Effective Mucus Clearance Is Essential for Respiratory Health

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Studies of the pathogenesis of cystic fibrosis (CF) and primary ciliary dyskinesia (PCD), as well as novel genetic mouse models, vividly illustrate that effective “mucus clearance” is a critical innate airway defense mechanism. Modern physics, physical chemistry, biochemistry, and cell and systems biology are revealing the structure of the mucus clearance apparatus and elucidating key parameters underlying its efficient function. New paradigms are evolving to describe the interaction of the near-cell surface environment with overlying mucus, the dominant role of adequate hydration for effective mucus clearance, the role of nucleotide and nucleoside signaling to regulate airway surface hydration, the physiochemical basis of mucus adhesion, and the pathophysiologic consequences of impaired mucus clearance. The recent success of hypertonic saline to restore surface hydration, the physiochemical basis of mucus adhesion, and the pathophysiologic consequences of impaired mucus clearance—The recent success of hypertonic saline to restore surface hydration, the physiochemical basis of mucus adhesion, and the pathophysiologic consequences of impaired mucus clearance—

ENVIRONMENT AND AN OVERLYING MUCUS LAYER

TWO LAYERS: A LUBRICATING PERICELLULAR ENVIRONMENT AND AN OVERLYING MUCUS LAYER

INNATE DEFENSE OF AIRWAY SURFACES AGAINST INHALED BACTERIA/YEAST/FUNGUS/PARASITES IS A MULTI-TIERED PROCESS, BUT MECHANICAL CLEARANCE IS PARAMOUNT

Molecular Biology. Rather than a comprehensive review of the entire field and its literature, we present eight topics that build on recent observations and also point toward future studies needed to advance this field. As background, it is important to understand that water transport across the apical plasma membrane of airway epithelial cells, and thus the hydration status of airway surface liquid (ASL), is principally regulated by Cl− ion export through both the cystic fibrosis transmembrane conductance regulator (CFTR) and Ca++ activated chloride channels (CaCC), and by Na+ influx through the epithelial Na+ channel (ENaC) (Figure 1).

Historical Perspective and Introduction

A coordinated system of epithelial water and ion transport, mucin secretion, cilia action, and cough, collectively termed “mucus clearance,” results in the continuous flow of fluid and mucus over airway surfaces. The study of mucus clearance has a fascinating history, and was investigated using “classical” particle clearance techniques 25–30 yr ago (reviewed in Ref. 1). Despite advances made by pioneering scientists, it was clear that “the physiologic techniques 25–30 yr ago (reviewed in Ref. 1). Despite advances made by pioneering scientists, it was clear that “the factors which normally control [airway surface liquid] secretion in such a manner as to prevent flooding or drying of the airways and facilitate mucociliary transport are still largely unknown” (2). Subsequently, mucus clearance per se has received less attention, as greater emphasis was placed on cellular and molecular aspects of pulmonary pathophysiology. Furthermore, the pathogenic importance of mucous transport was questioned due to minimal impact of mucolytic therapy on airway diseases. From her early work on the lungs’ secretory apparatus (3), to her generation of valuable tools to study mucous cells (4, 5), through her landmark cloning of the MUC5AC promoter (6), and recent studies of detailed signal transduction mechanisms in bacterial infection (7) and after smoke exposure (8), it is clear that Carol B. Bambaum recognized the importance of mucus clearance for respiratory health. We consider it an honor and privilege to summarize recent advances in the field of mucus clearance as a tribute to her in the American Journal of Respiratory Cell and Molecular Biology. Rather than a comprehensive review of the entire field and its literature, we present eight topics that build on recent observations and also point toward future studies needed to advance this field. As background, it is important to understand that water transport across the apical plasma membrane of airway epithelial cells, and thus the hydration status of airway surface liquid (ASL), is principally regulated by Cl− ion export through both the cystic fibrosis transmembrane conductance regulator (CFTR) and Ca++ activated chloride channels (CaCC), and by Na+ influx through the epithelial Na+ channel (ENaC) (Figure 1).

Keywords: chronic bronchitis; cystic fibrosis; primary ciliary dyskinesia

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Chronic bacterial infection of airways is usually associated with obstruction, which can be produced by tumors or foreign bodies, but is most commonly caused by mucus adhesion, formation of mucus plaques, and ultimately mucus plugs. Genetic diseases characterized by this phenomenon include CF and PCD (Figures 2A and 2B). In addition, recent evidence from the laboratory of Dr. Hogg and colleagues (9) suggests that mucus obstruction contributes to the pathogenesis of COPD (Figure 2C). These clinical observations are complemented by data from animal models of disease. Typically, chronic airway infection in a laboratory animal requires the administration of bacteria in a milieu designed to obstruct an airway lumen—for example, agarose beads (10). More recently, transgenic mice that overexpress the β subunit of ENaC revealed a phenotype in which depletion of ASL produced mucus adhesion, plaque and plug formation, and a reduced ability to clear inhaled bacteria (11) (Figure 2D). In contrast, diseases that affect other aspects of lung defense—for example, dysfunction of alveolar macrophages, neutrophils, or B-cells (both secretory IgA, and IgG)—tend to produce alveolar, rather than airway, pathology. These observations suggest that mechanical clearance is the dominant form of airway innate defense, and that failure of mucous clearance produces obstruction and predisposes to chronic bacterial infection.

THE MUCUS CLEARANCE SYSTEM REQUIRES TWO LAYERS: A LUBRICATING PERICELLULAR ENVIRONMENT AND AN OVERLYING MUCUS LAYER

 Efficient clearance from airway surfaces requires the coordinated interaction of two separate layers that together comprise ASL: an overlying transported mucus layer and a separate, distinct environment near the cell surface. The mucus layer must bind and entrap virtually all deposited particles. Its viscoelastic properties facilitate conversion of energy from beating cilia into vectorial mucus transport and should also be amenable to clearance by cough under periods of stress. The long-standing concept was that a low-viscosity aqueous environment, termed the “sol” or “periciliary liquid” layer, surrounded the cilia, in which they could freely beat. However, this concept never explained how the height of the layer varied from cell type to cell type, for
Figure 1. A depiction of ion transport across the apical plasma membrane of airway epithelial cells. Schema describing extracellular nucleotide/nucleoside biochemical networks, sensors (receptors), and regulatory paths controlling effectors (ion channels). Cl⁻ can egress through CFTR after adenosine stimulation of A2b-R or through CaCC after stimulation of P2Y2-R by ATP. Na⁺ transport is facilitated by ENaC. CF cells will not be able to mount a Cl⁻ response after adenosine due to the absence of functional CFTR. Finally, adenosine (ADO) and inosine (INO) are removed from the airway surface by concentrative nucleoside transporters (CNTs) (63).

example 7 μm over ciliated cells and 3 μm over goblet or nonciliated brush cells (12), or that electron microscopy after specific types of fixation revealed a complex architecture in this domain (Figure 3). Another conundrum emanated from studies of individuals with Type I pseudohypoaldosteronism (PHA), who fail to absorb liquid from airway surfaces due to genetic defects in ENaC (13). Previously, it was postulated that excess ASL would cause the mucus layer to “float off” cilia tips, reducing mucus transport. Strikingly, PHA patients with “excessively wet” airway surfaces also exhibited the fastest rates of lung particle clearance yet reported (13). Subsequent in vitro studies revealed that liquid added to airway surfaces selectively entered the mucus layer, causing it to swell and maintain a connection between cilia and mucus, such that mucus transport rates actually increased when liquid was added (14). The near cell ASL layer is likely comprised of tethered mucins and other molecules such as cell surface glycolipids, while the overlying layer consists of high-molecular-weight mucin dimers and trimers, 0.5–20 μM long, interacting with globular proteins (Figure 4). The nonmucin interacting proteins may serve as cross-links and/or provide other innate defense activities. The near-cell environment likely provides an architectural framework for the movement of water, imparts lubricating activity (15), and serves as a selective filter that restricts particle access to cell surfaces.

ADEQUATE HYDRATION OF THE MUCOSAL SURFACE IS ESSENTIAL FOR NORMAL MUCUS CLEARANCE

In general, the hydration of mucosal surfaces determines the efficiency of mechanical transport, for example, during blinking and swallowing. It was widely assumed that ciliary activity and mucin secretion were the major determinants of airway mucus clearance. While both are important, the balance of evidence suggests that hydration is the dominant variable governing mucus clearance. Individuals with CF, who exhibit a phenotype thought to reflect ASL volume depletion (16), develop more rapid and severe airway infection and destruction than patients with PCD, who have dysfunctional cilia. Asthma, which is clearly characterized by hypersecretion of mucin, is not associated with bacterial infection.

Further evidence for the dominant role of hydration status in mucus clearance comes from mouse models. A series of mice with either no cilia or dysfunctional cilia exhibited surprisingly
little or no pulmonary disease phenotype (17). Similarly, antigen- or cytokine-induced animal models resulting in hypersecretion of mucus exhibit relatively little airways obstruction or infection (18). In contrast, overexpression of the β subunit of ENaC in murine airways reduced periciliary volume, slowed mucus clearance, caused mucus adhesion (Figure 2D), and resulted in spontaneous mortality of ~60% by 30 d of age due to mucus obstruction/asphyxia (11).

As shown in Figure 5, an integrated model of the airway pericellular environment and overlying mucus layer, incorporating the functional requirement for adequate hydration, reconciles previous discrepancies related to mucus transport. In this model, the mucus layer acts as a fluid reservoir and, within physiologic limits, it accepts or donates liquid to maintain apposition of the mucus layer inner surface with the tips of the cilia. Thus, instead of excess fluid floating mucus off the cilia and slowing mucus clearance, mucus swells and clearance accelerates when liquid is added to the lumen, as confirmed in human airway epithelial cell cultures (14). Conversely, under conditions of relative dehydration, mucus can donate water to preserve periciliary hydration. However, when the airway surface becomes severely dehydrated, as in humans with CF and in βENaC transgenic mice, the ability of the mucus layer to “donate” water is exhausted. The periciliary layer collapses, and the viscous, adhesive mucus layer forcibly interacts with cell surface mucins (19), resulting in adhesion and decreasing mucus clearance, ultimately forming plaques and plugs of concentrated mucus (11).

**AIRWAY SURFACE HYDRATION IS REGULATED BY RELEASE AND METABOLISM OF NUCLEOTIDES**

Extracellular nucleotides (ATP, UTP, and UDP) and nucleosides (adenosine), acting on specific receptors (P2Y2–R [ATP/UTP], P2Y6–R [UDP], and A2b–R [adenosine]), stimulate Cl− transport when administered to the airway lumen (20). The observation that P2Y2–R activation not only initiates Cl− secretion, but also reciprocally inhibits Na+ absorption, strongly suggests the importance of these molecules to finely tune ASL height and volume. Indeed, recent studies using well-differentiated human airway epithelial cell cultures that recapitulate the morphology, ASL volume homeostasis, and mucus transport functions of the intact epithelium (Figure 6) suggest that the periciliary liquid layer approximates the height of the outstretched cilia (14). ATP and adenosine measurements in ASL, under static conditions, revealed ATP levels below those expected to activate P2Y2–R (~1 nM), whereas adenosine was in the concentration range expected to activate A2b–R (~40–50 nM). Addition of adenosine deaminase, which metabolically removes adenosine, or 8-SPT, an inhibitor of adenosine receptors, resulted in unrestrained Na+ absorption and ASL collapse (23). These results suggest that under static conditions, ATP is released onto the airway surface (estimated rate ~300–400 fmole/cm²/min), where it is metabolically converted into adenosine, which binds the A2b–R, in turn activating CFTR, which serves as a Cl− secretory channel and also inhibits ENaC. The net effect is to balance absorption and secretion to maintain an adequate volume of liquid for efficient mucus transport on the airway surface. We speculate that constitutive ATP secretion, perhaps via vesicle

**Figure 5.** The role of water in the structure and function of the “two-layer” mucus clearance system. Under normal conditions (*center panel*), there is sufficient water to hydrate the periciliary layer and mucus layer and mucus transport proceeds at normal rates (60 μm/s). Addition of water (and salt) to the surface selectively swells the mucus layer, maintaining apposition of the mucus and periciliary layers (*left panel*). Reduction in viscoelasticity may account for the observed acceleration of mucus transport (~100 μm/s) under highly hydrated conditions. Loss of water (and salt) from the airway surface collapses both the periciliary and mucus layers, producing mucus adhesion to cell surfaces (*right panel*).
release, is an intrinsic mechanism to hydrate airway surfaces and maintain basal mucus clearance.

Mechanotransduction is the process by which physical forces are translated to physiologic responses and is widely important in tissue homeostasis. Recent studies strongly suggest that phasic motion of the airway wall during lung inflation/deflation regulates ASL homeostasis via nucleotides and nucleosides (19). When normal human airway epithelial cells were subjected to phasic shear stresses similar to those achieved during tidal breathing, the height of ASL doubled, when compared with static conditions (Figure 8). Direct measurements revealed that ATP rose to \( \sim 30-40 \) nM in response to shear stress, which is sufficient to activate P2Y$_2$-R, and that adenosine concentrations doubled to \( \sim 160 \) nM (Figure 8). These studies confirm the importance of extracellular nucleotides and nucleosides to generate an ASL environment capable of mucus transport. Thus, normal human airway epithelia control ASL hydration via complementary pathways: ATP via P2Y$_2$-R, and adenosine, the metabolic product of ATP, via A$_3$-R. In CF, CFTR is not “available” for activation by adenosine, and ASL hydration is proportionate solely to ATP on airway surfaces. Thus, the CF lung is uniquely vulnerable to insults that reduce ATP on airway surfaces.

**Figure 6.** Cell culture/physiologic system to measure ASL volume homeostasis and mucus transport in well-differentiated human bronchial epithelial (HBE) cells. (A) Perfluorocarbon/osmium fixed HBE culture demonstrating excellent cellular differentiation and discrete periciliary (PCL) and mucus layers. (B) Living HBE culture with cellular (calcein-green) and ASL compartments (Texas-red dextran) labeled and visualized with x-z confocal microscopy. (C) En face view of culture with fluorescent beads trapped in mucus as viewed with time lapse fluorescence microscopy to measure rotational mucus transport. (D) Measurement of ASL height by confocal microscopy and transepithelial electric potentials (Vt) by microelectrodes to measure ASL volume and ion transport homeostasis.

**Figure 7.** ASL volume (height) regulation by normal HBE cultures under static conditions. (A) ASL height measured by x-z confocal microscopy at \( t = 0 \) after addition of 20 \( \mu \)l of PBS. (B) Mean data for ASL volume homeostasis after addition of PBS without (squares) or with (triangles) 8-SPT (10$^{-5}$ M) at 48 h. The blue area depicts normal periciliary liquid layer height. (C) Confocal images of ASL at 48 h without (top panel) and with (bottom panel) 8-SPT. The 3-\( \mu \)m level in the 8-SPT group represents the minimum volume on the airway surface, when liquid is trapped within flattened cilia. Adapted from Ref. 19, with permission.

**INADEQUATE HYDRATION CAUSES MUCUS ADHESION TO EPITHELIAL SURFACES, RESULTING IN OBSTRUCTION AND INFLAMMATION, AND SERVING AS A NIDUS FOR INFECTION**

Mucus adhesion likely contributes to the pathophysiology of lung disease in several different ways. Recent studies by Dr. Hogg and colleagues found that pack-years of cigarette smoking was directly correlated with epithelial hyperplasia and mucus obstruction (9). In the \( \beta \)ENaC mouse, mucus adhesion leads to the formation of mucus plugs that can be sufficiently severe to asphyxiate these animals (11). The failure of mucus clearance in \( \beta \)ENaC mice may directly produce chronic neutrophilic inflammation. Neutrophils have been observed in bronchoalveolar lavage fluid of patients with CF without apparent infection, which may be a consequence of viscous, static mucus, although an intrinsic CF airways hyper-inflammatory state or undiagnosed infection is also possible (24). It stands to reason that proinflammatory stimuli will become more concentrated and linger in a lung with ASL dehydration and static mucus. Thus, stimuli that are insufficient to induce inflammation in a normal lung, with adequate fluid transport over airway surfaces, may cause inflammation and self-reinforcing pathologic sequelae in a lung with impaired mucus clearance.
Perhaps the most important pathophysiologic effect of adherent mucus is that it is the site of chronic bacterial infection in the airways (Figure 9). Quantitative studies in CF airways show that infection centers on intraluminal mucus, with virtually no bacteria detected adherent to, or within, epithelial cells (25). Bacteria may adapt and survive in static mucus due to production of mucinases that help them gain nutrition. Indeed, the interaction of bacteria and static mucus is complex. For example, O2 gradients are generated in macroscopic mucus plaques, reflecting both long O2 diffusion distances and high rates of epithelial O2 consumption (25, 26). Bacterial communities in the airway lumens of patients with CF are likely thriving in an anaerobic environment (27). Physical chemical studies of mucus and calculations of O2 diffusivity and bacterial O2 consumption predict frankly anaerobic niches within mucus plaques. Mucus concentration (% solids) also may be important for the mode of bacterial growth. Specifically, the width of the mucin mesh or “pore size” varies dramatically with hydration status. In normal mucus, widths of 5–15 μm are observed, which foster bacterial motility and also enable quorum factors needed for biofilm formation to freely diffuse away (28). Both processes favor planktonic bacterial physiology, which will enhance bacterial exposure to antimicrobial substances and allow phagocytic cells to access, engulf, and kill bacteria (28). Conversely, in concentrated mucus, the apparent mesh size decreases below the size of a bacterium (<200 nm), limiting bacterial motility and egress of quorum factors. Coupled with the inability of soluble antimicrobial factors and neutrophils to penetrate and kill bacteria enmeshed in thick mucus, the thick mucus environment likely enhances biofilm growth. Finally, limited water availability may become a selective pressure for bacteria that thrive in relatively dry, anaerobic environments, including soil bacteria such as Burkholderia cepacia, resulting in the characteristic and unusual microbiology in the CF lung (27, 29). Similar phenomena, but accordingly less intense, may occur in the airways of patients with non-CF bronchiectasis and COPD.

**DISEASE EXACERBATIONS RESULT FROM INTERMITTENT CATASTROPHIC FAILURES OF MUCUS CLEARANCE, OFTEN TRIGGERED BY VIRAL INFECTIONS**

In asthma, viral infections are well-known triggers of bronchospastic episodes (30, 31). While there is no evidence that the incidence of viral infection is increased in CF, idiopathic bronchiectasis, PCD, or CB/COPD, it is highly likely that viruses “exacerbate” these diseases. Epidemiologically, in longitudinal studies, rhinoviruses, paramyxoviruses, and coronaviruses, as well as influenza and other viruses, are important triggers of COPD exacerbations (32). Although detailed longitudinal studies of viruses in CF are just emerging (33), several studies indicate a strong relationship between respiratory syncytial virus (RSV) and rhinovirus infection and pulmonary deterioration (34–36). Recent studies suggest an abnormal response of airway epithelial cells from individuals with asthma in response to rhinovirus (37). Similarly, viruses may trigger acute exacerbations in patients with other preexisting airway diseases, because the airways are already inflamed. Because the number and severity of exacerbations are key determinants of declining lung function in both CF and COPD, it is important to understand the pathophysiology of virus-induced exacerbations.

A key concept is that many chronic airway diseases are heterogeneously distributed throughout the lungs. It is likely that viruses are preferentially delivered to “normal” portions of diseased lungs due to airflow preservation to these regions. Many offending viruses, including RSV, infect “normal” ciliated epithelial cells, again biasing infection to more normal regions of the lung (38, 39). Viral infection upregulates ecto-ATPases, depleting extracellular ATP (19), which in turn is predicted to decrease Cl− secretion and inhibit mucus clearance. In this scenario, bacteria residing in “abnormal” regions will spread (“metastasize”) to these new areas of mucus adhesion/obstruction, resulting in more diffuse disease and a greater bacterial burden. Chemokines, cytokines, proteases, goblet cell hyperplasia, and mucus hypersecretion resulting from viral infection further contribute to a descending spiral of airway pathology. Therapy to maintain efficient mucus clearance represents a viable option to mitigate the deleterious effects of viral infection on lung function and to minimize exacerbation of chronic airway diseases.

**CF AND CB BOTH REFLECT FAILURE OF MUCUS CLEARANCE, BUT DIFFER IN INITIATING EVENTS, SEVERITY, EFFICACY OF COUGH, AND STIMULI PRODUCING CHRONIC INFLAMMATION**

There is evidence that mucus dehydration and adhesion occur in both CF and COPD. In CF, studies using well-differentiated...
airway epithelial cells in culture demonstrate failure to maintain adequate ASL hydration (16). In vivo, there is evidence for subtle inflammation and goblet cell hyperplasia in the nasal cavity of CF mice, the region that best mimics the human CF ion transport phenotype (12). In the lower airways of CF mice, CaCC mediates Cl⁻ transport rather than CFTR, and the mice also do not reprise the accelerated Na⁺ transport found in human CF airways, so there are ion transport correlates to explain the absence of a lower airway phenotype. However, as also noted above, overexpression of the β subunit of ENaC in the mouse airway depleted ASL volume and produced mucus adhesion, plaques, and plugs (11). Clinical studies of patients with CF suggest that the % solids concentration in CF secretions is increased (40). Finally, frozen sections of CF airways, a histologic technique that prevents artifactual splitting of luminal contents away from the airway wall, demonstrate close apposition of luminal mucus with the cell surface (Figure 10).

There is less direct data supporting dehydration and mucus adhesion in COPD. It is important to note that relative dehydration can be manifest as either less ASL or an increase in the % solids of materials present in the lumen. Goblet cell hypersecretion and mucus hypersecretion are prominent in COPD, and new data suggest that salt and water transport and mucin secretion are segregated to different cell types in the airway epithelium. Specifically, ENaC and CFTR are located within ciliated epithelial cells, whereas goblet cells secrete mucins but not ions or water (41). The data of Verdugo suggest that mucins are secreted after a pore is formed between the secretory granule and the plasma membrane and Na⁺ within ASL exchanges for Ca²⁺ in the granule, resulting in hydration and explosive exit from the granule (42, 43). Thus, relative dehydration of ASL in the CB airway can reflect a primary increase in the mass of solids on the airway surfaces due to mucin hypersecretion, or a relative paucity of liquid, due to abnormal Na⁺ absorption and/or Cl⁻ secretion, or both. Perhaps the most compelling data that mucus dehydration is a problem in COPD are those of Dr. Hogg et al. that describe mucus adhesion to airway surfaces and mucus obstruction in the small airways (see Figure 2 and Ref. 9).

Although both CF and COPD are characterized by relative dehydration and adhesion of mucus, the pathogenic sequence is very different in the two diseases. In CF, there is an intrinsic defect in the CFTR Cl⁻ secretory pathway and unregulated Na⁺ absorption. These two defects in CF deplete ASL volume, which is later worsened by chronic infection and mucin hypersecretion. As noted previously, CF airway epithelial cells in vitro under static conditions fail to auto-regulate ASL volume (Figure 7). This would predict rapid depletion of ASL volume and cessation of mucus transport under basal conditions in CF lungs within months of life. However, young patients with CF typically do well for significant intervals, and mucus clearance can be maintained, at least in certain regions, for substantial periods (44). One explanation lies in observations of in vitro ASL volume regulation under phasic motion conditions that mimicked the motion of tidal volume breathing in vivo (19). CF cultures under phasic motion conditions exhibited an increase in ASL volume, with two key differences from normal cultures (Figure 11). First, ASL height, although sufficient to maintain mucus transport, was less in CF, consistent with the absence of adenosine-regulated CFTR function. Second, addition of apyrase to CF cultures under phasic motion conditions led to the collapse of ASL, presumably due to the absence of ATP-stimulated purinergic receptor–mediated inhibition of ENaC and activation of CaCC. These data suggest that CF airways are critically vulnerable to insults that decrease ATP signaling. Consistent with the notion that CF exacerbations induced by viruses may be due to decreased ATP, RSV infection, which stimulated the breakdown of ATP, abolished the capacity of CF airway epithelia to maintain mucus transport at 48 h under static or phasic motion conditions. (D) Reduction in CF ASL height to below normal level (dashed blue line) after addition of apyrase to remove ATP but not after 8-SPT. Adapted from Ref. 19, with permission.
to maintain ASL volume under phasic motion conditions, whereas ASL volume was only moderately reduced by RSV in normal airway epithelial cells (19). Thus, one major difference between CF and COPD is that CF airways rely solely on ATP interaction with P2Y2-R to stimulate CaCC, whereas COPD cells can hydrate via both the ATP mechanism and via adenosine interaction with A2b-R to activate CFTR.

Mucus dehydration in COPD is likely multifactorial. As noted above, goblet cell hyperplasia and mucin hypersecretion per se may increase the % solids content of ASL, causing relative dehydration in COPD (45). It is also likely that defects in ion and water transport contribute. Kreindler and coworkers reported that cigarette smoke condensates had relatively few effects on Na+ transport rates, but inhibited both CFTR and CaCC-mediated Cl− secretion, possibly by inhibiting basolateral Na+−K+−2Cl− co-transport (46). More studies are necessary to define the specific mechanisms of cigarette smoke inhibition of ion transport and also whether nucleotide and nucleoside metabolism and levels are different in the airways of smokers or patients with COPD. Endogenous cell surface proteases such as prostasin activate ENaC and accelerate Na+ absorption (47), and soluble proteases, including elastase, have similar activity (48, 49). In the later stages of COPD, neutrophil-derived elastase may activate ENaC, leading to Na+ hyperabsorption. Thus, neutrophil inflammation due to cigarette smoking may produce sufficient free elastase to increase goblet cell number and mucin production and to elevate Na+ absorption, causing relative dehydration.

To summarize the differences and similarities between CF and COPD, we note that CF is the most aggressive form of chronic mucus obstruction and infection and likely exhibits the most dehydrated airway mucus. The prediction is that in COPD, mucus concentrations will be intermediate between normal and CF, and mucus adhesion less widespread and firm. Since COPD cells will be able to generate a fluid secretory response via both CFTR and CaCC, while the CF cell response will be limited to CaCC, the prediction is that the balance of beneficial versus harmful effects of chemokines, cytokines, ATP, and adenosine generated during inflammation will be different. Early in disease, increased ATP will benefit both CF and COPD by stimulating CaCC. Late in disease, ATP could be harmful in both, due to ATP-stimulated mucin release in lungs with existing goblet cell hyperplasia (50). Importantly, increased adenosine may be more detrimental in CF, due to potential cAMP-dependent acceleration of Na+ absorption (51) coupled with an inability to generate compensatory hydration through CFTR, whereas in COPD, negative effects of adenosine on ENaC can be offset by A2b-R activation of CFTR. Bacterial avoidance of host defense and biofilm formation will be more profound in more concentrated mucus, producing more aggressive disease in CF (28). Viral infections will trigger acute exacerbations in both diseases, but the ability to compensate for reduced mucociliary clearance by coughing will be less efficient in CF than in COPD. In CF, constant exposure to microbial and inflammatory products drives expansion of the airway epithelial cell endoplasmic reticulum compartment, causing greater Ca2+-activated cytokine release triggered by G protein–coupled receptors (52, 53). Constant exposure to bacteria and neutrophilic inflammation in CF, versus intermittent tobacco smoke and bacteria in COPD, likely results in stronger induction of the endoplasmic reticulum proinflammatory Ca2+ mechanism.

Mucolytic therapy with N-acetylcysteine has no proven benefit in either CF or COPD (54, 55). This may not be surprising, since cleaving mucins adherent to cell surfaces without coordinate rehydration may not be effective (imagine “gum stuck on a wall”—chopping the gum into pieces results in many stuck pieces!).

**RESTORATION OF AIRWAY SURFACE HYDRATION WITH HYPERTONIC SALINE IS EFFICACIOUS IN CF, AND STRATEGIES TO HYDRATE AIRWAY SURFACES, OR DRUGS TO RE-DIRECT ION FLOWS, MAY BE SIMILARLY USEFUL IN COPD**

Theoretically, inhaled hypertonic saline (HS) will generate transient NaCl osmotic gradients on airway surfaces that will draw water from the submucosal space onto the airway surface. The maximal effect and durability of HS treatment will reflect the rate at which NaCl is passively and actively absorbed from airway surfaces. Two clinical trials of inhaled HS in CF subjects have been recently reported (56, 57). Donaldson and colleagues found that 2 wk of inhaled HS (7%, 4 times/day) accelerated particle clearance from CF lungs (Figure 12), which was associated with a small improvement in pulmonary function and improved quality of life (56). Companion studies of Elkins and coworkers reported that 7% HS twice daily for 1 yr produced similar small improvements in pulmonary function, but larger (60%) reductions in acute exacerbations and improvements in quality of life (57). No serious adverse events were reported in either study, including significant bronchoconstriction, protracted cough, acquisition of new bacterial organisms, worsening of bacterial densities, or elevated sputum markers of inflammation. Taken together, the data suggest that HS eliminated or reduced mucus plugs from relatively few airways, producing only modest changes in pulmonary function, but that HS accelerated the rate of mucus clearance in the relatively normal areas of the CF lung.

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**Figure 12.** Mucus clearance in patients with CF under baseline conditions and after inhalation of 7% hypertonic saline (HS). (A) Whole lung mucus clearance over 60 min. (B) Twenty-four-hour clearance without (open bar) or with (shaded bar) HS. The shaded area with dashed line represents 24 h clearance ± 1 SD in normal individuals (n = 12 per group). * Different from basal (P < 0.05). Adapted from Ref. 56, with permission.
Thus, when areas of the CF lung with preserved function were subjected to insults such as viral infection, which are predicted to reduce ATP and decrease mucus transport (19), HS would sustain mucus clearance, minimizing spread of bacterial infection.

In COPD, HS has been used as a tool to induce sputum. Pre-treatment regimens and safety criteria for patients with COPD challenged with HS for investigative purposes have been established (58–60), and HS appears to be safe for most patients with COPD, including those with an FEV1 below 50%. There is one report of the effect of HS on mucociliary clearance in COPD. Clarke and colleagues used HS as a control arm for an active drug regimen consisting of hypertonic Na-acetylcysteine (61). Both regimens increased mucus clearance and maintained equal efficacy over 3 d. Thus, the effects of hypertonic Na-acetylcysteine were precisely mimicked by 7% HS. This study only assessed the acute effects of HS on mucus clearance and did not measure sustained effects, which is important, because in vitro studies suggest that HS in normal airways will have a shorter duration of action than in CF airways (12). The difference between CF and non-CF exists because NaCl can diffuse rapidly down the imposed chemical gradient through open Na+ and Cl– channels in normal airway epithelial cells, whereas diffusion is slowed in CF cells due to the absence of an active Cl– channel. Thus, additional clinical trials using more sophisticated approaches toward increasing ASL volume in normal airways are needed.

The positive results of HS in CF provide a rational basis for more sophisticated approaches toward increasing ASL volume for longer periods of time in both CF and COPD. One option is the use of nonionic osmolytes such as mannitol or xylitol. However, a common failing of both ionic and nonionic osmolytes is their action is directly proportional to the mass of compound delivered to the airway surface. Given current aerosol technologies, and assuming a relatively even distribution of aerosol deposited on the airway surface. Given current aerosol technologies, and assuming a relatively even distribution of aerosol deposited on the airway surface, the mass delivered to the small airways is likely small (62). Because the distal airways are a key site of disease in both CF and COPD (9), it will be necessary to develop improved therapies to treat these areas. The use of osmolyte therapy to validate airway surface rehydration as a therapeutic modality should stimulate the development and application of more potent pharmacologic agents that can be delivered in doses that exceed the EC50/IC50 of target receptors and/or channels.

Summary and Conclusion

Mucus clearance is an essential innate immune protective mechanism in the airways. Carol B. Basbaum’s lifetime of work contributed greatly to our knowledge of the respiratory tract mucus clearance system and its pathobiology. Recent advances have increased our understanding of the structure and function of the mucus clearance apparatus, its homeostatic regulation in normal airways, and how both genetic and acquired diseases, principally by affecting airway surface hydration, disrupt its function to ultimately degrade lung function. Recent clinical trials indicate that aerosolized hypertonic saline helps to rehydrate airway surfaces in patients with CF, improving mucus clearance, decreasing acute exacerbations, and increasing quality of life. This new therapy is a palpable reward of the advances in basic science and points the way toward even better treatments.

Conflict of Interest Statement: Neither author has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank their many colleagues at the UNC CF Center and elsewhere for sharing thoughts and data cited in this review. Lisa Brown provided outstanding graphics and editorial assistance.

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