Cystic fibrosis: a disease of vulnerability to airway surface dehydration

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Cystic fibrosis (CF) lung disease involves chronic bacterial infection of retained airway secretions (mucus). Recent data suggest that CF lung disease pathogenesis reflects the vulnerability of airway surfaces to dehydration and collapse of mucus clearance. This predisposition is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, resulting in (i) the absence of CFTR-mediated Cl⁻ secretion and regulation of epithelial Na⁺ channel (ENaC) function; and (ii) the sole dependence on extracellular ATP to rebalance these ion transport processes through P₂ purinoceptor signaling. Recent clinical studies indicate that inhalation of hypertonic saline osmotically draws sufficient water onto CF airway surfaces to provide clinical benefit.

Difficulties in translating mutations in CFTR into pathogenesis in CF lung disease

Research into the molecular pathogenesis of the syndrome of cystic fibrosis (CF) has evolved rapidly over the past 15 years. The identification and cloning of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (see Glossary) protein was a seminal event in this evolution [1,2]. Subsequently, the molecular and cellular pathogenesis of the most common CFTR mutation, ΔF508 CFTR, and other common severe CFTR mutations, was demonstrated to result in a failure of the mutated CFTR to fold properly and progress through maturational processes within the cell [3–5]. Hence, the mutated CFTR protein is degraded prior to insertion into the plasma membrane.

However, despite these great strides, there has been less insight into how mutations in the CFTR protein produce disease in CF-affected organs. Perhaps most perplexing has been the relationship between mutant CFTR function and the disease phenotype of CF in the lung, which is manifest as a failure of innate airways defense against inhaled bacteria that produces chronic bacterial infection of CF airway lumens and, ultimately, airway obstruction, bronchiectasis and death [6] (Box 1).

CF lung pathogenesis has been proposed to reflect problems in cell biology. One hypothesis has focused on a role for mutated CFTR to generate an epithelial inflammatory syndrome [7]. However, such a hypothesis fails to account for the predisposition to infection, and studies of primary cultures of airway epithelia have failed to detect differences in inflammatory phenotypes between CF and normal airway epithelia [8,9]. A second cell biological hypothesis has focused on the role of wild-type CFTR to function as a receptor to promote epithelial clearance and killing of bacteria, and a failure of CF airway epithelia to do so in the context of a mutant CFTR [10]. However, there is little evidence for epithelial killing of bacteria in the normal lung, and no good explanation for why mutant CFTR proteins that appear in the apical membrane (e.g. G551D) produce a CF phenotype similar to that of mutations with no protein in the apical membrane (e.g. ΔF508 CFTR) (see earlier).

There have been three attempts to link mutations in CFTR to failures of lung defense through the ion transport functions of CFTR. In the first, a role for CFTR in controlling airway surface liquid (ASL) salt composition was proposed. In this hypothesis, it was suggested that the absence of CFTR led to a failure to absorb Cl⁻ (and Na⁺), producing a relatively hypertonic ASL, and degradation of defensin-mediated antimicrobial activity [11,12]. However, it seems implausible to ‘trust’ the defense of the lung to antimicrobial activities because, for example, problems of resistance would probably soon emerge. Furthermore, most recent studies have failed to detect differences in tonicity of ASL between normal and CF airways [13,14]. In the second hypothesis, the role of CFTR in airway submucosal gland secretion was emphasized [15–20]. Evidence suggests that CF submucosal glands are defective in secreting liquid in response to cAMP-regulated agonists (e.g. vasoactive intestinal peptide) but not acetylcholine receptor agonists. It is likely that defective gland function

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**Glossary**

- **Airway surface liquid (ASL)**: the water contained in the periciliary and mucus layers.
- **Cystic fibrosis transmembrane conductance regulator (CFTR)**: protein product of the CFTR gene that exhibits, as its name implies, Cl⁻ channel and ENaC regulatory properties.
- **Epithelial Na⁺ channel (ENaC)**: heteromultimeric Na⁺ channel composed of products of three or four separate genes, including α, β, γ and, possibly, δ ENaC.
- **Mucus layer**: layer composed of unrestrained secreted mucins in tangled networks interacting with globular protein ‘stickers’.
- **Periciliary liquid layer (PCL)**: a domain surrounding the cilium and cell surface that is a grafted polyelectrolyte gel, composed, in part, of tethered mucins; it is not a liquid layer.
contributes to disease pathogenesis in the large airways by reducing their ability to respond to insults with secretion of antimicrobial agents and a modest amount of liquid. However, submucosal gland dysfunction is unlikely to contribute to disease pathogenesis in the CF small airways, the initial and major site of disease [6,21]. The third hypothesis proposed that CF airways disease results from a failure of the mechanical clearance system that constitutes the primary innate defense against bacterial infections – that is, mucus clearance [22]. This failure reflects the inability of CF superficial airway epithelia to maintain the hydration of CF airway surfaces required for efficient mucus clearance due to abnormal regulation of Na⁺ and Cl⁻ transport (see later) [23]. Based on mounting evidence from in vitro cell culture data, mouse models and human clinical trials with hydration agents, reviewed later, this hypothesis is preferred by the author, and constitutes the basis for this review.

**Mechanical (mucus) clearance as the primary innate defense mechanism for airways**

As shown in Figure 1, the effective clearance of particles deposited on airway surfaces requires the coordinated activities of a two-phase gel system on the airway surface: (i) the periciliary layer that extends from the cell surface to the height of the extended cilium; and (ii) the mucus layer that is positioned atop the cilia [24]. The layer that surrounds the cilia was originally thought to be a liquid but is more likely to be a grafted polyanionic gel [25]. Intriguingly, the properties of this type of gel provide an ideal low-friction environment for effective ciliary beat, and also provide a lubricant activity that prevents adhesion of the mucus layer to the cell surface [26]. The mucus layer is composed of extremely long, highly glycosylated polymers known as the secreted mucins. A combination of the mucins MUC5AC and MUC5B, secreted predominantly in health, by goblet cells and submucosal glands, respectively, constitute this gel layer [27]. Biophysical studies suggest that the mucins are organized as a mesh, with a pore size optimal for trapping and retaining virtually any inhaled particle [28].

Recent evidence, primarily from mouse models, suggests that hydration of the two-phase ASL compartment is the primary determinant of the efficacy of mucus transport [29]. As shown in Figure 1, the hydration of this compartment is determined by the net activities of active ion transport systems. Normal airway epithelia have the capacity both to absorb and to secrete salt, with water moving osmotically in response to the generated salt gradients [30,31]. Under normal conditions, active Na⁺ transport is the dominant ion transport pathway. The dominance of this process reflects the large volume of ASL funneling up airways with converging surfaces that must be absorbed to maintain a thin film of ASL on airway surfaces. Active Na⁺ transport is mediated by a rate-limiting channel in the apical membrane, the epithelial Na⁺ channel (ENaC), and Na⁺ is extruded from the cell through the basolaterally located Na⁺/K⁺-ATPase. Under conditions when there might be too little ASL on the airway surface, the ENaC is inhibited, electrochemical driving forces for Cl⁻ secretion are generated, and Cl⁻ is secreted through two apical membrane Cl⁻ channels. It seems that the Cl⁻ channel that mediates the basal Cl⁻ channel activity of the airway epithelium is the CFTR Cl⁻ channel, whereas a second Cl⁻ channel, the calcium-activated Cl⁻ channel (CaCC), responds to stresses [32]. It should be noted that airway epithelia are relatively ‘leaky’, so the counter-ion for absorption (Cl⁻) or secretion (Na⁺) moves through the paracellular path.

The CFTR protein exerts a major influence on the capacity of the airways to maintain normal hydration, with CFTR functioning both as a Cl⁻ channel and as an inhibitor of the ENaC. Hence, as shown in Figure 1b, it is predicted that in CF airway epithelia, with an absence of either molecular or functional CFTR in the apical membrane, there will be unregulated Na⁺ absorption and a decreased capacity to secrete Cl⁻. Both defects combine to produce dehydration of the airway surface, with a collapse of the periciliary layer (PCL), concentration of mucins within the mucus layer, and adhesion of mucus to the airway surface [22]. It is predicted that the adhesion of mucus to airway surfaces initiates the airflow obstruction, inflammation and chronic infection that are features of CF. Thus, the simplest form of the ASL volume depletion and dehydration hypothesis involves unremitting and inappropriate absorption of ASL.

**Evidence for ASL volume depletion in CF**

Evidence emanating from three systems favoring the low volume and dehydration hypothesis can be summarized as follows. First, observations from air–liquid interface cultures maintained under static conditions have demonstrated that normal airway epithelia maintain adequate ASL volume on airway surfaces (defined as liquid height being equal to the extended cilium – i.e. ~7 μm) for extended periods of time, whereas CF cultures do not [22,32] (Figure 1c,d). Bioelectric studies revealed that the normal airway epithelia adjust the rates of Na⁺ and Cl⁻ secretion to maintain adequate salt, and hence water, on airway surfaces for normal hydration, whereas CF airway epithelial cells are locked into an unregulated Na⁺ absorptive mode, with little capacity to secrete Cl⁻ ions. These studies demonstrated that the failure to maintain adequate hydration on CF airway cell culture surfaces led to a complete failure of mucus transport and, ultimately, adhesion of mucus to cell surfaces [22,41].

Second, mouse models have provided important in vivo data. The normal mouse lung expresses little CFTR and, hence, mice with targeted disruptions of the murine CFTR gene (i.e. ‘CF mice’) exhibit little or no pulmonary disease phenotype [33]. Consequently, to test the importance of ASL dehydration in the pathogenesis of airways disease,
Mall et al. [29] engineered murine airways to accelerate Na\(^+\) absorption. Transgenic overexpression of a single ENaC subunit, the \(\beta\)ENaC subunit, produced an approximate 2.5-fold increase in Na\(^+\) absorption; a depletion of ASL, as measured morphometrically in the PCL and physicochemically in the mucus layer (percentage solids content of the mucus layer); and mucus obstruction. Indeed, the mucus obstruction was so severe that \(\sim 50\%\) of the transgenic mice died owing to asphyxia within the first 30 days of life. An unexpected but important result was that the failure to clear mucus was associated with a sterile, neutrophilic inflammation mediated by the
production of proinflammatory cytokines. Finally, these mice had difficulty in clearing bacteria, and recent studies have shown that they exhibited a robust inflammatory response to respiratory viruses. Thus, these studies have strongly suggested that ASL depletion can produce major features of CF lung disease in vivo.

Third, evidence for ASL volume depletion and dehydration emanates from several types of human clinical studies. Perhaps the first data indicating the importance of dehydration and mucus plugging were reported by Zuelzer and Newton [21] in the 1940s, in infants who had died from meconium ileus. These pathologists demonstrated that the earliest lesions in the CF lung, as early as 2–4 days after birth, were ‘bland’ mucus plugs in the bronchioles. Subsequent experiments in the late 1960s from Matthew et al. [34] and Potter et al. [35,36] revealed that the water content of CF secretions, expressed as percentage solids, was reduced as compared with disease controls and normal subjects. Although the subject of much debate, studies from the 1990s indicated that this dehydration is an isotonic dehydration — that is, CF and normal airway secretions both have a concentration of NaCl that is approximately isotonic [13,14,37].

The collective data indicating that CF airways might be dehydrated led to therapeutic trials with aerosolized hypertonic saline (HS) [38,39]. As discussed in detail later, the concept tested was that the inhalation of solutions containing high concentrations of salt (~7%) would produce transient hypertonicity on airway surfaces (note: plasma and interstitial salt concentrations are ~0.9%) to ‘rehydrate’ these surfaces osmotically. In brief, short-term (two-week) inhalation of HS four times daily increased mucus transport both acutely and chronically, and this response correlated with improvements in lung function and quality of life [38]. In parallel, long-term studies of HS in Australia revealed that there was a sustained improvement in lung function in CF patients on HS therapy (7%, twice daily) as compared with vehicle, and, importantly, there was a major reduction in infectious exacerbations in HS-treated subjects [39]. These data suggest that rehydration of CF airway surfaces is indeed beneficial, consistent with the in vitro culture and mouse model data supporting the low volume and dehydration hypothesis.

‘Key caveats’ to the notion of Na+ hyperabsorption and failed Cl− secretion in the pathogenesis of CF airways dehydration

Recent studies have suggested that the notion of simple, persistent Na+ hyperabsorption, coupled with failure to secrete Cl−, is an oversimplified version of what might happen in the CF patient [32]. Indeed, these studies were stimulated by predictions from in vitro studies (see earlier) that CF airways should rapidly and homogeneously fill with a mucus gel soon after birth, in contrast to the clinical data that demonstrated that CF airways disease becomes manifest many months to years after birth [6,22,40]. Study of this apparent incongruity led to two major modifications of the simple volume hyperabsorption hypothesis.

First, it seems that CF airways disease reflects a vulnerability to collapse of ASL volume in response to exogenous stresses (e.g. viral infections or aspiration). The data that led to this concept emerged from studies in which the normal shear stresses that occur during tidal breathing in vivo were applied to the cell culture preparations described earlier [32,41]. These studies revealed the importance of signaling molecules within the ASL itself in controlling ASL volume [42]. Although this signaling system is not understood in its totality, it seems that two extracellular signaling molecules, ATP and adenosine (ADO), are vital for determining ASL volume and hydration in a state optimal for normal host defense. As shown in Figure 2, normal airway epithelia release ATP into the luminal compartment by both a constitutive (probably vesicular mediated) and a shear stress-regulated process. ATP released into the luminal compartment has two important fates. (i) ATP interacts with luminal P2Y2 purinoceptors that mediate inhibition of the ENaC and activation of both the CFTR and CaCC [43]. (ii) ATP is metabolized by cell surface ectoenzymes to produce ADO [44]. ADO interacts with luminally positioned A2a receptors that, through cAMP-dependent mechanisms, activate CFTR, and, by an as-yet-unknown mechanism, induce CFTR to inhibit the ENaC [45]. The volume of ASL seems to be regulated by the magnitude of shear stress-induced ATP release, and subsequent studies have shown that both ATP and ADO are important for supporting the physiologic volume of ASL on normal airway surfaces.

By contrast, CF airway epithelia fail to express a CFTR protein, whether functional or otherwise, at the apical membrane (Figure 2b). CF airway epithelia release ‘normal’ amounts of ATP in response to shear stress, and the released ATP effectively regulates the ENaC and the CaCC through P2Y2 receptor signaling [41,42,46,47]. However, in contrast to normal airway epithelia, luminally formed ADO does not activate Cl− secretion and inhibit the ENaC because of the absence of the target of A2b signaling (i.e. CFTR). Thus, CF airways epithelia are vulnerable in two respects from an ASL volume and hydration perspective: (i) they are predicted to have an ASL volume that is less than normal but probably sufficient for mucus transport in times of health; and (ii) they depend solely on ATP for the production of sufficient ASL to maintain mucus transport.

A mechanism by which an exogenous insult can lead to collapse of CF mucus transport defenses in response to a reduction in ATP levels in the ASL compartment has recently been described [32]. As shown in Figure 3, an early manifestation of respiratory syncytial virus (RSV) airways infection is an upregulation of a cell surface ectoenzyme that metabolizes ATP and reduces ASL ATP concentrations. Lesser P2Y2 receptor activation reduces ENaC inhibition and stimulation of Cl− secretion, the net effect being ASL volume depletion and collapse of mucus transport. Thus, the ASL low volume hypothesis predicts a sensitivity to viral respiratory infections, consistent with the observations that CF patients have a more severe clinical response to RSV infections than do normal individuals [48].

Second, it is important to emphasize that CF lung disease is heterogeneous — that is, the CF phenotype does not appear and progress homogeneously throughout the lung. Perhaps the first evidence of this notion emanated from pathology studies that showed that the upper lobes of
CF subjects are typically first affected, whereas the lower lobes could be normal [49]. More recently, CT scanning studies of young infants have also shown a predilection for early bronchiectasis in the upper lobes of the lung, with relatively normal structures in the lower lobes [50]. Clinically, it has long been known that CF exacerbations are often associated with ‘new clinical findings’ in circumscribed areas of the lung, suggesting that progression is not uniform but heterogeneous. Finally, the recent mucociliary clearance data from Donaldson et al. [38] support the notion that mucus clearance in the CF lung is also heterogeneous (Figure 4).

There are two interesting speculations with regard to the heterogeneity of CF lung disease. First, there is the simple question of why this happens, given the fact that the CF lung is homogeneously deficient in CFTR function. One speculation is that with normal tidal breathing, shear stresses are greater in the lower lobes, where, by virtue of gravitational forces, more than two-thirds of the normal tidal ventilation occurs [32]. Thus, it is predicted that there is more shear stress in the lower lobes and hence more ATP release, producing more P2Y2-mediated inhibition of Na+ absorption and induction of CaCC-mediated Cl−/C0 secretion, and a relative increase in ASL volume. The
second speculation is that it might be possible to arrest the progression of CF lung disease if the stimuli that drive the progression are identified and if effective therapies can be appropriately delivered to these regions (see later).

Thus, it seems that the inclusion of compensatory mechanisms to regulate the balance of Na\(^+\) absorption and Cl\(^-\)/HCO\(_3^-\) secretion – that is, extracellular ATP signaling (Figures 2, 3) – provides a more accurate description of the pathogenesis of CF lung disease than the simpler model depicted in Figure 1.

**Events ‘downstream’ of ASL volume depletion in CF airways pathogenesis**

What is unique about the CF bacterial bronchitis phenotype is the persistence of the bacterial infection. For example, CF patients can be infected virtually life-long with...
the original *Pseudomonas* organism that colonizes and infects their lungs [51]. The acquisition of this persistent infection and the responses to it are complex but many aspects seem to be congruent with the dehydrated, adherent mucus plaques predicted by the low volume hypothesis.

One reason for CF airways infections being so persistent is that bacteria grow in biofilm-type physiologies and morphologies [52,53]. Bacteria growing as biofilms are difficult to eradicate by secondary host defense and by antimicrobial agents [54]. Recent studies suggest that *Pseudomonas* interacts with the dehydrated mucus of CF airways in a manner that favors biofilm formation [28]. A normally hydrated mucus layer exhibits a mucin-dependent mesh size of ~0.5–10.0 μm in diameter. Bacteria deposited on a normally hydrated mucus gel (~2% solids) can ‘swim’ through the gel and remain in a planktonic state within it. By contrast, with depletion of salt and water, the mucin-dependent meshwork in a CF mucus gel becomes ‘tight’ (mesh size of <100 nm). The small mesh size restricts the mobility of *Pseudomonas* that deposit on the surface of CF mucus, resulting in the bacteria replicating at the site of deposition faster than they migrate from it. This phenomenon leads to accumulation of bacteria and a high local production of autoinducers that produce biofilm formation [55,56]. This motility restriction, coupled with a reduced permeation of autoinducers from the site of their production through concentrated mucus, triggers biofilm formation in CF, but not normal, mucus.

Airways have secondary defense mechanisms against bacteria that are not normally cleared by mucus clearance. These defenses include soluble antimicrobial agents (e.g. lactoferrin and lysozyme) and neutrophils that can migrate into the airway lumen and capture and kill bacteria. Recent studies have shown that the concentration of mucus that occurs in CF airways reduces the ability of lactoferrin and lysozyme to permeate this environment to inhibit bacterial growth [28]. Similarly, the reduction in the mesh size of the mucin network in concentrated CF mucus limits the ability of neutrophils to penetrate mucus and subsequently capture and kill bacteria [57].

An interesting and important consequence of persistent intraluminal bacterial infection is the host inflammatory response. Again, the unusual characteristic of CF is the persistence of this bacterial stimulus, with patients having >10⁶ microorganisms/ml of intraluminal mucopurulent material in some airways for the majority of their lives. Recent data suggest that the persistent intraluminal bacterial infection upregulates the normal host response to luminal bacterial pathogens [9,58]. One response is to increase the production of proteins that have antimicrobial and anti-inflammatory properties, in addition to proteins involved in the repair of bacterial- and inflammation-induced cellular damage. This increased protein synthetic response initiates a form of the ‘unfolded protein response’, which is associated with expansion of the endoplasmic reticulum (ER) compartment and increased capacity to fold and synthesize proteins [59–61]. One consequence of ER expansion is to increase the inositol trisphosphate-releasable Ca²⁺ stores. Thus, Ribeiro et al. [58] have reported that freshly excised CF cells, or indeed normal cells chronically exposed to CF airway luminal mucopurulent material, exhibit increased Ca²⁺-dependent interleukin-8 release in response to inflammatory mediators that signal through Ca²⁺-dependent processes (e.g. bradykinin). At one level, this amplified inflammatory response might be beneficial to the host, particularly if the host has the capacity to clear the offending agent. However, in CF, where the bacteria persist within the immobilized mucopurulent material and mucus plaques, the amplified inflammatory response might damage airway walls, producing the bronchiectasis typical of CF. Unresolved are questions of whether there are other mechanisms that produce hyperinflammatory responses, and, indeed, if CF airway epithelia are intrinsically hyper-inflammatory consequent to CFTR defects [62–64].

Thus, it seems that many of the heretofore perplexing problems of infection and inflammation in CF pathogenesis can be explained by interactions of infectious agents and host inflammatory processes with the abnormally thick, adherent mucus that characterizes the CF airways.

Therapies directed at airway surface rehydration in CF

Mammalian airway surfaces are relatively permeable to water [65]. Thus, rehydration therapies require the addition of salt to airway surfaces, to draw water onto airway surfaces osmotically. In general, there are two ways to ‘add salt’ to CF airway surfaces. The first is for patients to inhale HS (see earlier). The second is to administer agents to the CF airway surface that will redirect ion transport towards the secretory direction.

As described earlier, HS seems to have a therapeutic benefit over the short term (pulmonary function) and over the long term (reduction in exacerbations) in CF patients [39,66–68]. Interestingly, HS might be more therapeutically beneficial in CF than in normal airways (Figure 5). In normal subjects, when HS is deposited on airway surfaces, a transepithelial chemical gradient for Na⁺ and Cl⁻ is generated that can be dissipated through movement of Na⁺ through the ENaC and Cl⁻ through the CFTR channel. Indeed, there is a ‘kinetic horse race’ between NaCl absorption and water transport in the direction of the osmotic (salt) gradient – that is, towards the lumen. In the normal airway, NaCl is absorbed sufficiently rapidly that osmotically driven water transport to the airway surface is modest. By contrast, when HS is deposited on CF airway surfaces, Na⁺ can theoretically permeate the epithelium through the open ENaC and Cl⁻ through the CFTR channel. Nevertheless, it seems that HS increases the production of proteins that have antimicrobial and anti-inflammatory properties, in addition to proteins involved in the repair of bacterial- and inflammation-induced cellular damage. This increased protein synthetic response initiates a form of the ‘unfolded protein response’, which is associated with expansion of the endoplasmic reticulum (ER) compartment and increased capacity to fold and synthesize proteins [59–61]. One consequence of ER expansion is to increase the inositol trisphosphate-releasable Ca²⁺ stores. Thus, Ribeiro et al. [58] have reported that freshly excised CF cells, or indeed normal cells chronically exposed to CF airway luminal mucopurulent material, exhibit increased Ca²⁺-dependent interleukin-8 release in response to inflammatory mediators that signal through Ca²⁺-dependent processes (e.g. bradykinin). At one level, this amplified inflammatory response might be beneficial to the host, particularly if the host has the capacity to clear the offending agent. However, in CF, where the bacteria persist within the immobilized mucopurulent material and mucus plaques, the amplified inflammatory response might damage airway walls, producing the bronchiectasis typical of CF. Unresolved are questions of whether there are other mechanisms that produce hyperinflammatory responses, and, indeed, if CF airway epithelia are intrinsically hyper-inflammatory consequent to CFTR defects [62–64].

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activating Cl⁻ channels in the absence of inhibiting the ENaC might paradoxically increase absorption rather than induce secretion. Alternatively, there are agents that selectively block the ENaC to conserve liquid on airway surfaces and perhaps generate driving forces for CaCC-mediated Cl⁻ secretion. These agents include small-molecule open channel ENaC blockers (e.g. PS552) and agents that inhibit the cell surface proteases that activate the ENaC when it appears on the cell surface (e.g. Aerovance 152) [70]. Finally, there are agents that both inhibit ENaC and initiate Cl⁻ secretion. The most advanced of these compounds is INS37217, a metabolically stabilized UTP derivative that

**Figure 5.** Effects of inhalation of HS (1000 mM NaCl, 7%) on ASL volume in normal and CF airway epithelia. (a) Deposition of droplets containing 7% HS on a normal airway (mucus) surface that is isotonic (~150 mM NaCl). Note that the interstitium is, by definition, isotonic (0.9%). (b–d) Normal airway epithelia. (b) The NaCl chemical gradient generated by deposition of 7% HS is rapidly dissipated by transepithelial absorption through the basally activated ENaC and CFTR channels. Water moves towards the airway surface from the interstitium in response to an osmotic gradient generated by HS deposition. (c) Modest expansion of the ASL volume that is ‘stored’ in the expanded mucus layer reflects rapid absorption of NaCl and, hence, a relatively smaller gradient for H₂O permeation. (d) The added ASL is gradually reabsorbed by active Na⁺ transport. (e–g) CF airway epithelium. (e) The NaCl chemical gradient generated by HS deposition is not dissipated quickly because Cl⁻ cannot permeate rapidly through the relatively Cl⁻-impermeable CF cell. Hence, the HS-generated osmotic (NaCl) gradient persists for a larger interval than on normal airway surfaces, and, hence, a larger amount of water permeates to the apical surface. (f) The large water flow to the airway surface produces a larger increase in ASL volume on CF than on normal airway surfaces; compare this with (c). (g) The added ASL is absorbed by active Na⁺ transport but the greater initial volume expansion produces a larger ASL volume expansion at t = 60 than normal [compare height of mucus layer in (d) with that in (g)].
interacts with the P2Y_2 receptor to inhibit Na^+ absorption and initiate CaCC-mediated Cl^- secretion [71]. Mol. 1901, A152 and PS552 are currently in Phase II testing, and INS37217 is currently in Phase III testing. Of note, it might be most rational to combine molecules that have ENaC-blocking activity with those that have Cl^-secretory activity or HS, to hydrate airway surfaces maximally.

**Concluding remarks**

Data derived from multiple experimental systems suggest that CF airways are vulnerable to dehydration-induced loss of mechanical (mucus) clearance of airway surfaces. The dehydration that characterizes CF airway surfaces reflects the inability to regulate Na^+ and Cl^- transport coordinately owing to the absence of the CFTR function in the apical membrane of airway epithelia. New directions of research to understand this proximate component of CF airways disease pathogenesis should involve gaining a physicochemical understanding of the competition for water between the grafted polyanionic gel constituting the PCL, and the unrestrained mucin gel constituting the mucus layer. A corollary is to elucidate the mechanisms by which mucus adheres to airway surfaces when water is limited – for example, in CF. New research is also required to translate these concepts into new therapies. A major hurdle is to develop sensitive and reproducible assays that measure the hydration of airway surfaces. Technologies using exhaled breath condensates, measuring the ratio of protein biomarkers to urea, might be feasible. Finally, although hypertonic saline seems to be surprisingly effective at treating CF lung disease, its effectiveness in treating small airways is at best modest, and it produces only a ~40–60% reduction in exacerbation frequencies. Thus, strategies must be developed to improve the hydrating capacity of inhaled therapies and increase their durability. In parallel, it will be useful to identify approaches to detach mucus from airway surfaces (e.g. through inhaled detergents) to rescue lung function, in addition to protecting it. The next few years will see intensive efforts to optimize rehydration therapies to develop the most effective and convenient forms of what might be chronic but potentially ‘curative’ therapies for CF lung disease.

**References**

3 Cheng, S.H. et al. (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827–834.
57 Matsui, H. et al. (2005) Reduced 3-dimensional motility in dehydrated airway mucus prevents neutrophil capture and killing bacteria on airway epithelial surfaces. J. Immunol. 175, 1090–1099

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