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CFTR-ENAC INTERACTIONS

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# Regulation of airway surface liquid volume by human airway epithelia

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**Abstract** Mucus clearance on airway surfaces is a primary form of pulmonary defense. The efficiency of mucus clearance in large part depends on the volume of the airway surface liquid components, including both the periciliary liquid (PCL) layer and the mucus layer. Studies with in vitro model systems suggest that the mucus layer acts as a passive reservoir to redistribute water to and from, as needed, the PCL layer. In contrast, the overall volume of airway surface liquid is determined by active transepithelial salt transport. Data from in vitro systems suggest that airway epithelia have the capacity to both absorb and secrete liquid in response to the volume requirements on the apical surface. At present, the nature of the signals that transmit information about airway surface liquid volume to epithelia and their sensors are unknown. However, progress in elucidation of this system is important, because it appears that these systems are deranged in the genetic disease cystic fibrosis, which is characterized by airway surface liquid volume depletion, mucus stasis, and chronic infection. Thus, insights into these systems may offer novel therapeutic opportunities to correct this physiologic dysfunction of airway epithelia.

**Keywords** Airway epithelia · Airway surface liquid · Periciliary liquid · Mucus layer · $Na^+$ absorption · $Cl^-$ secretion · Cystic fibrosis

## Introduction

Mechanical clearance of mucus from the intrapulmonary airways is the primary form of innate airways defense against inhaled pathogens. Although the mucociliary clearance apparatus has been described previously in qualitative terms [12], it was with the advent of well-differentiated cell culture models interfaced with confocal microscopy that a detailed understanding of the clearance process has evolved [8]. Analyses performed in these cell culture systems, which exhibit mucus transport in vitro, have revealed an airway surface liquid (ASL) divided into a well-defined periciliary liquid (PCL) layer and a mucus layer. During mucus transport, both layers are transported on airway surfaces at approximately equal velocities [8]. Thus, these studies have allowed investigation of the important properties of both the PCL and the mucus layers for effective mucus clearance.

Clearance of mucus from the conducting airway system of the lung faces impressive "geometric" problems [3]. At a macroscopic scale, the surface area of the small airways is ~2 $m^2$, which converges to a "choke point" at the third-generation bronchial region (~50 $cm^2$). Because the height of ASL is approximately the same in distal and proximal airway regions, and the velocities of mucus transport in the respective regions are within an order of magnitude of one another, large volumes of liquid must be absorbed in the transit from distal to proximal airways to preserve ASL heights at levels optimal for mucus transport. At a finer scale, when two small airways converge to form one larger airway, the effective airway surface area (diameter) is reduced. Thus, there is a "volume load" imposed at the point of convergence of two smaller airways to form a single airway.

Airway epithelia have evolved a number of mechanisms that effectively control ASL height for efficient mucus transport. These mechanisms involve "passive" ones in series with active ion transport mechanisms located within the airway lining cells, i.e., airway epithelia. The goal of this review is to describe the passive and active mechanisms for regulating ASL volume and how they are integrated to provide efficient mucus transport in the healthy lung.

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"Passive" water transport on airway surfaces – the role of the mucus layer as a liquid reservoir

The mucus layer is operationally defined as the liquid layer that resides atop the tips of cilia that is occupied by the "gel-forming" mucins. In human airways, the predominant gel-forming mucins in health are MUC5AC, thought to be produced mainly by goblet cells, and MUC5B, thought to be mainly produced by submucosal glands. It also appears likely that some of the cell surface mucins, e.g., MUC1 and MUC4, are shed into the airway lumen and accumulate within the mucus layer.

Recent studies with cell culture systems have shown that liquid can be added to or removed from the mucus layer without altering the height of the PCL layer (Fig.1) [10]. Specifically, these studies showed that removing ~50% of the liquid from the ASL compartment led to selective reduction in the volume of the mucus layer, with preservation of the PCL layer, and at least partial preservation of mucus transport. When the converse experiment was performed, i.e., the ASL volume was effectively doubled, all of the added liquid entered the mucus layer, the PCL layer maintained its volume, and interestingly, mucus transport rates increased. Further studies revealed that when more volume was removed from the ASL compartment, e.g., more than 70%, the mucus layer could no longer effectively "donate" liquid to the PCL layer, volume was lost from the PCL, and mucus transport ceased. The upper limit of volume addition to the mucus layer was not determined in these studies.

One implication of these findings is that as liquid in vivo converges onto a single airway from two smaller airways, the excess liquid deposited on the single airway from the two distal airways may be transiently "stored" in the mucus layer, with preservation of PCL layer height and mucus transport. Presumably, the excess liquid (salt and water) will be removed by transepithelial ion transport (see below) from the mucus layer as the ASL moves up the single airway. Conversely, when water loss occurs from airway surfaces, in proximal airways due to exercise-induced evaporation, the volume is selectively lost from the mucus layer, with preservation of PCL and mucus transport during periods of exercise.

Despite our better understanding of the role of the mucus layer as a reservoir, there are still surprisingly large gaps in our knowledge of the physiology and biophysics of this layer. For example, it is still not clear how this layer is formed in vivo, nor what are the processes that maintain it as a physical layer. Older theories, suggesting that the mucus layer was created by the thixotropic actions of beating cilia, appear to be inaccurate. Perhaps more likely, there are important phase transitions that occur as a function of the concentration of the gel-forming mucins in ASL. Further, as noted above, it is not known what the water storage capacity of the mucin layer is, nor, more importantly, what are the biophysical principles that govern the capacity of this layer to absorb or donate liquid. Finally, it is not yet clear how the addition of liquid to airway surfaces increases mucus transport, as the biophysical properties of the mucus layer may be less important than the properties of the PCL layer. Thus, future studies will be required to study the complex interrelationships among the hydration status of the mucus layer, the viscosity of the PCL, and the regulation of ciliary beat frequency.

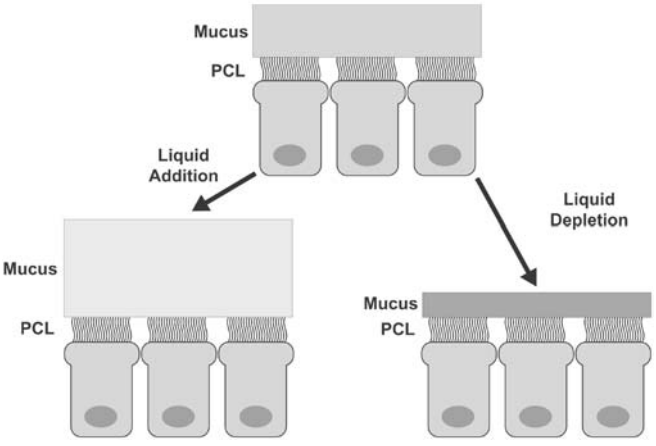


**Fig. 1** The mucus layer as a liquid reservoir. Upper panel depicts normal anatomy of mucus and periciliary liquid (PCL) layers. Added liquid selectively enters into and expands the mucus layer (*lower left*), whereas the mucus layer volume can be reduced by ~50% without perturbing PCL volume (*lower right*)

## The role of active airway epithelial ion transport in determining ASL volume

The principal determinant of ASL volume is the mass of salt on the airway surface [1]. This fact reflects the highly water permeable nature of the airways epithelium, that rapidly restores/maintains ASL volume in a near-isotonic state [9]. The mass of salt on an airway surface is determined in part by the movement of liquid along airway surfaces and in part by the active ion transport mechanisms located within that airway region. Recent studies have emphasized the importance of the role of the PCL layer volume (height) in the maintenance of effective mucus clearance. Again, with the advent of well-differentiated airway model systems coupled to confocal microscopy, it is has been possible to study the metabolism of the PCL layer under physiologic conditions.

A useful model for studying PCL physiology has been the well-differentiated culture that has had the mucus layer removed and a volume of liquid that mimics the properties of PCL placed on its surface. A convenient paradigm has been to start experiments with an "excess" of PCL that might mimic the height found at the convergence point of two airways. As shown in Fig.2, normal human airway epithelia absorb rapidly the excess PCL, but as the PCL layer approaches the physiologic height (7 μm), active volume absorption ceases and a steady state is achieved.

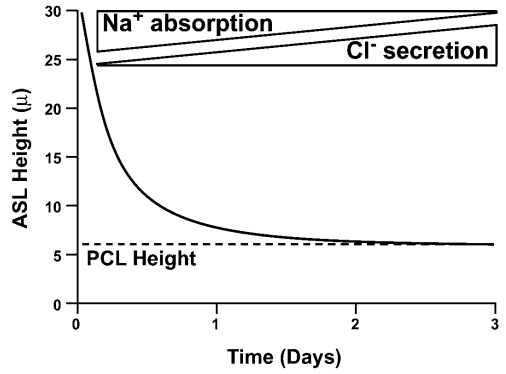


**Fig. 2** Regulation of PCL volume by active ion transport. Maintenance of PCL volume at physiologically relevant height (7 μm, as defined by height of extended cilium) is accomplished by a mix of $Na^+$ absorption and $Cl^-$ secretion

A series of studies employing measures of transepithelial potential difference (PD) as an index of active ion transport and selective inhibitors of $Na^+$ (amiloride) and $Cl^-$ (bumetanide) transport has been used to understand the regulation of PCL absorption as a function of PCL layer height (volume) [10]. In brief, the rapid absorption of excess PCL is mediated by transepithelial amiloride-sensitive $Na^+$ absorption. As PCL volume approaches the physiologic height, the amiloride-sensitive PD ($Na^+$ absorption) is reduced, which slows the rate of volume absorption. Importantly, inhibition of the apical membrane $Na^+$ conductance also generates driving forces for $Cl^-$ secretion, which induce $Cl^-$ secretion-mediated volume flows [13]. One implication of this finding is that the channel that mediates $Cl^-$ secretion, i.e., likely the cystic fibrosis transmembrane conductance regulator (CFTR), is partially activated in the basal state. This finding is consistent with reports from studies of nasal PD in humans in vivo [4].

The mechanisms that control the activity of the apical membrane $Na^+$ channel (ENaC) and the apical membrane $Cl^-$ channel [CFTR, and possibly the $Ca^{2+}$-activated $Cl^-$ channel (CaCC)] as a function of PCL volume are currently unknown. It seems possible that information that controls the activity of these channels is encoded within the PCL layer itself. Thus, candidates that would control the activity of the apical membrane $Na^+$ channel might include extracellular nucleotides, e.g., ATP, which have been shown to inhibit ENaC via interactions with the $P2Y_2$ receptor ($P2Y_2$-R) [6]. The transduction mechanism for $P2Y_2$-R-mediated inhibition of ENaC was previously thought to be due to activation of protein kinase C (PKC), although more recent data suggest that there may be a role for PLC in controlling the local concentrations of membrane phosphatidylinositol-4,5-bisphosphate (PIP2) that interact directly with the N-terminus of ENaC subunits [5]. In addition, it is also possible that there may be a role for the channel activating protein (CAP) and endogenous CAP inhibitors in regulating the activity of ENaC [11]. However, it has been difficult thus far to identify the inhibitors, the actual targets of CAP, and the physiologic role for this system in regulating volume transport in model systems.

With respect to regulators of $Cl^-$ transport, the nucleotide/nucleoside system is again an attractive candidate and may signal through concentrations of ATP and adenosine in PCL. ATP, through purinoceptor activation, can activate CFTR through a PKC-dependent mechanism and activate CaCC through a $Ca^{2+}$-dependent mechanism. Similarly, recent data suggest that the local formation of adenosine, interacting with apical membrane adenosine $A_{2b}$ receptors, $G_S$ proteins, and adenyl cyclase, regulates the activity of CFTR [2].

With respect to the epithelial sensors that detect PCL volume, certainly for information encoded within the PCL layer, e.g., nucleotides/nucleosides and the CAP system, purinoceptors and presumably the CAP protein itself are likely candidates. However, it is also quite likely that there may be other sensors in the airway epithelium that sense other properties of PCL that may relate to volume. Thus, it is possible that as PCL volume shrinks, the concentration of solutes that impart viscosity to the PCL is increased. As PCL moves along airway surfaces, there would be increased shear on airway surfaces that may sensed by stretch- or shear-activated ion channels within the apical membrane.

## Future studies

As noted throughout this narrative, there are still surprising gaps in our knowledge of fundamental aspects of the mucociliary system. In parallel with our lack of knowledge of the biophysical forces that initiate and form a mature mucus layer, we also know little about the processes that form and define the PCL. Interestingly, over the ciliated cell, normal PCL height is operationally and morphologically defined as height of the extended cilium. However, over other cell types, e.g., goblet or Clara cells, the PCL (perhaps not so aptly named) approaches 1–3 μm in height. Thus, it would appear that the physical attributes of the cell surface, i.e., cilia and/or microvilli, are important in determining the mucin-free "periciliary" liquid layer volume (height). Further, based on preliminary studies with cultures from patients with immotile cilia, it appears that a well-defined PCL layer is formed in the absence of ciliary beating. Consequently, it would seem that we may need an entirely different theoretical formulation to describe the formation of this layer.

Finally, it will be important to understand the derangements of the homeostasis of PCL on airway surfaces in patients with lung disease. Clearly, the prime disease candidate for abnormalities in PCL is cystic fibrosis (CF). It remains to be elucidated whether the defects in PCL in CF reflect primarily abnormalities in $Na^+$ transport, $Cl^-$ transport, or a combination of the two [7]. Similarly, it appears that there will be other diseases that are associated with the absence of PCL and

adherence of mucins to the cell surface, e.g., chronic bronchitis. The task for the future will be to determine whether chronic bronchitis reflects primarily depletion of PCL due to ion transport defects or excessive secretion of mucus that creates a water-depleted environment. Regardless, it does appear that a unifying theme for therapy of obstructive airways diseases is the need for proper hydration on airway surfaces to generate effective mucus clearance. Thus, insights into the physiology of this system in health and disease should lead to novel ways of dealing therapeutically with important human diseases.

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