxide production by airway epithelial cells in response to flagellin was dependent on interaction of TLR5 with TLR4. TLR2 also was shown to be involved in signaling induced by flagellin in human airway epithelial cells, suggesting a possible TLR2/TLR5 heterodimer interaction [67]. Biotinylation of surface proteins of airway epithelial cells followed by co-immunoprecipitation experiments demonstrated that both TLR2 and TLR5 were associated with the ganglioside, asialoGM1, in the plasma membrane [71]. IRAK1 and TRAF6 were also found in the co-receptor complex, whereas TLR4 was not. Furthermore, treatment of airway epithelial cells with *Pseudomonas aeruginosa* pili or flagella mobilized asialoGM1, TLR2, and TLR5 to the apical surface of the cells leading to Ca⁺²-associated activation of mitogen-activated protein kinases (MAPKs), nuclear translocation of NF- κ B, and production of IL-8 [67]. These combined results indicate that TLRs link the asialoGM1 glycoconjugate to intracellular signal transduction leading to a proinflammatory host response following interaction with bacterial components. Other groups, however, have questioned the role of asialoGM1 as a cell surface receptor for *P. aeruginosa*, particularly clinical isolates of the bacterium [72].

Neutrophil Elastase in the Airways

Neutrophils and macrophages constitute a third layer of defense in the clearance of bacteria from the lungs. The anti-microbial function of these immune cells is directly mediated through phagocytosis, and indirectly by the release of anti-microbial agents [73]. Among the soluble mediators released by neutrophils is the serine protease, neutrophil elastase (NE). Studies using NE knockout mice showed that this protease is required for host defense against experimental infection by Gram-negative bacteria [74]. However, the role of NE in the normal lung response to spontaneous bacterial infection needs to be more firmly established. Some evidence suggests that NE promotes neutrophil migration into the lung by degradation of the extracellular matrix, but this remains controversial [75]. In general, NE is considered as a proinflammatory molecule, and NE chemical inhibitors decrease inflammation and lung edema in animal models [76]. Part of the mechanism through

which NE mediates its anti-microbial effects is up-regulation of mucin secretion by goblet cells [77]. Using a co-culture system containing neutrophils and primary tracheal epithelial cells, Kim et al. [78] demonstrated that activation by fMLP/cytochalasin B resulted not only in increased NE production by neutrophils, but also greater mucin release from the epithelial cells. Both effects were blocked in a dose-dependent fashion by pretreatment with α 1-protease inhibitor, implicating a proteolytic effect of NE on the epithelial cells. Kohri et al. [79] reported that NE treatment of NCI-H292 airway epithelial cells stimulated the production of MUC5AC mucin through transforming growth factor- α (TGF- α)-dependent activation of the epidermal growth factor receptor (EGFR). Park et al. [80] showed that NE treatment of well-differentiated primary normal human bronchial epithelial (NHBE) cells cultured at an air-liquid interface (ALI) increased the release of MUC5AC and MUC5B mucins via an intracellular signaling pathway involving protein kinase C δ (PKC δ). To date, NE is the most potent mucin secretagogue described.

Control of Airway Inflammation

Given the intricate and diverse host airway inflammatory mechanisms, a critical balance between these processes and the counter-regulating anti-inflammatory pathways is absolutely required to maintain a homeostatic environment in the airways. This balance ensures that harmful environmental insults are effectively neutralized without excessive bystander tissue damage. Although a large body of literature has characterized the microbial-stimulated pro-inflammatory pathways summarized above, relatively less is known about the compensatory anti-inflammatory responses. Nevertheless, it is hypothesized that failure to down-regulate airway inflammation results in the development of acute or chronic respiratory diseases, including COPD, CF, ARDS, and asthma [25]. A number of anti-inflammatory molecules have been shown to play an important role in controlling the normal inflammatory response in the lung, including IL-10, transforming growth factor- β (TGF- β), peroxisome proliferator activating receptor (PPAR)- γ , and Mucin-1 (MUC1) [25, 81, 82]. However, what is less clear is whether defective expression and/or structure/function of these, or related, anti-inflammatory mediators is responsible for the etiopathogenesis of inflammatory lung diseases. The following sections briefly describe each of these key anti-inflammatory mediators with the goal of stimulating further basic and clinical research on their role in airway inflammatory diseases.

Interleukin-10

IL-10 down-regulates the expression of proinflammatory cytokines, including interferon- γ (IFN- γ), IL-2, and TNF- α , major histocompatibility complex (MHC) class II antigens, and leukocyte co-stimulatory molecules [83]. IL-10 also enhances

B cell survival, proliferation, and antibody production. These pleiotropic effects are mediated through interaction of the IL-10 homodimer with its cognate IL-10 receptor α subunit (IL-10R α), and subsequent binding of this ligand-receptor complex to the IL-10R2 co-receptor. An accumulating body of evidence points toward a role for IL-10 in chronic inflammation during COPD and asthma [84, 85], although a direct causal effect for IL-10 in the pathogenesis of these disorders is unclear. In the case of CF, airway secretions from afflicted patients, as well as CFTR^{-/-} mice, have decreased IL-10 levels compared with secretions from normal individuals or CFTR^{+/+} mice [82].

Transforming Growth Factor- β

TGF- β is an anti-inflammatory cytokine that exists in three isoforms, TGF- β 1, - β 2 and - β 3. TGF- β knockout mice are embryonic lethal as a result of profound multi-organ inflammation. TGF- β ^{+/-} heterozygous mice have reduced levels of the cytokine and exhibit exacerbated airway inflammation compared with wild type animals, suggesting a role for endogenous TGF- β in suppressing the development of allergic airway disease [86]. Additional evidence supporting an anti-inflammatory role for TGF- β comes from the observation that intratracheal delivery of TGF- β suppressed allergen-induced airway inflammation in a murine model of asthma [87]. Increased airway inflammation also was evident upon inhibition of TGF- β -dependent intracellular signaling [88]. Genetic studies have demonstrated an association between gene polymorphisms of the TGF- β locus and COPD [89]. Finally, a possible role for TGF- β in CF comes from the report that CF human cell lines and cells from CFTR^{-/-} mice have decreased Smad3 levels and decreased responses to TGF- β [90].

Peroxisome Proliferator Activating Receptor- γ

PPAR- α , - β , and - γ are members of the steroid hormone receptor family of ligand-activated transcription factors [82]. PPARs form heterodimers with retinoid X receptors that regulate gene transcription. PPAR γ is expressed as two isoforms, PPAR γ 1 and PPAR γ 2, that differ by the presence of a unique 30 amino acid segment in the latter [91]. PPAR γ 2 is primarily expressed in adipose tissue, while PPAR γ 1 is expressed in the lung, heart, skeletal muscle, intestine, kidney, pancreas, spleen, breast, and lymphoid tissues [92]. Both PPAR γ molecules are activated by prostanooids, a subclass of eicosanoids consisting of prostaglandins, thromboxanes, and prostacyclins. Synthetic PPAR γ ligands, such as the thiazolidinediones [93], have been developed that suppress inflammation both in vitro and in vivo [94, 95], including in response to lung infection with *Pseudomonas aeruginosa* [96], the major bacterial species that is responsible for the morbidity and mortality of CF. In the case of CF, at least three lines of evidence have been reported for an anti-inflammatory

role for PPAR- γ . First, PPAR- γ inhibits airway inflammation by competitively inhibiting NF- κ B binding to gene promoters, thereby blocking the activation of pro-inflammatory cytokines [97]. Second, PPAR- γ expression is decreased in lung of CFTR^{-/-} mice compared with CFTR^{+/+} mice [98]. Finally, CF airway epithelial cell lines have reduced PPAR- γ levels compared with normal cells [99]. Thus, decreased PPAR- γ expression likely contributes to defective NF- κ B signaling that favors increased airway inflammation in CF, and possibly other inflammatory airway diseases. However, the exact mechanisms by which PPAR γ down-regulates inflammatory responses in CF and other lung diseases remain to be clarified.

MUC1 Mucin

Of the 20 known mucin genes, MUC1 was the first to be cloned [100, 101]. MUC1 is a single pass, transmembrane glycoprotein located on the apical surface of airway epithelial cells and is composed of two polypeptide chains, a large molecular weight (>250 kDa) subunit containing glycosylated variable number of tandem repeats (VNTR) and a SEA (sea urchin sperm protein, enterokinase, agrin) domain, and a 25 kDa subunit comprised of the transmembrane and intracellular COOH-terminus (CT) regions of the molecule [25]. The two polypeptide structure of MUC1 arises as a consequence of proteolysis within the SEA domain [102]. MUC1 is unique among the membrane-bound mucins because its CT region constitutes a signal transduction domain. The CT contains multiple amino acid sequence motifs predicted as binding sites for Shc, c-Src, Grb-2, β -catenin, and phosphoinositide 3-kinase (PI3K) [25]. These motifs are evolutionarily conserved and undergo tyrosine phosphorylation. The presence of CT phosphorylation sites associated with signaling cascades that have been characterized for other membrane receptors has suggested that MUC1 is functionally analogous to cytokine and growth factor receptors [103].

Anti-Inflammatory Role of MUC1 in Airway Epithelia

Identification of a functional role for MUC1 in the airways was made possible by the generation of Muc1 knockout (Muc1^{-/-}) mice [104]. Early experiments demonstrated that Muc1^{-/-} mice were predisposed to developing spontaneous eye inflammation due to infections by *Staphylococcus*, *Streptococcus*, or *Corynebacterium* compared with wild type animals with an intact Muc1 gene [105]. Subsequent studies by Lu et al. [106] using an experimental model of bacterial lung infection showed that Muc1^{-/-} mice exhibited reduced lung colonization by *P. aeruginosa*, greater recruitment of leukocytes and higher levels of TNF- α and KC (mouse IL-8) in BALF compared with their wild type littermates. In vitro and in vivo mechanistic studies have indicated that MUC1/Muc1 plays an anti-inflammatory role during *P. aeruginosa* airway infection by suppressing TLR5 signaling [107–110].

More interestingly, the anti-inflammatory effect of MUC1/Muc1 was not limited to TLR5, but also included TLR2, 3, 4, 7 and 9, suggesting that this cell surface mucin may be a universal, negative regulator of TLR signaling [110]. Given that the host responses to lung pathogens involves the expression of multiple PAMPs, which must be activated and regulated in response to infection, this finding suggests a crucial role for MUC1/Muc1 in the resolution of inflammation, and perhaps in the genesis of chronic inflammatory disorders, such as COPD, CF and asthma.

Regulation of MUC1 Expression by TNF- α

Given the anti-inflammatory role of MUC1 in the airways, it is crucial to understand the mechanisms by which MUC1 gene expression is regulated. Several proinflammatory cytokines have been shown to up-regulate MUC1 expression. Noteworthy in this regard is TNF- α . Skerrett et al. [111] reported that TNFR1^{-/-} mice treated intranasally with *P. aeruginosa* showed significantly increased airway inflammation compared with wild type mice, as measured by enhanced bacterial clearance from the lungs, increased numbers of neutrophils in BALF, and higher levels of TNF- α in BALF. Subsequently, TNF- α was demonstrated to stimulate MUC1 expression in A549 lung epithelial cells [107, 112]. The molecular mechanism of TNF- α -induced MUC1 up-regulation has been described in detail using a combination of biochemical, pharmacological, and molecular biological approaches [107]. The requirement for TNF- α in increased MUC1 expression has also been observed in A549 cells infected with respiratory syncytial virus (RSV) [109], as well as in mice infected with *P. aeruginosa* [113]. Thus, these results suggest that TNF- α may play a key role in controlling inflammation during airway infection, from the initiation phase of bacterial exposure to the final resolution of inflammation, the latter likely by inducing the expression of key anti-inflammatory molecules, such as MUC1, with possible assistance by IL-10 and/or PPAR- γ .

A Proposed Model for the Anti-Inflammatory Role of MUC1 in the Airways

Based on the accumulated published literature, we propose the following model to account for MUC1, TLRs, and TNF- α in the airway epithelial response to respiratory infection [25]. Normally, transiently inspired pathogens are quickly removed by mucociliary clearance and phagocytosis by resident leukocytes in the airway lumen. With abnormally high pathogen load, for example due to a predisposing condition such as CF, microbial PAMPs activate TLRs resulting in the production of proinflammatory mediators (IL-8 and TNF- α), thereby promoting leukocyte influx into the airways. During the early stage of infection, MUC1 expression is sufficiently low and TLR signaling is not antagonized. However, after invading pathogens have been

cleared, increased levels of inflammatory products such as neutrophil elastase and TNF- α up-regulate MUC1 expression which, in turn, suppresses the release of TNF- α , thus inhibiting TLR-dependent airway inflammation. The net effect facilitates pathogen removal and returns the lungs to homeostasis. Future experiments are needed to provide additional support for this proposed negative feed-back loop model system.

MUC1 Mucin and Chronic Inflammatory Lung Disease

TNF is the major pro-inflammatory molecule during airway infection. Ulich et al. [114] demonstrated that intratracheal LPS-induced neutrophilic inflammation in rats can be inhibited by intratracheal administration of soluble TNFR, suggesting that TNF/TNFR interaction plays a key role in LPS-induced airway neutrophilic inflammation. Interestingly, TNFR1 deficient mice not only failed to control either LPS or *Pseudomonas aeruginosa*-induced neutrophilic inflammation [111] but showed greater neutrophilic inflammation to the contrary. Recently Choi et al. [113] showed that Muc1^{-/-} mice behaves exactly the similar way as TNFR deficient mice in response to airway *Pseudomonas aeruginosa* infection, i.e., an increased neutrophilic inflammation, compared with their WT Muc1^{+/+} mice. The relationship between TNFR and Muc1 can be explained by Koga et al. [107] who demonstrated that the levels of MUC1 are controlled by the TNF/TNFR signaling pathway. Thus, MUC1/Muc1 seems to be controlled mainly by TNF both in vivo [113] and in vitro [107]. This timely regulation of inflammation and its resolution has also been demonstrated *in vivo* between TNF and IL-10, in which the former induces the latter, one of the major anti-inflammatory molecule [115]. Thus, failure to induce sufficient levels of anti-inflammatory molecules in a timely manner during the course of lung inflammation will result in lung tissue damage, and the subsequent repair processes will result in lung remodeling, a major characteristic of inflammatory lung diseases such as COPD and CF. Whether other anti-inflammatory molecules are also controlled through the similar mechanism remains to be elucidated.

One of the interesting questions that arise from this study is why there are multiple anti-inflammatory molecules and how they interact with each other during airway infection. For example, it has been shown that MUC1 induces IL-10, an anti-inflammatory cytokine, in dendritic cells [116] and that the levels of IL-10 in the BALF of Muc1 KO mice were significantly greater following *Pseudomonas aeruginosa* infection as compared with those of Muc1^{+/+} mice (unpublished data), suggesting the possible collaboration between the two during inflammation. The same question may be applied to other anti-inflammatory molecules in the lung that have been reported recently, including CD44 [117], aryl hydrocarbon receptor [118] and various lipid mediators [119, 120]. Further studies are required to understand the functional relationships between the known anti-inflammatory molecules during the resolution of airway inflammation.

Summary and Conclusions

In summary, airway epithelial cells play a critical role in the pathogenesis of chronic inflammatory lung disease. Their primary role in the process of host defense becomes dysregulated, and the excess inflammation causes increased mucus production and hypersecretion, resulting in mucostasis, airway obstruction, and tissue remodeling from several downstream events. Clinical consequences include an accelerated decline in lung function, greater respiratory symptoms, exacerbations of underlying lung disease, recurrent lower respiratory tract infection, and higher mortality. Multiple complex interactions between inflammatory cytokines and epithelial cells exist, and the precise roles of each in the generation of mucins and the amplification of lung inflammation remain unclear. There is, however, emerging evidence that the role of MUC1 mucin is essential to the airway epithelium's response to environmental toxic agents, and therefore essential to the development of chronic and persistent inflammation. Further studies are required to better understand the roles of this mucin as well as others in the pathogenesis of inflammatory lung disease.

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