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On the Pathogenesis of Acute Exacerbations of Mucoobstructive Lung Diseases

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Abstract

Mucoobstructive lung diseases have highlighted the importance of a proper description of the normal mucus clearance system. A useful description of the normal mucus clearance apparatus requires the presence of two gels on the airway surface (i.e., a mucus layer gel and a periciliary gel). Importantly, most mucoobstructive lung diseases are distributed heterogeneously in the lung, and exacerbations may reflect spread of the disease to previously normal areas. The spread may reflect disturbances in the balance of water between the two gel layers, producing heterogeneous mucus adhesion and infection within the lung. Ultimately, spread can produce losses of lung function that may be associated with acute exacerbation frequency.

Keywords: mucus clearance; osmotic pressure; periciliary gel layer

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Chronic bronchitis can be described as a chronic mucoobstructive lung disease. A common feature of both genetic and environmental forms of chronic bronchitis (e.g., cystic fibrosis [CF] and smoke-induced chronic obstructive pulmonary disease [COPD], respectively) is that they are characterized by chronic cough and sputum production. Importantly, the frequency and severity of acute exacerbations contribute to the overall loss of lung function in mucoobstructive lung diseases (1). Therefore, a comprehensive understanding of the natural history of chronic bronchitis requires an analysis of both the underlying mucoobstructive chronic bronchitis and the pathophysiology of acute exacerbations.

Normal Mucus Clearance Mechanisms

In health, mucus clearance reflects the aggregate activities of (*1*) ciliary beat, (*2*) mucin secretion, (*3*) globular protein secretion, and (*4*) transepithelial ion and water transport. All four components of this system appear to have basal and variable rates. Most attention has been devoted to the regulation of cilial beat frequency and mucin secretion rates. However, more recently, it has become apparent that an understanding of the maintenance of normal mucus clearance requires knowledge of the integrated activities of the transepithelial ion (and water) transport and mucin secretion rates that control the concentration of mucus on airway surfaces.

Traditionally, mucus transport has been envisioned as a mucus layer of undefined concentration moving over a periciliary environment that is occupied by liquid. Recent data suggest that the airway surface topology is better characterized as a two-gel system, with a mucus layer moving atop a second gel layer (i.e., the periciliary gel layer or PCL) (Figure 1) (2). The mucus layer consists of the secreted mucins, MUC5AC and MUC5B, whereas the periciliary gel layer is occupied by the tethered mucins, MUC1, MUC4, MUC16, and perhaps MUC20. The recognition of the second gel layer on the airway surface in the periciliary environment is more than a "detail." Indeed, the presence of two gels on the airway surface is of immense importance with respect to the distribution of water between the two compartments, which ultimately regulates the efficiency of the mucus transport system. For example, in health, it is important that the periciliary environment be well hydrated. This requirement reflects the fact that hydration serves to provide lubricant activities to the PCL, allowing the mucus layer to move with low frictional energy over the PCL surface.

Because each of the two gels is composed of mucins in concentrations in which the mucin molecules interpenetrate (i.e., as "semidilute" solutions), the osmotic water-drawing power of each gel layer can be described in terms of simple

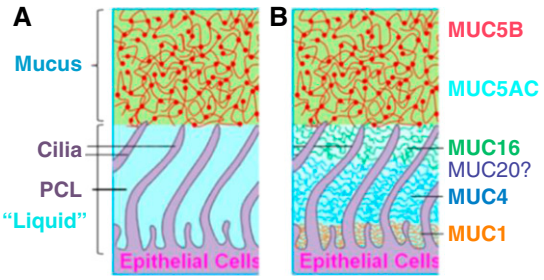

Figure 1. Topography of mucus clearance systems. (*A*) Classic mucus layer "floating" on periciliary liquid layer. (*B*) Two-gel formulation, with secreted mucins (MUC5B and MUC5AC) interpenetrating and residing over a brush-like periciliary gel layer (PCL) composed of MUC1, 4,16, and possibly 20, tethered to cilial and epithelial surfaces. Adapted by permission from Reference 2.

polymer physics equations. Specifically, the water-drawing power of each gel (i.e., the osmotic pressure, π) is proportional to the Boltzmann's constant times absolute temperature divided by the mucus concentration to the third power ($\pi \cong kT/[\text{mucus}]^3$). In health, the mucin concentration of the periciliary environment appears to be higher than the normal mucus layer (i.e., the PCL osmotic pressure is ~500 Pa, whereas the osmotic pressure of the mucus layer is ~100 Pa).

The mucus layer concentration can be described most simply in terms of the percentage of solids content, which correlates tightly with mucin concentrations and osmotic pressures (3). To maintain a "normal" mucus concentration (2% solids, 100 Pa) and efficient mucus transport, there is active sensing of the mucus concentration by the airway epithelium. Recent data suggest that motile cilia sense the concentration of the mucus layer by sensing the mechanical properties of the mucus layer that are hydration dependent (e.g., shear-stress and the elastic modulus) (4). When cilia interact with hyperconcentrated mucus, increased stresses are applied to the cilial shaft that produce cilial strain, and strain ultimately releases ATP from the cell into the extracellular environment in a strain-dependent fashion. Extracellular ATP itself, and/or its extracellular metabolite, adenosine (ADO), activates luminal purinoceptors including P2Y2-R and A_{2b}. Both of these receptors act to inhibit absorption through the epithelial Na^+ channel, accelerate chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) and calcium-activated chloride channels, and serve to increase fluid secretion onto the airway surface. Thus, there is a dynamic feedback mechanism to maintain normal mucus layer hydration in health.

Mucoobstructive Disease

Airways diseases can disrupt the regulatory pathways that maintain normal mucus layer concentrations. The two-gel model predicts that in disease, the concentration of the mucus layer rises to values equal to that of the PCL (2). This rise can reflect either net volume depletion and/or mucin hypersecretion. When disease produces a condition in which the mucus layer and the PCL concentrations become similar, removal of water from the airway surface by epithelial ion transport mechanisms will remove water from both compartments, producing progressive osmotic compression of the periciliary environment by the mucus layer with slowing of mucus clearance. As disease progresses, the depletion of airways surface liquid become sufficiently severe that the mucus layer virtually flattens the cilia, producing mucus stasis and ultimately adhesion. This PCL collapse is observed with what may appear to be initially surprisingly small changes in the mucus layer concentration (i.e., rising from 2 to 6% solids content). However, the polymer physics equations for mucus layer osmotic pressure, describing the osmotic pressure as a third power function of mucus concentration, juxtaposed to the resting PCL (500 Pa) vs. mucus layer (100 Pa) osmotic pressures, make it apparent that seemingly small changes in mucus concentration can produce compression and mucus adhesion.

Increased osmotic pressures have been correlated with slowed mucus clearance in *in vitro* studies under controlled conditions that are consistent with the two-gel polymer physics formulation (5). More recently, evidence of increased mucus layer concentration has been achieved in clinical samples from subjects with CF and subjects with cigarette smoke–induced chronic obstructive lung disease (3). In CF, the increased concentrations almost undoubtedly reflect the imbalance between chloride secretion and sodium absorption that is a feature of this disease (6). In COPD, the imbalance may reflect the abnormal regulation of fluid transport, which appears to result in part from depletion of ATP and ADO on airway surfaces because of up-regulation of airway surface ecto-ATPases and adenosine deaminase (5). In COPD, the increased mucus concentration observed *in vivo* was related to a decrease in both mucociliary transport rates and FEV_1.

Animal Models

A key question is whether mucus hyperconcentration *per se* can drive the full pathogenesis of mucoobstructive lung diseases. This question is difficult to resolve in cross-sectional or even longitudinal studies in human subjects. Therefore, it has been addressed best in animal models.

Perhaps the most direct test of this hypothesis is by using the beta sub-unit of the epithelial sodium channel (βENaC) transgenic overexpressing mouse (7). This mouse model features an airway-specific promoter to increase the expression of the murine βENaC transgene, which accelerates the basal rate of sodium absorption. Importantly, this mouse does not exhibit defective chloride secretion, but the net effect of accelerating sodium absorption without disturbing chloride secretion is increased volume absorption. The depletion of airway surface liquid produces mucus hyperconcentration, which is associated with early neonatal mortality in βENaC overexpressing mice.

The βENaC mouse as an adult also exhibits most of the features of chronic bronchitis (8). For example, the βENaC mouse expresses heterogeneous airways mucus obstruction, typically associated with high levels of MUC5B in airway lumens. We have speculated that it is the mucus adhesion that drives the

pathogenesis of the downstream events of chronic bronchitis. For example, the mucus adhesion in the βENaC mouse is associated with chronic inflammation, characterized by an increased number of neutrophils and activated airway macrophages. Importantly, this inflammation persists in βENaC mice raised in gnotobiotic facilities, suggesting that mucus *per se*, and not bacterial infection, is driving inflammation (9). Furthermore, the inflammation is associated with airways epithelial remodeling with evidence of epithelial hyperplasia and mucus cell metaplasia. Finally, the phenotype is associated with evidence of air space enlargement, consistent with a developmental form of emphysema.

Over the past decade, many interesting aspects of mucoobstructive diseases have been investigated in this mouse model. For example, the mucoobstructive disease phenotype is related to mucus adhesion with concentrated MUC5B. Other studies revealed that the βENaC mice exhibit routine airways bacterial infection in the neonatal and transient bacterial infections in the adult period (9). It appears that the predominant infectious load originates from the oral cavity, with aspiration and poor clearance likely being the portal for infection. In contrast, the precise mechanisms linking the airways inflammation to alveolar disease have not yet been elucidated. Finally, the βENaC mouse has been useful for evaluating potential therapies for mucoobstructive disease, including the possibility of eliminating/drastically reducing mucin secretions as tested in crosses between the βENaC mouse and MUC5B and MUC5AC knock-out mice.

Mechanisms of Acute Exacerbations

Epidemiologically, acute exacerbations occur mostly in the fall and winter seasons. This observation, coupled with numerous studies using modern molecular virology techniques, has suggested that respiratory viruses are often the initiating stimulus for acute exacerbations in patients with CF and COPD. Similarly, both subjects with CF and subjects with COPD exhibit associations between gastric reflux and exacerbation frequencies (10). Thus, it appears that the exacerbations that plague both populations result from aspirations of gastric/infectious insults presented to mucoobstructed lungs with heterogeneous disease and abnormal host defense.

We have speculated that acute exacerbations can be triggered by viral events that affect the concentration of the mucus layer. Previous *in vitro* experimental data demonstrated that CF airway cultures infected with RSV failed to maintain airway surface liquid (ASL) volumes adequate to promote transport (11). The pathophysiology of this response reflected the fact that respiratory syncytial virus (RSV) up-regulated ecto-ATPases, removing the sole signaling molecule (ATP) that maintains airway hydration in CF. More recent studies have also suggested that RSV also up-regulates the synthesis and secretion of MUC5AC and MUC5B. Thus, the combined effect of depleted airway surface liquid volume and increased mucins synthesis is predicted to produce an increase in mucus layer concentrations that would slow transport and produce mucus plugging.

A key feature of mucoobstructive lung diseases (e.g., CF and COPD) is the heterogeneous nature of disease. We have speculated that the heterogeneity of disease reflects the heterogeneity of lung involvement during exacerbations superimposed upon a relatively uniform host defense defect. Furthermore, we have speculated that the heterogeneity of lung involvement during an exacerbation reflects the heterogeneity of the distribution of the insult (e.g., the heterogeneous distribution of inhaled viruses [from the nasopharynx]) and the heterogeneous distribution of aspirated gastric/pharyngeal contents. The general notion is that the stimulus for an exacerbation is distributed preferentially to the areas of the lung that receive the most air flow (i.e., those that are the least damaged), so the exacerbation will reflect the spread of the disease to previously nondiseased areas (Figure 2). Thus, an exacerbation can be viewed as spread of disease to previously nondiseased areas rather than intensification of disease in previously affected areas. A corollary of this hypothesis is that the bacteriology of an exacerbation will typically reflect the migration of bacteria by an intrapulmonary aspiration from areas of fixed disease to areas of newly developed disease. Thus, the bacteriology of an exacerbation may not, and usually does not, reflect major changes from basal bacteriology.

Preliminary data in a small number of subjects with CF who were studied under stable conditions and during exacerbations are consistent with this notion (12, 13). For example, in subjects with CF and an average FEV_1 of ~55%, spontaneous exacerbations as defined by the Rosenfeld exacerbation criteria produced a drop in FEV_1 that was modest (i.e., ~2%). Coincident with the exacerbation, both quantitative bacterial cultures and community structure (microbiome studies)

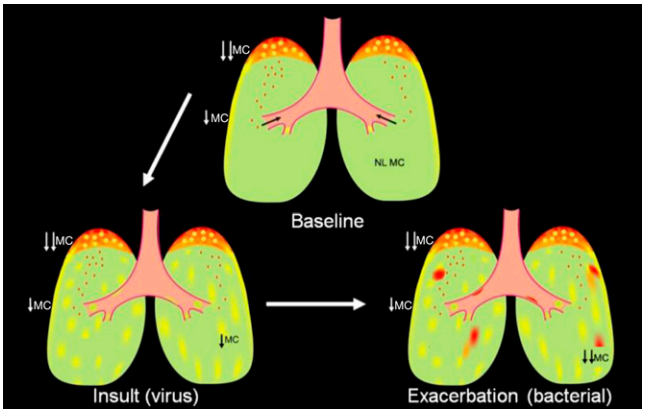

Figure 2. Anatomy of spread of disease during acute exacerbations in the context of heterogeneous, mucoobstructive lung disease. A sequence occurs whereby a heterogeneously damaged lung (*top panel*) is exposed to a virus that heterogeneously infects previously "normal" lung areas, reflecting the distribution of airflow. The virus concentrates airway mucus and reduces local mucus clearance (MC) (*yellow areas*, *lower left panel*). Intrapulmonary aspiration of bacteria from diseased areas is deposited on areas of virus-induced mucus adhesion, setting the stage for the spread of potentially chronic bacterial infection (*red areas*, *lower right panel*). Adapted by permission from Reference 6.

were performed. Interestingly, no differences in quantitative bacterial cultures, quantitative numbers of *Pseudomonas* or *Staphylococcus*, or changes in16-S–defined bacterial communities were detected between the baseline and exacerbation states. In contrast, modest increases in mucin concentrations were observed, suggesting mucus dehydration. Strikingly, dramatic reductions in mucociliary clearance were observed (i.e., reflecting an approximate 30–40% decrement in mucus clearance rates). Importantly, the decrements in mucus clearance were not homogenously distributed throughout the lung. Indeed, the focal decrements in mucus clearance were observed in areas of the lung that had previously exhibited normal clearance. Thus, these data are consistent with the hypothesis that at least some CF exacerbations are a reflection of spread of disease rather than disease intensification.

Conclusions

Mucoobstructive lung diseases that are characterized clinically as chronic bronchitisc appear to be caused by regional failures of mucus clearance. Collapses in mucus clearance appear to reflect an increased concentration of the mucus layer that produces compression and mucus adhesion to the periciliary gel and/or epithelial surface. The causes of mucus hyperconcentration are multiple, including intrinsic ion transport defects, malregulation of extracellular signals controlling airway-surface hydration (ATP/ADO), and/or mucin hypersecretion. Mucoobstructive lung diseases appear to evolve in response external stresses that can produce exacerbations, including viruses and gastric/pharyngeal aspirations. Importantly, the aspirations appear to be distributed heterogeneously within the lung, producing exacerbation phenotypes that are often characterized by spread of infection/inflammation, rather than intensification. Therapies to treat exacerbations may be focused profitably on strategies that might reduce the concentration of mucus and treat the underlying stimulus (i.e., viral infections). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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