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Cystic Fibrosis: The Mechanisms of Pathogenesis of an Inherited Lung Disorder

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Abstract

Cystic fibrosis patients exhibit lung disease consistent with a failure of innate airway defense mechanisms. The link between abnormal ion transport and disease initiation and progression is not fully understood, but airway mucus dehydration seems paramount in the initiation of CF lung disease. New therapies are currently in development that target the ion transport defects in CF with the intention of rehydrating airway surfaces.

Introduction

There are more than 30,000 Americans with cystic fibrosis (CF), the most common autosomal recessive genetic disorder of Caucasians. CF patients born in 1970 had only a 50% chance of reaching age 15, but in little more than 2 decades the chances of reaching that age had increased to 95% [1]. An increased understanding of normal airway physiology and the pathophysiology of CF has spawned new treatments that have driven this impressive increase in patient survival.

CF lung disease is characterized by persistent bacterial infection that is usually acquired in childhood and maintained throughout the patient's life. The infection is usually restricted to the airway lumen and persists despite intensive antimicrobial therapies. Clinical data suggests that the disease is typically initiated following viral infection or aspiration [2] and that throughout adult life the disease progresses with discrete acute exacerbations, also potentially linked to viral infection [3]. This review will focus on the mechanisms thought to produce CF lung disease and the novel agents that are in development as specific therapies.

Airway hydration

The *CFTR* gene ([NM 000492](#)) and mutations identified with the syndrome of CF were identified in 1989 [4]. Within two years, the cDNA was cloned and the protein identified as a plasma membrane bound cyclic-AMP sensitive anion channel [5]. Subsequent experiments suggested that CFTR regulated other ion channels, *e.g.*, ENaC [6], consistent with its name as the CF transmembrane conductance regulator. The $\Delta F508$ mutation, the deletion of phenylalanine at position 508 of the polypeptide, is present in 70% of CF chromosomes, although more than 1000 other mutations have been documented in the CF gene

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(www.genet.sickkids.on.ca/cftr). The $\Delta F508$ mutation results in abnormal biogenesis and premature degradation of CFTR protein by the cells quality control system and, as a result, there is a paucity/absence of CFTR in the apical membrane of CF epithelial cells [7]. This results in a decreased permeability for chloride ions across CF epithelia [8]. Research has focused, therefore, on how defective ion transport can result in a disease that is exemplified by a defective innate defense system.

Initial studies suggested that the absence of CFTR led to a change in the composition of the airway surface liquid (ASL), namely an increase in $[NaCl]$. The 'high salt' hypothesis postulated that this increased salt concentration inhibited the actions of a naturally secreted, salt-sensitive, antimicrobial protein (hBD-1), leaving CF epithelia susceptible to infection [9, 10]. In support of this, recent studies have demonstrated a difference in the osmolarity between CF and normal ASL, although in both of these cases CF ASL was found to be isotonic or hypotonic overall, rather than hypertonic [11, 12].

In contrast, an alternative hypothesis suggested that CF ASL is isotonic but has a low volume. CFTR was suggested to coordinate the rate limiting steps for both secretion and absorption, namely Cl^- secretion by CFTR, and Na^+ absorption by the epithelial sodium channel (ENaC). The 'low volume' hypothesis suggested that the absence of CFTR meant that the total mass of $NaCl$ in CF ASL was reduced, and that the osmotic force for water transport to the lumen was lessened. As a result, the airway surface became dehydrated and mucociliary clearance (MCC) retarded. In support of this hypothesis, data have emerged that normal and CF ASL is not compositionally different in Na^+ and Cl^- concentrations, *i.e.*, both are isotonic [13], and that the ASL volume/depth of CF epithelia *in vitro* is much lower than normal (3-4 μm vs. $\sim 7 \mu m$ in normal [14,15,16]. Over the past decade an accumulation of evidence from cultured human bronchial cells, mouse models and human clinical studies have supported the low volume hypothesis. One important prediction of this hypothesis is that increasing the mass of salt on the epithelial surface would increase the osmotic driving force for water to enter the luminal space, and this effect would be therapeutically beneficial (see later section).

The key mechanisms in the development of CF lung disease are shown in Figure 1. In normal airway epithelia, CFTR-mediated Cl^- secretion and ENaC mediated Na^+ absorption are coordinated to maintain ASL at a depth that allows cilia to beat effectively and move the mucus blanket across the surface of the epithelium. The absence of CFTR protein and/or function in CF airway epithelium produces two defects in this process. First, the reduced permeability of CF epithelia to Cl^- results in reduced Cl^- secretion and hence less mass of salt on the apical surface. The result is a reduced driving force for water to enter the lumen. Secondly in the absence of CFTR, ENaC activity is unchecked, resulting in an unregulated and inappropriate absorption of salt from ASL. The overlying mucus blanket, consequently, becomes dehydrated and compresses the cilia, resulting in the arrest of MCC.

Interestingly it was widely believed that if abnormalities of ion transport produced the opposite effect, *i.e.*, increased salt mass on airway surfaces, the ASL depth would increase above the height of the cilia, the mucus would 'float off' from the ciliary tips, and MCC would decline. However, a deeper understanding of the properties of mucus and the disease type I pseudohypoaldosteronism (PHA) have changed that perception. PHA patients have defective ENaC function and, as a result, fail to absorb fluid from the ASL. These patients display the predicted excessively 'wet' airway surfaces. However, rather than reduced MCC, they display a marked increase in lung clearance, $\sim 4x$ faster than normal [17]. This condition has been modeled in cultured systems where addition of ASL volume leads to an increase in the velocity of mucus transport [18]. In the cultured system, it was shown that the mucus blanket expands with additional hydration, taking up water like a sponge, and as a result the height of the mucus layer increased. Crucially, the mucus blanket kept contact with the cilia, and this 'looser'

expanded mucus was more readily moved by the beating cilia. The regulation of the height/volume of ASL has become central to understanding how airways maintain a hydrated and healthy mucus at the apical surface.

How is ASL height regulated?

One of the first steps in investigating how CFTR may regulate ASL is to examine where CFTR is expressed in the airways. Surprisingly, there is still controversy over where CFTR is predominantly expressed. Initial studies demonstrated that CFTR immunoreactivity and mRNA was found predominantly in the serous cells of the sub-mucosal glands [19]. This led to the assumption that sub-mucosal glands were the primary structure responsible for ASL secretion and that CF lung disease was essentially a disease of the submucosal glands. This would be important since drug delivery for correction/potential of CFTR in mucus plugged submucosal glands would be restricted to systemically active drugs. However, a more recent study employing high affinity monoclonal CFTR antibodies has suggested that the ciliated cells of the superficial epithelium are the primary site for CFTR expression [20]. In this study, CFTR was consistently observed (immunoreactivity, in situ hybridization and functionally) in the ciliated cells of the surface epithelium from, the nasal epithelium to the proximal bronchioles. CFTR was also consistently expressed in the ciliated duct cells of the submucosal glands, however, less consistently (6 out of 26 subjects) in the acinar cells. These data suggest that CFTR expression is predominant in the ciliated cells of the superficial epithelium. In terms of ASL, it is evident that secretion from both the surface epithelium and the submucosal glands contribute to its volume but the surface epithelium, representing the largest surface area in close contact with the ASL, must be the main site for modification of ASL volume.

Measurement of ASL regulation *in vivo* is almost impossible due to the very shallow depth of ASL (~25 μm) and the 3 dimensional architecture of the airways. However, cultured human bronchial cells (HBCs) and animal models have allowed insight into the regulation of ASL volume. Using confocal microscopy to examine ASL height of cultured HBCs it was demonstrated that cells maintain an ASL height of ~7 μm , under static culture conditions, allowing full extension of the cilia during beating and mucus flow over the culture surfaces [21,14] (see Figure 2). Addition or removal of isotonic fluid to the ASL is sensed, and the cells restore ASL height, over a period of hours, back to ~7 μm .

How changes in ASL are sensed is not fully known. However, the secretion of soluble mediators, by the cells themselves, is known to play a role. In normal airway epithelia, adenosine in the ASL is known to regulate ASL height *via* apically located adenosine receptors (likely A2b). For example, inclusion of an adenosine receptor blocker (8-SPT) in ASL results in a much diminished ASL height, inconsistent with MCC [22]. Examination of CF cultures shows that ASL is reduced compared to normal (~3 μm vs. ~7 μm) under static conditions. However, maintaining CF cells under phasic motion/shear stress conditions (a dynamic force designed to mimic the forces experienced by the epithelium during tidal breathing) resulted in an increase in CF ASL height and restoration of MCC [23]. Since ATP is known to be released by cells in response to mechanical stress [24], ASL [ATP] was measured under shear stress conditions and found to increase over static levels. Next, CF ASL height during shear stress was measured under conditions in which ATP concentrations were reduced (*i.e.*, inclusion of the enzyme apyrase in ASL to drive ATP $\rightarrow$ AMP reaction). Under such conditions, ASL height declined and MCC stopped, suggesting that ATP is a second soluble mediator present in ASL that regulates ASL height. ATP-mediated ASL secretion in CF is modulated both by ENaC inhibition and activation of a calcium activated chloride channel (CaCC), an alternative secretory pathway that represents another therapeutic target. CaCC has been reported in native tissues [25], note that in normal airway epithelia ATP regulates ASL secretion *via* both CaCC and CFTR chloride channels. The persistent effectiveness of shear stress ATP signaling in CF

may explain why CF subjects can do relatively well for long intervals. Indeed, this mechanically induced secretion may help explain why percussion of the chest wall is effective in CF therapy, where physiotherapy or high frequency oscillating vests are used to stimulate mucus expectoration.

In addition, soluble mediators may regulate Na^+ absorption *via* ENaC in airway epithelial cells. A recent wealth of data suggest that ENaC is activated by serine proteases [26] present on the apical membrane of airway epithelial cells. Furthermore, ENaC activity is regulated by the interaction of soluble protease inhibitors, released by the cell into the ASL, and the membrane bound proteases expressed at the cell surface (for a comprehensive review of this emerging field, see [27]). Soluble mediators, then, are known to play an important part in the regulation of ASL volume, by controlling secretory ion transport *via* CFTR and CaCC, and the reabsorptive pathway, *i.e.*, ENaC.

Understanding how ASL is regulated, whilst giving important insights into normal airway function, still does not fully explain how an ion transport defect results in a failure of innate lung defense. However, a number of studies have demonstrated how ion transport may result in poor mucus clearance. Disruption of anion secretion, either by blocking basolateral anion uptake or blocking apical anion secretion, in pig bronchi resulted in mucus plugs in the submucosal ducts [28] and a thickened mucus that collapsed the cilia in the airway lumen [29]. However, one of the most important studies linking defective ion transport to a failure of innate lung defense was the development of the Scnn-1b (ENaC) transgenic mouse in which the β subunit of ENaC was over-expressed in airway epithelia with a clara cell specific promoter [30]. The Scnn-1b mouse displays lower ASL, with collapsed cilia and adhesive mucus plugs in the upper and lower airways. In addition, the mucus plugs display elevated levels of macrophages and neutrophils, and lung lavage has increased cytokine concentrations, suggesting that inflammation is initiated in the absence of bacterial infection. This important study reveals that CF-like lung disease can be initiated by inducing hyperabsorption alone and suggests that airway dehydration is the initiating factor in the development of CF-like lung disease.

Mucociliary Clearance

Airway hydration is crucial to maintain healthy flowing mucus. Mucus represents the main barrier between the airway epithelium and the external environment and is, thus, the primary defense mechanism of the airways. However, little is known about the substance, structure, development, and maintenance of this primary barrier. Traditionally, models have depicted the mucus layer as a discrete blanket that sits atop the cilia, whilst the cilia beat within a watery layer termed the sol or peri-ciliary liquid (PCL). More recent work suggests that the ASL consists of two mucin gels, with different types of mucins playing different roles in the lower phase (PCL) or upper phase (mucus blanket). Salt and water are the solvents of both gels, and indeed, each layer may compete for solvent. EM microscopy reveals that there is an organized structure within the periciliary space likely consisting, in part, of tethered mucins. The overlying mucus layer is a tangled polymer gel formed predominantly by the secreted high molecular weight mucins MUC5AC and MUC5B, emanating from the goblet cells and submucosal glands respectively. The viscoelastic properties of this upper gel phase are determined by the mucins themselves, other constituent macromolecules *e.g.*, globulins, proteins and DNA, and importantly the level of hydration of the gel.

A healthy mucus layer consists of around 1% salt, 1% mucins and 98% water [31], it behaves rheologically predominantly like a viscous liquid with a small elastic component [30]. This is important since the mucus layer must display liquid-like behavior to spread over the airway once it is secreted, but also some elastic behavior, so that the kinetic energy from ciliary beating

is translated into movement. In contrast, the >8% mucus characteristic of CF patients displays highly elastic behavior and shear thickening [31]. Shear thickening describes a property of the mucus that results in thickening of the mucus as force is applied to it, suggesting that ciliary beating may further solidify the CF mucus. Importantly, CF mucus is adhesive and the combination of low viscosity, high elasticity and high adhesivity makes CF mucus especially hard to clear, even during cough [32]. It is this property of CF mucus that leads to mucus stasis and adhesion, which can be viewed as the initiating lesions in CF airway disease pathogenesis.

Polymer gels, like mucus, form a mesh-like net. The pore size of this mesh is important, since it defines the permeation of different sized particles. Normally hydrated mucus has a pore size of around 0.2-1 μm , whereas in dehydrated CF-like 8% mucus, the pore size is not fully resolved but is <100 nm. The decreased pore size of CF mucus may explain why the secondary defense function of neutrophils is defective in CF, *i.e.*, neutrophils cannot penetrate CF mucus to capture and kill bacteria [30].

The reduced mesh size of CF mucus may also have important effects on bacterial infection. *P. aeruginosa* forms macrocolonies in CF secretions, and these macrocolonies, or 'biofilms', are resistant to antimicrobial agents since they form a dense encasing matrix [33]. Macrocolonies of *P. aeruginosa* form readily in 8% mucus but not normal mucus, likely reflecting reduced bacterial motility in the dense net of 8% mucus (see Figure 3).

Another component of innate lung defense are soluble antimicrobial substances. Lactoferrin, a secreted and abundant component of ASL [34] is a major such substance on airway surfaces. *P. aeruginosa* in abiotic flow chambers display increased migration in the presence of lactoferrin, a mechanism that has been postulated to prevent macrocolony formation. However, inclusion of lactoferrin in *P. aeruginosa* infected 8% mucus did not reduce macrocolony formation, suggesting that even though lactoferrin may increase bacterial twitch response, that motility is still limited by mucus mesh size. Thus, macrocolonies can still form in dehydrated mucus in the presence of lactoferrin [35]. Thickened mucus coupled with lactoferrins cleavage and reduced activity in CF patients [36, 37] may together severely limit the airways natural host defense mechanisms.

A deeper understanding, then, of the properties of the formation and maintenance of mucus at the airway surface should help in the fight against CF lung disease, and indeed many therapies are aimed at improving the viscoelastic properties of CF mucus, either by rehydration or by enzymatic modification of mucus components. The maintenance of properly hydrated mucus is paramount in providing the correct rheological properties for efficient mucus clearance.

As stated earlier, one prediction of the low salt hypothesis is that increasing the mass of salt in ASL will provide an osmotic gradient for the entry of water to the airway lumen, rehydrating mucus and potentially allowing more MCC. Recent clinical data has provided an insight into how the simple act of rehydrating mucus can increase MCC and, even in the short term, lead to improved lung function. CF patients treated with nebulized hypertonic saline (HS 7%, 5 ml 4 times per day) showed increased rates of mucus clearance and an improvement in lung function [38] (see Figure 4). Longer term studies in which CF patients were treated with HS twice a day for up to 1 year suggest that, whilst the increase in lung function is modest, there was a 50% reduction in the CF patients' exacerbations [39]. The disparity in the modest increase in lung function but large reduction in exacerbations may reflect the heterogenous nature of CF lung disease. In totally blocked airways HS probably fails to make much difference since salt deposition will be limited/absent. In less affected areas of lung, HS probably corrects MCC to a level where further viral insult/aspiration can be effectively countered by the lungs innate defense mechanisms. These clinical studies demonstrate further that hydration status of airway mucus is key to understanding the mechanisms of CF lung disease.

New Therapies to Correct Airway Hydration

Figure 5 and Table 1 summarize the main channels and receptors present in airway epithelium that represent potential therapeutic targets for the treatment of CF. Because CF is characterized by an absence of the CFTR protein at the apical membrane, ‘corrector’ drugs are currently in development which aim to promote the delivery of $\Delta F508$ protein to the cell surface. Since $\Delta F508$ CFTR is known to be active as a cAMP-regulated chloride channel [40], the aim is to direct mutated CFTR to the apical membrane to partially restore ion transport. Another class of drugs in development are termed ‘potentiators’, which are designed to increase the function of mutated CFTR in the apical membrane. As well as targeting $\Delta F508$, these compounds have been demonstrated to increase the activity of at least one other common CFTR mutation, namely G551D [41].

Since airway dehydration results from both a paucity of secretion and inappropriate absorption, a series of drugs that target both pathways are in development. Drugs that reduce absorption, *e.g.*, Parion 552-02, coupled with residual ATP-mediated secretion may help re-instate some MCC themselves. Whilst inhibition of absorption alone may restore MCC, the use of these drugs in concert with HS or activation of alternate secretory pathways, *i.e.*, CaCC, would perhaps further increase MCC. Indeed drugs targeting the activation of the CaCC pathway are already in phase II clinical trials (Moli 1901). Alternatively, drugs that both increase secretion and inhibit absorption across airway epithelium are being developed. INS37217 a stabilized nucleotide which mimics the effect of shear stress released ATP is in phase III trials and INO-4995, an inositol derivative, has completed pre-clinical testing. All of these drugs aim to correct airway hydration status and, if started before disease initiation, may prevent/delay or at least slow CF airway disease progression. To complement these approaches there are a number of drugs in development that are aimed at treating established CF airway disease, namely anti-infective/anti-inflammatory drugs, and drugs that target mucus rheology. A summary of the Cystic Fibrosis Foundations therapeutic pipeline can be found at <http://www.cff.org/research/DrugDevelopmentPipeline/>.

In conclusion, an accumulation of research data and clinical trials over the past decade has suggested that CF airway disease is initiated as a consequence of airway dehydration. The improvements in lung function, and reduction of exacerbation frequency as a consequence of airway rehydration by HS, are an exciting development since this approach may be the first to target the initial defect in airway disease pathogenesis. Consequently, it may be possible to avert disease progression in patients with established CF lung disease and prevent disease onset in infants diagnosed with CF at birth.

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References

1. Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947-2003. *Eur Respir J* 2007;29:522–6. [PubMed: 17182652]
2. Abman SH, Ogle JW, Butler-Simon N, Rumack CM, Accurso FJ. Role of respiratory syncytial virus in early hospitalizations for respiratory distress of young infants with cystic fibrosis. *J Pediatr* 1988;113:826–30.
3. Punch G, Syrmis MW, Rose BR, Harbour C, Bye PT, Nissen MD, Elkins MR, Sloots TP. Method for detection of respiratory viruses in the sputa of patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2005;24:54–7. [PubMed: 15616837]

4. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066–73.
5. Rich DP, Anderson MP, Gregory RJ, Cheng SH, Paul S, Jefferson DM, McCann JD, Klinger KW, Smith AE, Welsh MJ. Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. *Nature* 1990;347:358–63.
6. Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. *Science* 1995;269:847–50. [PubMed: 7543698]
7. Ameen N, Silvis M, Bradbury NA. Endocytic trafficking of CFTR in health and disease. *J Cyst Fibros* 2007;6:1–14. [PubMed: 17098482]
8. Cotton CU, Stutts MJ, Knowles MR, Gatzky JT, Boucher RC. Abnormal apical cell membrane in cystic fibrosis respiratory epithelium. An *in vitro* electrophysiologic analysis. *J Clin Invest* 1987;79:80–5. [PubMed: 3793933]
9. Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996;85:229–36. [PubMed: 8612275]
10. Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* 1997;88:553–60. [PubMed: 9038346]
13. Knowles MR, Robinson JM, Wood RE, Pue CA, Mentz WM, Wager GC, Gatzky JT, Boucher RC. Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects. *J Clin Invest* 1997;100:2588–95. [PubMed: 9366574]
14. Matsui H, Grubb BR, Tarran R, Randell SH, Gatzky JT, Davis CW, Boucher RC. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell* 1998;95:1005–15. [PubMed: 9875854]
15. Tarran R, Grubb BR, Gatzky JT, Davis CW, Boucher RC. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J Gen Physiol* 2001;118:223–36. [PubMed: 11479349]
16. Tarran R, Grubb BR, Parsons D, Picher M, Hirsh AJ, Davis CW, Boucher RC. The CF salt controversy: in vivo observations and therapeutic approaches. *Mol Cell* 2001b;8:149–58. [PubMed: 11511368]
17. Kerem E, Bistrizter T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, MacLaughlin E, Barker P, Nash M, Quittell L, Boucher R, Knowles MR. Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. *N Engl J Med* 1999;341:156–62. [PubMed: 10403853]
18. Tarran R. Regulation of airway surface liquid volume and mucus transport by active ion transport. *Proc Am Thorac Soc* 2004;1:42–6. [PubMed: 16113411]
19. Engelhardt JF, Yankaskas JR, Ernst SA, Yang Y, Marino CR, Boucher RC, Cohn JA, Wilson JM. Submucosal glands are the predominant site of CFTR expression in the human bronchus. *Nat Genet* 1992;2:240–8. [PubMed: 1285365]
20. Kreda SM, Mall M, Mengos A, Rochelle L, Yankaskas J, Riordan JR, Boucher RC. Characterization of wild-type and deltaF508 cystic fibrosis transmembrane regulator in human respiratory epithelia. *Mol Biol Cell* 2005;16:2154–67. [PubMed: 15716351]
21. Tarran R, Boucher RC. Thin-film measurements of airway surface liquid volume/composition and mucus transport rates *in vitro*. *Methods Mol Med* 2002;70:479–92. [PubMed: 11917544]
22. Tarran R, Button B, Picher M, Paradiso AM, Ribeiro CM, Lazarowski ER, Zhang L, Collins PL, Pickles RJ, Fredberg JJ, Boucher RC. Normal and cystic fibrosis airway surface liquid homeostasis. The effects of phasic shear stress and viral infections. *J Biol Chem* 2005;280:35751–9. [PubMed: 16087672]
23. Tarran R, Button B, Boucher RC. Regulation of normal and cystic fibrosis airway surface liquid volume by phasic shear stress. *Annu Rev Physiol* 2006;68:543–61. [PubMed: 16460283]
24. Lazarowski ER, Tarran R, Grubb BR, van Heusden CA, Okada S, Boucher RC. Nucleotide release provides a mechanism for airway surface liquid homeostasis. *J Biol Chem* 2004;279:36855–64. [PubMed: 15210701]

25. Mason SJ, Paradiso AM, Boucher RC. Regulation of transepithelial ion transport and intracellular calcium by extracellular ATP in human normal and cystic fibrosis airway epithelium. *Br J Pharmacol* 1991;103:1649–56. [PubMed: 1718521]
26. Tarran R, Trout L, Donaldson SH, Boucher RC. Soluble mediators, not cilia, determine airway surface liquid volume in normal and cystic fibrosis superficial airway epithelia. *J Gen Physiol* 2006;127:591–604. [PubMed: 16636206]
27. Chambers, LA.; Rollins, BM.; Tarran, R. Liquid movement across the surface epithelium of large airways. In: Scheid, P., editor. *Respiratory Physiology and Neurobiology*. Elsevier; 2007.
28. Inglis SK, Corboz MR, Taylor AE, Ballard ST. Effect of anion transport inhibition on mucus secretion by airway submucosal glands. *Am J Physiol* 1997;272:L372–7. [PubMed: 9124389]
29. Trout L, Townsley MI, Bowden AL, Ballard ST. Disruptive effects of anion secretion inhibitors on airway mucus morphology in isolated perfused pig lung. *J Physiol* 2003;549:845–53. [PubMed: 12702745]
30. Matsui H, Verghese MW, Kesimer M, Schwab UE, Randell SH, Sheehan JK, Grubb BR, Boucher RC. Reduced three-dimensional motility in dehydrated airway mucus prevents neutrophil capture and killing bacteria on airway epithelial surfaces. *J Immunol* 2005;175:1090–9. [PubMed: 16002710]
31. Dawson M, Wirtz D, Hanes J. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport. *J Biol Chem* 2003;278:50393–401. [PubMed: 13679362]
32. Rubin BK. Mucus structure and properties in cystic fibrosis. *Paediatr Respir Rev* 2007;8:4–7. [PubMed: 17419972]
33. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 2000;407:762–4. [PubMed: 11048725]
34. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. *Nature* 2002;417:552–5. [PubMed: 12037568]
35. Matsui H, Wagner VE, Hill DB, Schwab UE, Rogers TD, Button B, Taylor RM 2nd, Superfine R, Rubinstein M, Iglewski BH, Boucher RC. A physical linkage between cystic fibrosis airway surface dehydration and *Pseudomonas aeruginosa* biofilms. *Proc Natl Acad Sci U S A* 2006;103:18131–6. [PubMed: 17116883]
38. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006;354:241–50. [PubMed: 16421365]
39. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006;354:229–40. [PubMed: 16421364]
40. Pasyk EA, Foskett JK. Mutant (delta F508) cystic fibrosis transmembrane conductance regulator Cl-channel is functional when retained in endoplasmic reticulum of mammalian cells. *J Biol Chem* 1995;270:12347–50. [PubMed: 7539001]
41. Illek B, Zhang L, Lewis NC, Moss RB, Dong JY, Fischer H. Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. *Am J Physiol* 1999;277:C833–9. [PubMed: 10516113]
42. Van Goor F, Straley KS, Cao D, Gonzalez J, Hadida S, Hazlewood A, Joubbran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, Negulescu P. Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1117–30. [PubMed: 16443646]
43. Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Glockner-Pagel J, Canny S, Du K, Lukacs GL, Caplan MJ. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004;304:600–2. [PubMed: 15105504]
44. Mall M, Kunzelmann K. Correction of the CF defect by curcumin: hopes and disappointments. *Bioessays* 2005;27:9–13. [PubMed: 15612029]
45. Zaman K, Carraro S, Doherty J, Henderson EM, Lendermon E, Liu L, Verghese G, Zigler M, Ross M, Park E, Palmer LA, Doctor A, Stamler JS, Gaston B. S-nitrosylating agents: a novel class of

- compounds that increase cystic fibrosis transmembrane conductance regulator expression and maturation in epithelial cells. *Mol Pharmacol* 2006;70:1435–42. [PubMed: 16857740]
46. Yang H, Shelat AA, Guy RK, Gopinath VS, Ma T, Du K, Lukacs GL, Taddei A, Folli C, Pedemonte N, Galiotta LJ, Verkman AS. Nanomolar affinity small molecule correctors of defective Delta F508-CFTR chloride channel gating. *J Biol Chem* 2003;278:35079–85. [PubMed: 12832418]
 47. Pedemonte N, Lukacs GL, Du K, Caci E, Zegar-Moran O, Galiotta LJ, Verkman AS. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. *J Clin Invest* 2005;115:2564–71. [PubMed: 16127463]
 48. Hirsh AJ, Molino BF, Zhang J, Astakhova N, Geiss WB, Sargent BJ, Swenson BD, Usyatinsky A, Wyle MJ, Boucher RC, Smith RT, Zamurs A, Johnson MR. Design, synthesis, and structure-activity relationships of novel 2-substituted pyrazinoylguanidine epithelial sodium channel blockers: drugs for cystic fibrosis and chronic bronchitis. *J Med Chem* 2006;49:4098–115. [PubMed: 16821771]
 49. Moody M, Pennington C, Schultz C, Caldwell R, Dinkel C, Rossi MW, McNamara S, Widdicombe J, Gabriel S, Traynor-Kaplan AE. Inositol polyphosphate derivative inhibits Na⁺ transport and improves fluid dynamics in cystic fibrosis airway epithelia. *Am J Physiol Cell Physiol* 2005;289:C512–20. [PubMed: 15857902]
 50. Deterding RR, Lavange LM, Engels JM, Mathews DW, Coquillette SJ, Brody AS, Millard SP, Ramsey BW. Phase 2 Randomized Safety and Efficacy Trial of Nebulized Denufosal Tetrasodium in Cystic Fibrosis. *Am J Respir Crit Care Med*. 2007
 51. Cloutier MM, Guernsey L, Mattes P, Koeppen B. Duramycin enhances chloride secretion in airway epithelium. *Am J Physiol* 1990;259:C450–4. [PubMed: 2169195]
 52. Macvinish LJ, Cope G, Ropenga A, Cuthbert AW. Chloride transporting capability of Calu-3 epithelia following persistent knockdown of the cystic fibrosis transmembrane conductance regulator, CFTR. *Br J Pharmacol* 2007;150:1055–65. [PubMed: 17339840]
 53. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME. The Pulmozyme Study Group. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med* 1994;331:637–42. [PubMed: 7503821]
 54. Frederiksen B, Pressler T, Hansen A, Koch C, Hoiby N. Effect of aerosolized rhDNase (Pulmozyme) on pulmonary colonization in patients with cystic fibrosis. *Acta Paediatr* 2006;95:1070–4. [PubMed: 16938752]
 55. Robinson M, Daviskas E, Eberl S, Baker J, Chan HK, Anderson SD, Bye PT. The effect of inhaled mannitol on bronchial mucus clearance in cystic fibrosis patients: a pilot study. *Eur Respir J* 1999;14:678–85. [PubMed: 10543292]
 56. Walker NM, Simpson JE, Levitt RC, Boyle KT, Clarke LL. Telniflumate increases survival in a cystic fibrosis mouse model of distal intestinal obstructive syndrome. *J Pharmacol Exp Ther* 2006;317:275–83. [PubMed: 16354791]

Figure 1.

Schematic diagram of the mechanisms leading to dehydrated mucus and poor MCC in the CF airway. *Left panel*, in pseudohypoaldosteronism (PHA) impaired ENaC function results in unbalanced net secretion and 'inappropriately' wet airways. The upper mucus layer expands its volume, and this looser mucus is cleared rapidly by ciliary beating. By expanding its volume the mucus maintains contact with the ciliary tips and MCC remains effective. *Middle panel*, in normal airways ASL depth is balanced by secretory and absorptive processes, mediated by CFTR and ENaC respectively. ASL height is maintained at a depth compatible with effective MCC, but slower than that seen in PHA patients. *Right panel*, in CF airways the absence of CFTR leads to defective Cl^- secretion and 'inappropriate' Na^+ absorption. This results in dehydration of airway surfaces, and thickened mucus that collapse the cilia. The adherent static mucus can trigger an inflammatory response *per se*, but also provides an ideal environment for bacterial colonization.

Figure 2.

Regulation of ASL depth on cultured human bronchial epithelial cells by soluble mediators determines MCC rates. *A*, after addition of isotonic fluid to normal airway epithelia, under static conditions, the rates of Na⁺ absorption and Cl⁻ secretion are regulated to reset and maintain ASL height at ~7 μm. CFTR represents the main Cl⁻ secretory pathway and is stimulated by adenosine present in the ASL. *B*, CF epithelia are predominantly absorptive. The absence of CFTR means that not only has the apical membrane lost its main Cl⁻ secretory pathway, but that CFTR's inhibition of ENaC is lost. This results in absorption of water from the periciliary layer (PCL) and the collapse of cilia. Adenosine is present in CF ASL but fails to regulate ASL volume. *C*, normal airway epithelia under phasic motion (shear stress) have increased levels of nucleosides/nucleotides and respond by increasing Cl⁻ secretion through CFTR and CaCC. This results in increased ASL height. *D*, CF epithelia under phasic motion

release enough ATP to stimulate CaCC and increase ASL height, despite the absence of Cl^- secretion through CFTR. *E and F*, Fluorescently labeled beads present within the mucus of CF epithelial cultures are tracked over time to assess MCC rates (pictures in *E* are 5 s exposures). CF cultured cells are exposed to either static conditions or phasic motion for the preceding 24 h before bead velocities are measured. Cultures maintained in static conditions display a low bead velocity, but cultures maintained under phasic motion show increased bead velocity (~10 fold) as a result of CaCC-mediated fluid secretion.

Figure 3.

The properties of healthy and CF mucus and how they lead to bacterial colonization and ‘biofilm’ development. **A**, The composition of normal (2.5%) and CF-like (>8%) mucus is summarized. CF-like mucus has ~4 times the mucin content of normal mucus and displays increased protein/DNA content. **B**, The velocity of GFP-expressing *P. aeruginosa* was found to be reduced by ~17 times in CF-like 8% mucus *cf.* normal 2.5% mucus. **C**, *P. aeruginosa* forms ‘biofilms’ in CF-like 8% mucus but not in normal 2.5% mucus. **D**, CF airways contain mucus plugs that contain macrocolonies of *P. aeruginosa* (e.g., clear patch within box). **E**, Mice overexpressing β -ENaC demonstrate airways mucus plugs resembling those observed in CF.

Figure 4.

Hypertonic saline improves lung function and MCC rates in CF patients. *Left panel*, the MCC rates were determined (radioisotope clearance) during a baseline period before patients were administered hypertonic saline (triangles), then again after 13 days of hypertonic saline therapy (circles). Increased MCC rates were observed in CF patients after administration of hypertonic saline. *Right panel*, CF patients experienced an increase in lung function (FEV₁) of ~5% over the period that hypertonic saline was administered.

Figure 5.

Summary of the receptors and channels that represent therapeutic targets for the treatment of CF. Mutated CFTR is absent from the plasma membrane of CF cells, and is instead broken down within the cell. ‘Corrector’ drugs are under development that aim to allow mutated CFTR to move to the plasma membrane (1 – Vertex, UCSF, Seer). Once mutated CFTR is in place in the plasma membrane, ‘potentiator’ drugs aim to increase mutated CFTR activity and restore ion/water transport (2 – Vertex, UCSF). Alternative Cl⁻ channels in the apical membrane have also been targeted, by focusing on the pathways that lead to channel activation, *e.g.*, inositol phosphates/polycyclic peptides (3 – Lantibio, Inologic) or receptors that activate these pathways, *e.g.*, P2Y₂ (4 – Inspire). Alternative strategies target ENaC for inhibition, to reduce absorption (5 – Parion), or inhibit ENaC while activating CaCC (5 – Inologic).

Table 1

Summary of the novel agents in the research pipeline for the treatment of CF. # research/pre-clinical, * phase I trial, ** phase II trial, *** phase III trial, • available to patients. † multiple groups published on drug.

Target	Strategy	Expected outcome	Who?	Therapies in trial	Ref
CFTR	To translocate mutated protein to the apical membrane	Potentially, partial restoration of chloride secretion	Vertex	Vertex Corrector#	42
			Seer Pharm.	Curcumin*	43
			†	GSNO#	44
			UCSF	UCSF compound#	45
CFTR	To increase activity of mutated protein at the apical membrane	Increase chloride secretion and airway hydration	Vertex	Vertex Potentiator*	46
			UCSF	UCSF compound#	47
ENaC	To inhibit hyperabsorption	Reduce absorption and increase airway hydration	Parion Sciences	Parion 552-02**	48
Alternative Cl ⁻ channels	To activate endogenous alternative chloride secretory pathways	Increased secretion and increased airway hydration	Inologic	INO-4995	49
			Inspire Pharmaceuticals	Denufosol****	50
			Lantibio	Moli-1901**	51
			Sucampo	SPI-8811**	52
Mucus	To change the viscoelastic properties of mucus by rehydration or enzymatic modification	Make mucus easier to clear by normal ciliary activity	Genentech	Pulmozyme•	53
			†	Hypertonic saline•	54
			Pharmaxis	Bronchitol***	38
			Genaea Corp.	Lomucin**	39
					55
					56