Epithelial–Immune Cell Interactions for Drug Discovery in Chronic Obstructive Pulmonary Disease

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Abstract

New studies of chronic obstructive pulmonary disease (COPD) are revealing the key role of airway epithelial cells and innate immune cells in the initiation, exacerbation, and progression of airway disease. An emerging scheme focuses on expansion of airway progenitor epithelial cells that feed forward for a type 2 immune response and consequent IL-13–driven mucus production that is linked to the morbidity and mortality of COPD. Analysis of human airway progenitor epithelial cells and airway tissue shows that IL-13 signaling to MUC5AC mucin gene expression relies on specific activation of mitogen-activated protein kinase 13, providing a

druggable target for attenuating mucus production in the setting of viral infection and other inhaled stimuli of airway inflammation. Moreover, structure-based drug design is delivering highly potent, selective, and nontoxic small-molecule kinase inhibitors of mitogen-activated protein kinase 13 that offer a therapeutic strategy to downregulate excess mucus production to a physiological level and thereby achieve a precision medicine solution to the major health care problem of COPD and related airway diseases.

Keywords: airway disease; airway epithelial cells; chronic obstructive pulmonary disease; drug discovery; innate immune cells

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Chronic lower respiratory diseases and in particular chronic obstructive pulmonary disease (COPD) have emerged as leading causes of morbidity and mortality in the United States and worldwide. Moreover, there is growing recognition that the progression of these diseases is linked to inflammatory mucus obstruction of the airways. In particular, studies have demonstrated that inflammatory mucus production is significantly associated with decreased survival in patients with advanced COPD including a subgroup that underwent surgery for emphysema (1, 2). In addition, there is considerable evidence that the chronic bronchitis component of COPD is responsible for exacerbations of this disease and thereby serves as an early predictor of the development and progression of COPD (3, 4). Thus, excess inflammatory mucus

production is connected to early and late stages of the pathogenesis of COPD and the consequences for functional disability.

Despite the evidence that excess production of airway mucus is key to COPD-related debility, there are no specific and potent drugs for directly and effectively blocking excess mucus production in chronic bronchitis or any other form of respiratory airway disease. There is some evidence that macrolides (e.g., azithromycin), phosphodiesterase-4 inhibitors (e.g., roflumilast), antioxidant/reducing agents (e.g., *N*-acetylcysteine), and glucocorticoids might decrease the number of exacerbations of COPD, presumably based on attenuating chronic bronchitis (5). However, these drugs do not appear to have a specific and direct effect on mucus production *in vivo* at the level of airway

epithelial cells. It appears more likely that these agents act via the immune system to downregulate immune cell–derived cytokines that might stimulate epithelial cell mucus production, and even these indirect effects might be relatively weak. Similarly, new biologics are generally based on anti-cytokine/cytokine receptor monoclonal antibodies (mAbs) that are also directed at the immune response versus direct action on airway epithelial cells that are the source of mucus production (6). Thus, at present, there are no specific, direct, safe, and effective therapeutics and especially low-molecular-weight compounds to attenuate airway mucus production without the likelihood of considerable off-target effects distant from the mucous cell.

Here we develop the case for a new signal transduction pathway for the control

of inflammatory mucus production based on understanding epithelial-immune cell interactions and the special roles of stem/progenitor epithelial cells and innate immune cells in three key areas: 1) the pathogenesis of airway disease and excess mucus production in humans with COPD; 2) the pathogenesis of comparable airway disease in animal models of this process, triggered by viral infection and other inflammatory stimuli including tobacco smoke; and 3) the translation of pathogenesis to drug discovery and development as a means to control airway disease and mucus production based on what we have learned in humans and animal models.

Pathogenesis of Airway Disease in Humans

Patient-oriented research has brought major paradigm shifts to the pathogenesis of chronic airway disease. These modifications include 1) the transition from lung physiology to cell biology and immunology, with the recognition that “airway inflammation” is a key component of the disease process; and 2) the subsequent recognition that the innate immune response, and particularly the type 2 immune response, is a key driver of the airway inflammation, mucus production, and hyperreactivity that are characteristic of this type of disease. The next challenge for the field of airway research is to define the basis for the initiation and persistence of the immune response, and in particular how the innate immune response can remain activated or at least more susceptible to activation to drive a long-term inflammatory disease. This concept will likely need to fully reset the conventional view that the innate immune response is programmed for only an acute and transient response to inflammatory stimuli versus the long-lasting memory of the adaptive immune response.

Indeed, this additional paradigm reset has been at least partially addressed in a series of research advances that provide a mechanism for persistent activation of the innate immune response in airway disease. Some of the first insights came from observations that sentinel airway epithelial cells could respond to inhaled inflammatory stimuli such as air pollutants and in turn direct immune cell infiltration into the

airways with consequences for airway hyperreactivity and mucus production (7, 8). Subsequent studies showed that airway epithelial cells also respond to other inhaled stimuli, particularly viral infection, by producing bioactive lipids and cytokines that control immune cell traffic, activation, and survival (9). However, it remained difficult to understand how a relatively transient epithelial cell response could account for chronic activation of innate immune cells that would be required for long-lasting and often progressive airway disease.

A key insight to the persistence mechanism came with the finding that airway disease in COPD was associated with an increase in airway progenitor epithelial cell (APEC) function. In particular, airway epithelial cells isolated from patients with COPD exhibited increased levels (reflecting increased efficiencies) for spheroid/organoid formation in three-dimensional culture compared with non-COPD control subjects (10). The capacity of these airway epithelial cells for growth and differentiation defined them as multipotent APECs, and their isolation using a basal cell marker (integrin subunit α_6 [ITGA6]) further identified them as a subset of basal epithelial cells. Moreover, the increase in APEC function was associated with increased numbers of IL-33-high basal cells in

patients with COPD in concert with an *IL-13-IL-13R* to *MUC5AC* mucin gene expression signature (10) that could also be marked with TREM-2 (triggering receptor expressed on myeloid cells-2)-positive M2 macrophages (11). Because the ATP danger signal caused IL-33 release from basal airway epithelial cells (10), the findings provided a pathway from APEC expansion to increased basal cell expression and release of IL-33 to trigger a type 2 immune response. Together, these data provided a provisional scheme for how a renewable population of airway progenitor/stem cells might provide an ongoing upstream susceptibility to chronic innate immune activation (as depicted in Figure 1). However, given the limitations of human studies, this scheme still left major questions over 1) whether and how initial inhaled stimuli led to APEC expansion; and 2) whether APEC-dependent release of IL-33 or related cytokines caused the airway hyperreactivity and mucus production that are central features of COPD and related airway diseases.

Pathogenesis of Airway Disease in a Mouse Model

To address these new questions about cause and effect in chronic airway disease, it was

Figure 1. Scheme for airway progenitor epithelial cell (APEC) expansion, interaction with immune cells, and differentiation into mucous cells in the pathogenesis of chronic airway disease. Sequential steps include the following: 1) initial environmental stimulation (primarily by viral infection) causes APEC expansion; 2) epithelial danger signals such as ATP cause release of IL-33 from airway epithelial basal cells; 3) secreted IL-33 activates innate immune cells (such as ILC2s, NKT cells, and M2 macrophages), leading to IL-13 production; and 4) activation of IL-13R on APECs drives differentiation into airway mucous cells that are characteristic of airway disease. IL-13R = IL-13 receptor; ILC2s = type 2 innate lymphoid cells; NKT cells = natural killer T cells. Adapted by permission from Reference 10.

critical to develop animal models of the disease process that maintained high fidelity to the observations in humans with COPD. In that regard, studies of a postviral mouse model of chronic airway disease have provided significant insights into these issues. In particular, a mouse model was developed using infection with the natural pathogen mouse parainfluenza virus, also known as Sendai virus (SeV), that replicates with high efficiency (e.g., 1×10^6 -fold increase in viral titer). Under these conditions, severe infection triggers a postviral process that leads to long-term airway inflammation, hyperreactivity, and mucus production (12). Moreover, postviral disease appears to be exaggerated by exposure to other inflammatory stimuli, including tobacco smoke, suggesting a connection to COPD exacerbations. Similar postviral disease develops after severe infection with influenza A virus (IAV) infection (13), consistent with evidence that any type of respiratory virus infection can lead to airway disease in humans (14). In addition, both SeV and IAV initiate airway disease that depends at least in part on a type 2 immune response with IL-13 induction and consequent MUC5AC-dominant mucus production (13, 15, 16). The study of IAV also reveals that chronic airway disease develops at focal sites of transcriptionally active viral remnants, suggesting a relationship to initial locations of infection.

Further analysis of the SeV mouse model of airway disease has also provided additional corroboration of the epithelial-immune cell scheme proposed for human COPD. In particular, a combination of genetic deficiency and antibody-blocking strategies has shown that IL-33–IL-33 receptor signaling is required for the development of postviral airway disease (10). In addition, the disease depends on downstream activation of innate immune cells, notably natural killer T cells, TREM-2⁺ M2 macrophages, and type 2 innate lymphoid cells (10, 16–18). Ongoing studies are defining the precise role of individual immune cell subsets and their dependence for activation on IL-33 and related epithelial cell–derived cytokines such as thymic stromal lymphopoietin (TSLP). These studies depend on new tools to specifically track and delete each of these immune cell populations while maintaining an otherwise intact immune system. Similarly, the availability of single-cell analysis allows for the definition of rare cell

populations that have been difficult to track by conventional techniques in both animal and human lung cell profiling. These experiments will be critical to defining markers of immune cell activation to monitor this type of response in humans with airway disease.

Ongoing studies also aim to define the cellular source of IL-33 and in turn the nature of the APEC population that might differentiate into IL-33–high airway or alveolar epithelial cells in the postviral mouse model of airway disease. The development of new *Il33*-transgenic reporter mice and more precise methods to isolate mouse APECs will soon provide this information. Nonetheless, the findings to date already suggest that a renewable epithelial cell population is programmed for expansion to drive chronic inflammatory disease after viral infection as a paradigm for what might also happen in humans with COPD with virus-induced exacerbations. The persistence of progenitor cells could thereby mediate ongoing susceptibility to airway disease. We anticipate that the isolation of a tissue progenitor cell population with a specific molecular requirement in experimental models and humans will provide previously undefined approaches and tractable therapeutic targets to preserve normal tissue homeostasis and prevent remodeling disease in the lung.

Drug Discovery for COPD and Related Airway Disease

Although questions remain over the precise nature of the epithelial-immune cell pathway to COPD and related airway diseases, there is already a useful substrate to discover and develop new therapeutic strategies based on the existing framework. As noted earlier, the targets found in this pathway have been pursued successfully with the development of blocking mAbs aimed at downregulating the type 2 immune response. In particular, mAbs directed against IL-5 (mepolizumab and reslizumab), IL-5 receptor (benralizumab), IL-4 receptor α chain that is common to IL-13 and IL-4 receptors (dupilumab), and TSLP (tezepelumab) are proving effective in decreasing exacerbations in patients with refractory eosinophilic asthma. Each of these strategies is also under consideration for treatment in COPD (6). In that regard, at least some utility is found in tracking airway

eosinophilia in COPD, likely reflecting the overlap between COPD and asthma (19, 20) and the consistent increase in eosinophils in postviral airway disease. Moreover, anti-IL-5 mAb showed effectiveness in a subset of patients with COPD with eosinophilia (21), thereby validating activation of the type 2 immune response in at least a subset of patients with COPD as noted above in human studies and reported by others as well (22). Alternatively, there remains the longstanding and ongoing pursuit of conventional components of the immune response. For example, neutrophils are linked to airway disease separate from their role in pulmonary emphysema (7, 23, 24). In turn, neutrophil connections to IL-17–related pathology raise consideration of treatment with an available mAb directed against the IL-17 receptor A chain (brodalumab), although this approach was not effective in postviral airway disease (13). Related strategies to block other conventional targets such as tumor necrosis factor- α , IL-1 β , or IL-6 with biologics or small molecules remain similarly uncertain, particularly in relation to excess mucus production.

Given the complexity and possible toxicity of blocking the immune response, we reasoned that it might be useful to consider a therapeutic target that is closer to the end-organ dysfunction, that is, the step wherein progenitor epithelial cells differentiate into mucous cells in response to immune signals, especially derived from IL-4/IL-13–dependent receptor activation. This approach required a better understanding of the molecular pathway leading from IL-4/IL-13 signaling to the development of mucous cells. In that regard, seminal studies identified chloride channel accessory 1 (*Clca1*) as the most prominently induced lung mRNA in the setting of excess airway mucus production after SeV infection in mice (25). Given the mouse *Clca1* sequence homology to ion channels, the corresponding Clca1 protein became an attractive target for drugs to modify mucus production. However, the absence of a transmembrane domain indicated that Clca1 could not function as an ion channel. Indeed, subsequent study suggested that Clca1 might instead be coupled to airway fluid secretion via self-cleavage and consequent accessory activity for the major calcium-activated chloride channel in airways (26). In addition to the miscue over ion-channel function, we also learned that

newly generated *Clca1*-deficient mice manifested the same excess production of inflammatory mucus as wild-type control mice after viral infection (25). This finding was explained when we recognized that the mouse *Clca* gene locus contained at least two additional IL-13-responsive *Clca* genes, allowing for functional redundancy and compensation for *Clca1* deficiency.

Given these unexpected complexities in *Clca* biology, most research groups abandoned drug discovery based on *CLCA1* control of mucus production. However, we recognized that the mouse *Clca* gene locus was significantly more complicated than the corresponding locus in humans. Thus, the observed failure in the mouse model did not necessarily predict the lack of selective *CLCA1* function in humans. Indeed, we next found that human *CLCA1* (but not the three other human *CLCA* genes) was responsive to IL-13 stimulation and was induced in concert with *MUC5AC* mRNA and corresponding *CLCA1* and *MUC5AC* proteins with subsequent packaging of both proteins into mucin granules in human airway epithelial cells (27). Moreover, *CLCA1* expression was required for IL-13-induced *MUC5AC* expression based on short hairpin RNA-mediated gene knockdown in human airway epithelial cells and was sufficient for *MUC5AC* induction based on studies of an inducible *CLCA1* gene system in NCI-H292 cells (27). These findings raised the utility of searching for a druggable target in the IL-4/IL-13 to IL-13 receptor to *CLCA1*/*MUC5AC* pathway to excess mucus production. In that regard, phosphokinase-antibody array analysis of *CLCA1*-induced cells revealed the unexpected activation of mitogen-activated protein kinase 13 (MAPK13), in addition to previous kinase signaling candidates such as MAPK14 that were the subject of earlier studies. Validation of these findings in patients with excess mucus production due to COPD came from studies of airway tissue. Thus, morphology-directed sampling of airway tissue in lung explants showed increased expression of *CLCA1* and *MUC5AC* at the mRNA and protein levels, colocalization of *CLCA1* and *MUC5AC* to mucous cells, and increased expression and activation of MAPK13 in airway tissue from patients with COPD compared with non-COPD control subjects (27). The most recent data further suggest that MAPK13 is also localized to mucin granules in airway epithelial cells (28), consistent with coordinated function of the

MAPK13-*CLCA1*-*MUC5AC* components of mucus production.

Together, these findings identified the potential for a new druggable pathway for excess mucus production. In particular, the observations suggested an approach to downregulate excess, inflammatory mucus to a normal, physiological level through the discovery and development of a heretofore unavailable small-molecule kinase inhibitor directed against MAPK13 (as depicted in Figure 2). To address this goal at the level of medicinal chemistry, we identified a previous MAPK14 inhibitor, BIRB-796 (MAPK14 IC_{50} , 5 nM), that also showed weak activity against MAPK13 (MAPK13 IC_{50} , 500 nM). Molecular modeling of BIRB-796-MAPK13 binding and subsequent cocrystal structures of additional chemical analogs served as a basis for structure-based drug design of more potent MAPK13 inhibitors based on enzyme inhibition assays and cell-based mucus inhibition assays (27). Ongoing studies are providing new compounds with even greater potency and selectivity for MAPK13 inhibitors that lack cytotoxic effects, thereby providing high therapeutic ratios in cell-based assays of mucus inhibition. Further development of MAPK13 and combined MAPK13/MAPK14 inhibitors is being guided by pharmacokinetic analysis to effectively and safely attenuate inflammatory mucus production *in vivo*.

The approach requires new animal models given the difference in both *Clca1* and *Mapk13* function in mice compared with humans (25, 27) as well as analysis of the combined MAPK13/MAPK14 inhibitor to capture the benefit of blocking conventional inflammatory signals found in some studies of patients with COPD (29). Further study of fully and partially selective inhibitors *in vivo* will also be useful in defining the drivers for inflammatory mucus production and the functional biology of airway mucus for host defense versus airway disease. We also recognized that type 2 immune activation along with MAPK13 overexpression and activation is found in secretory cell-derived carcinomas (30). These data suggest that MAPK13 inhibitors might prove useful to interrupt epithelial progenitor/stem cell participation in carcinoma as well as mucus production. In both cases, treatment with these inhibitors might be delivered more precisely using companion diagnostics to track MAPK13, *CLCA1*, and *MUC5AC* as biomarkers of stem/progenitor cell expansion and differentiation.

Summary and Future Directions

It has been difficult to understand how an abnormal immune response leads to the development of chronic inflammatory

Figure 2. Therapeutic strategy for downregulation of excess inflammatory mucus to normal physiological mucus levels, using a small-molecule kinase inhibitor of MAPK13. Left box: Depiction of the formation of excess inflammatory mucus in airway disease based on IL-4 and/or IL-13 activation of the IL-13 receptor (IL-13R) with activation of STAT6 and MAPK13 that in turn drives expression of *CLCA1*, *MUC5AC*, and MAPK13 for subsequent packaging into mucin granules as APECs differentiate into airway mucous cells. Right box: Depiction of the formation of normal physiological levels of airway mucus when the same pathway is downregulated with a MAPK13 inhibitor. APEC = airway progenitor epithelial cell; *CLCA1* = chloride channel accessory 1; MAPK13 = mitogen-activated protein kinase 13; *MUC5AC* = mucin 5AC; SMKI = small-molecule kinase inhibitor; STAT6 = signal transducer and activator of transcription 6. Adapted by permission from Reference 27.

disease in general, and this has also been the case for chronic airway disease in the form of COPD. Progress in human studies and corresponding animal and cell models suggests that the innate immune response can drive the initiation, exacerbation, and progression of this type of disease in response to inhaled inflammatory stimuli and infections, particularly with respiratory viruses. Major insights include the following: first, that APECs may be programmed for growth and differentiation to provide an ongoing susceptibility to a type 2 immune response; and second, that APECs or APEC-derived cells might use cytokines such as IL-33 to “ping” the immune cells for cytokines (such as IL-13) as a feed-forward mechanism for APEC differentiation into mucous cells. Further data on the regulation of APEC programming

should provide for understanding how airway disease develops and how to control it. However, these breakthroughs in pathogenesis should even now translate into new therapeutic strategies to prevent chronic airway disease. Current biologics against the type 2 immune response may prove effective in this regard, but at present there are no small-molecule drugs that specifically, directly, potently, and safely target APECs to attenuate airway mucus production and thereby prevent the morbidity and mortality of airway disease in COPD. In that regard, we identify MAPK13 as a master regulator of APEC transition to mucous cells and define a strategy for MAPK13 inhibitors as the best candidates to date to downregulate this key step toward inflammatory mucus production. The participation of APECs in airway disease

appears to overlap with the activation of comparable progenitor/stem cells in carcinomas, raising the possible effectiveness of a broader strategy for MAPK13 inhibitors to control epithelial stem cell growth and differentiation. The combined and multidisciplinary pursuit of disease pathogenesis and drug discovery should thereby deliver new precision medicine for controlling epithelial-immune cell interactions that drive disease due to inflammation and cancer. ■

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