Cystic Fibrosis: Pathogenesis and Future Treatment Strategies

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Since the detection of the underlying gene defect, our knowledge of how the genetic mutations in cystic fibrosis cause lung disease has increased substantially, but we still lack a complete understanding of some of the pieces in the puzzle. Nevertheless, the information gained has led to new therapeutic approaches that address key factors of cystic fibrosis pathophysiology. Past therapeutic successes were largely based on targeting the consequences of the cystic fibrosis transmembrane regulator dysfunction, such as phlegm retention, infection, and inflammation, but new therapies may be able to address the underlying abnormality rather than its downstream effects. The efficacy of these treatments still needs to be established, but early studies look promising for several compounds. This review summarizes our current understanding of the pathophysiology and treatment of cystic fibrosis lung disease. *Key words: cystic fibrosis, airway surface liquid, cystic fibrosis transmembrane regulator, CFTR, pharmacotherapy, chloride secretion, gene therapy, osmotic therapy.*
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Introduction

Cystic fibrosis (CF) can serve as a paradigm of how a better understanding of the underlying disease process can translate into new and potentially more causative treatment approaches. As in many areas of research, more detailed studies of the molecular structure and function of the CF transmembrane regulator (CFTR) protein have shown us that its functions and interactions may be much more complex than initially anticipated. Therefore, while many aspects of CF pathophysiology have been clarified, there are still multiple areas of ongoing debate that need to be

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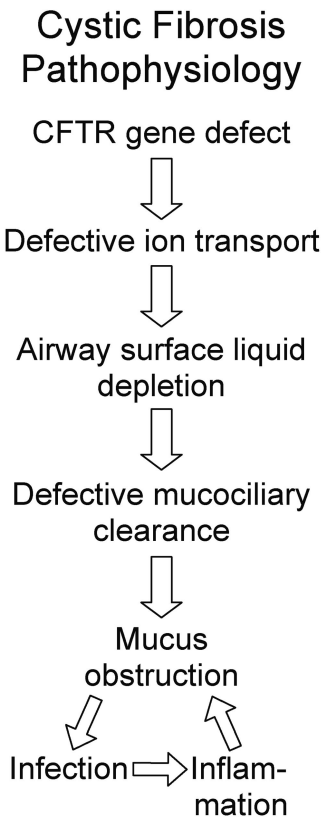

Fig. 1. Cascade of pathophysiology in cystic fibrosis lung disease.

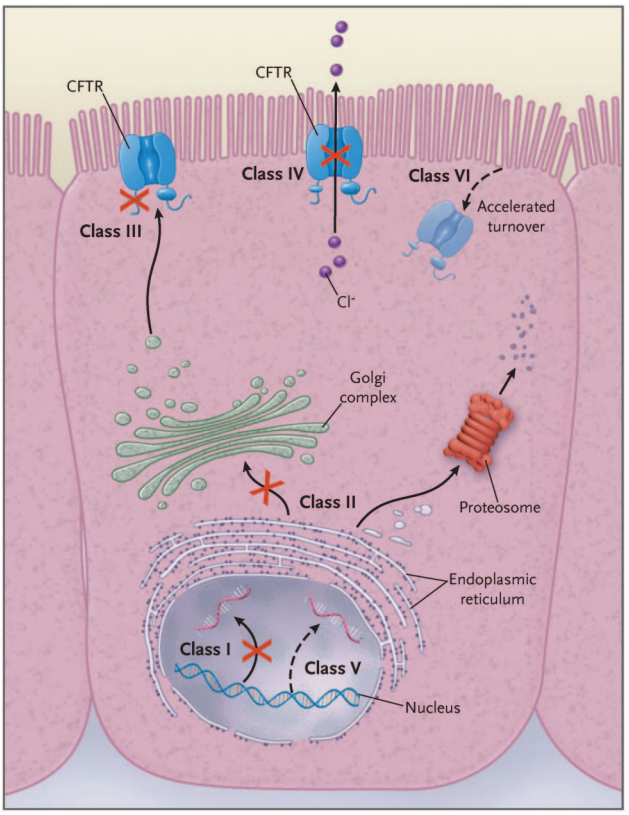

Fig. 2. Classes of cystic fibrosis transmembrane regulator (CFTR) mutations (see text). (From Reference 5, with permission.)

clarified. Resolving these controversies will be important to identifying the most promising treatment strategies. This review will summarize the current knowledge on how CF gene mutations cause disease and will focus largely on the respiratory tract. In addition, new therapeutic targets and compounds that have arisen from this improved understanding will be discussed.

Pathophysiology

A brief and simplified cascade of the current concept of how lung disease evolves in CF is summarized in Figure 1. The CF gene defect leads to an absent or malfunctioning CFTR protein, which results in abnormal chloride conductance on the apical membrane of the epithelial cell. In the lung this results in airway surface liquid depletion and, since airway surface liquid is essential to support ciliary stability and functioning, ciliary collapse and decreased mucociliary transport. The consequence of this is a vicious circle of phlegm retention, infection, and inflammation. Though this broad concept is largely undisputed, controversies exist on multiple aspects of this cascade. Topics that have specific relevance to the development of new therapies are highlighted below.

Genetics

After the CFTR gene defect was detected in 1989, it was expected that a limited number of disease-causing mutations would cause CF.[1,2] By now more than 1,500 different mutations have been described, but it is important to understand that the functional consequences of many of these mutations are poorly understood and the majority of these mutations are rare.[3] In fact, less than 10 mutations occur with a frequency of more than 1%, whereas the most common mutation worldwide, caused by a deletion of phenylalanine in position 508 (DF508) is found in approximately 66% of CF patients. CFTR mutations can be grouped into different classes based on their functional consequences on the CFTR within the cell: CFTR is either not synthesized (I), inadequately processed (II), not regulated (III), shows abnormal conductance (IV), has partially defective production (V), or has accelerated degradation (VI) (Fig. 2).[4,5] The class I, II, and III mutations are more common and associated with pancreatic insufficiency, whereas patients with the less common class IV, V, and VI mutations often are pancreatic sufficient. So far, the prognostic knowledge of the genetic mutation is of limited clinical value, but this changed recently because of the

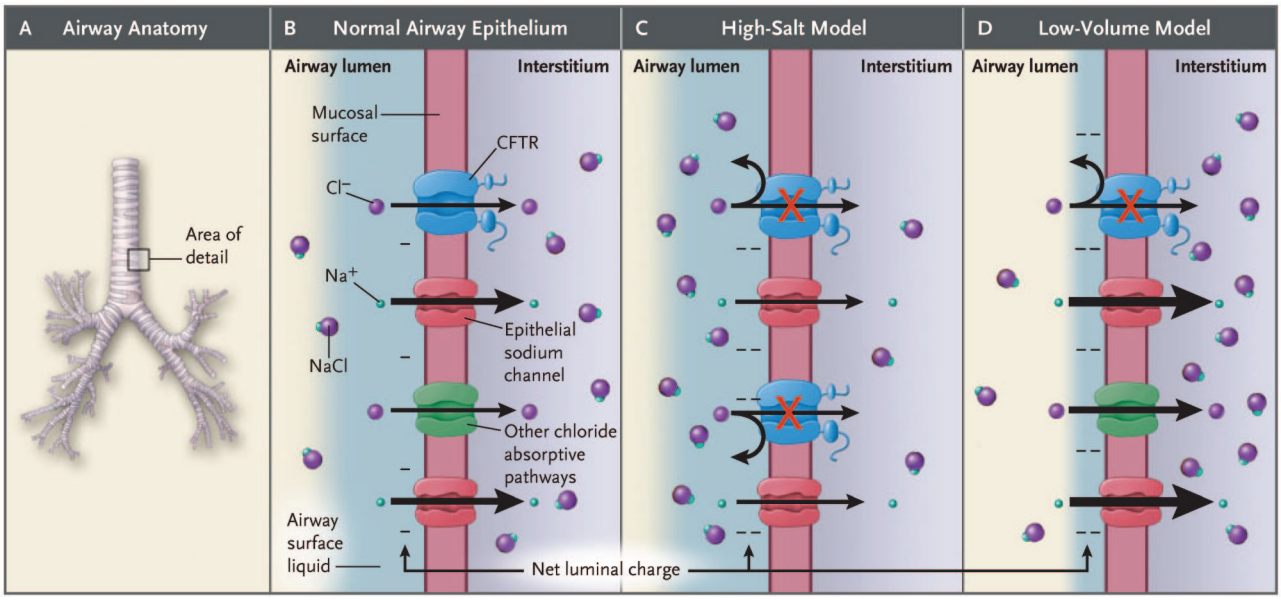

Fig. 3. Current models of cystic fibrosis transmembrane regulator (CFTR) functioning in the airways. (From Reference 5, with permission.)

development of new treatment approaches that address specific aspects of CFTR dysfunction.[6] A detailed understanding of the underlying genetic abnormalities is therefore essential to define the best targets for therapies, For instance, DF508 CFTR is misfolded and is recognized as abnormal protein in the endoplasmic reticulum, and is consequently proteolytically degraded in the proteasome.[7,8] Small amounts of DF508 CFTR reach the plasma membrane of epithelial cells, and even DF508 CFTR has functional activity.[9] However, DF508 CFTR, though it is functionally active, has a greatly reduced half life in the plasma membrane,[10] which suggests that DF508 CFTR rescue from endoplasmic-reticulum degradation or interference with pathways that recycle CFTR in the plasma membrane may be therapeutic strategies. Drugs to correct the intracellular trafficking of DF508 CFTR are currently in development and described below.

Cystic Fibrosis Transmembrane Regulator Function

CFTR belongs to a family of transmembrane proteins called adenosine triphosphate (ATP) binding cassette transporters, and functions as a chloride channel in apical membranes.[11] This fits with the clinical and laboratory observations prior to the detection of the CF gene, that the underlying defect in CF is one of chloride secretion.[12] However, CFTR possesses other functions in addition to being a chloride channel. CFTR has been described as a regulator of other membrane channels, such as the epithelial sodium channel and the outwardly rectifying chloride channel.[13] CFTR also transports or regulates HCO_3^- transport through epithelial cell membranes and may act as a channel for other proteins, such as glutathione.[14,15] Aside from these known functions, newer proteomic approaches have shown that CFTR interacts with many other intracellular proteins, and the pathophysiologic relevance of these interactions has not been fully elucidated.[16] However, this understanding is important for therapeutic interventions, since drugs that induce chloride secretion but do not affect other CFTR functions may not address all relevant aspects of CF pathophysiology.

Airway Surface Liquid and Mucociliary Clearance

There is ongoing discussion on how CFTR causes disease, but the most widely accepted model is that CFTR acts as a chloride channel that pumps chloride from the intracellular space to the extracellular space. This seems to be a rather basic statement, but it has in fact been rather difficult to clearly demonstrate the direction of ion flux in CF epithelium. Lack of CFTR activity will therefore cause less chloride secretion, and, since ion transport also creates an osmotic gradient, less water transport into the epithelial surface layer (Fig. 3).[17] This is not the only mechanism, since, as mentioned above, one of CFTR's physiologic roles is to inhibit sodium absorption, and loss of CFTR therefore causes excessive sodium (and water) absorption through the epithelial sodium channel.[18] Which of the 2 mechanisms (reduced chloride secretion or sodium hyperabsorption) is more relevant in the development of CF lung disease is unclear, and it is likely that both events are important in the development of airway-surface-liquid depletion. Airway-surface-liquid depletion is believed to cause ciliary collapse and loss of mucociliary clearance.[19,20]

According to this model, mucociliary transport should be completely absent in CF, which is not the case.[21] Local mediators secreted onto the airway surface liquid play an important role in the dynamic regulation of the airway surface liquid volume, because they can induce both CFTR-dependent and CFTR-independent chloride secretion.[22] The P2Y receptor is activated by ATP in both CF and non-CF epithelium, and mediates chloride secretion independent of CFTR through an alternative chloride channel.[23] This mechanism is triggered by movement and is important for increasing airway surface liquid in CF cells in vitro. This is relevant for understanding specific disease scenarios and for developing treatments. Respiratory syncytial virus and possibly other viruses increase ATPase activity in respiratory epithelium, which results in breakdown of ATP and loss of this compensatory mechanism, which may partially explain their negative impact on airway clearance in CF patients.[22] On the other hand, ATP analogues increase mucociliary clearance in CF and are being tested in patients with CF (see below).

Infection and Inflammation

Bacterial lung infections (eg, *Pseudomonas aeruginosa* or *Staphylococcus aureus*) are typical of CF lung disease.[23] Several hypotheses may explain the high affinity of *P. aeruginosa* for the CF lung and the failure of the mucosal defense system to clear these organisms. Most of the hypotheses assume that there are disease-specific explanations for the high rate of bacterial infection, but that concept has been challenged because other patient groups that have poor mucociliary clearance, such as individuals with primary ciliary dyskinesia, also have infections with organisms typical in CF, including mucoid *P. aeruginosa*, although this is generally seen at a later age.[24] Comparative studies may clarify which aspects of bacterial infection are disease-specific and which can be considered an unspecific consequence of reduced mucociliary clearance.

Earlier studies suggested that *P. aeruginosa* binds to CF airway epithelial cell membranes in higher density than they do to cells from normal individuals, because of a higher density of the asialo-GM1 receptor.[25] Others proposed that CFTR acts as a receptor for *P. aeruginosa*, which mediates intracellular uptake of bacteria and *P. aeruginosa* killing—a process that is defective in CF.[26] However, more recent studies revealed that both *P. aeruginosa* and *S. aureus* are mainly located in the mucus layer on respiratory epithelial cells, rather than directly on cell membranes, which makes it less likely that CF cell-specific changes are a key factor in the development of *P. aeruginosa* infection.[27,28]

An alternative concept is based on the assumption that the luminal side of CF respiratory epithelium has an increased sodium chloride concentration, which leads to inhibition of salt-sensitive cationic antimicrobial peptides (defensins) in the airway surface liquid.[29] However, not all defensins are salt-sensitive, it is difficult to prove or disprove that the airway surface liquid in CF is hypertonic, and most studies have found that airway surface liquid is isotonic in CF. CF mucus contains very little intact mucin,[30,31] and since mucins bind bacteria and aid in clearing bacteria from the airway, the mucin deficit may increase exposure of the CF epithelium to bacteria or their products and thereby trigger inflammation.

Though the debate continues on the pathophysiologic relevance of some of these factors, the bulk of the evidence suggests that dehydration of airway surface liquid is a key factor that impairs cilia functioning and mucociliary clearance in CF, so inhaled bacteria are not cleared.[28,32] Also, CF sputum has a below-normal oxygen concentration, which may trigger *P. aeruginosa* to switch from nonmucoid to mucoid cell types, which is the predominant *P. aeruginosa* phenotype in CF lungs[28] and is resistant to host defenses.

The vicious circle of sputum retention, infection, and inflammation perpetuates itself, because inflammatory products (eg, elastase) released by neutrophils stimulate mucus secretion and mucus breakdown. There is also evidence that inflammation is dysregulated in CF airways, but a complete discussion of this topic would be beyond the scope of this review. Neutrophilic airway inflammation has been detected in infants with CF in the first months of life, and in CF fetal lung tissue.[33,34] Whether inflammation is directly related to the CFTR defect is still disputed, but an exaggerated, sustained, and prolonged inflammatory response to bacterial and viral pathogens is an accepted feature of CF lung disease. There is also sufficient evidence that the persistent endobronchial inflammation is deleterious on the course of lung disease, so anti-inflammatory therapy may be relevant.[35]

New Therapies

The increased knowledge of how CFTR dysfunction causes lung disease has resulted in new exciting targets for treatment. CF care has largely focused on the downstream effects of CFTR dysfunction (sputum retention, infection, and inflammation) and there have been many advances on those problems, but this review will primarily focus on therapies that aim to treat the underlying abnormality in CF, either directly or through mechanisms that are closely related to the basic defect.

Cystic Fibrosis Transmembrane Regulator Replacement Therapy

After the CFTR gene defect was discovered, there was considerable enthusiasm that gene therapy could be rap-

idly developed, and CF has had among the highest number of gene-therapy trials.[36] Several vector (ie, gene-transfer) systems have been tested in human trials; adenoviruses, adeno-associated viruses, and cationic lipids have been the most commonly used vectors. The initial in vitro and in vivo studies looked quite promising, with successful gene transfer into airway epithelial cells, but in most studies CFTR expression has been rather short-lived, and it is difficult to establish a link between the relatively small improvements in CFTR functioning and clinical manifestations in patients.[37] One of the challenges is that we still lack sufficient knowledge of how much improvement in CFTR function is needed to make a clinical difference.[38] That information is also crucial for selecting the appropriate vector, because viral vectors seem to be more efficacious but are also more likely to have adverse effects, compared to liposomal vectors, which generally have lower transfection efficiency. The transient effect on gene expression makes it likely that gene therapy will require repeated dosing, which is especially problematic with viral vectors, because virus-specific immune response develops with recurrent dosing.

Few multi-dose trials have been conducted. An adeno-associated-virus-based gene therapy looked promising in an early-phase trial because it successfully transferred the gene and improved forced expiratory volume in the first second (FEV_1) and sputum interleukin 8 concentration,[39] but a subsequent trial powered to test clinical efficacy failed to reproduce those results.[40] The most ambitious gene-therapy trial is underway in the United Kingdom, with a cationic-lipid-based vector.[41] Other vector systems are being optimized in preclinical studies and may lead to further clinical trials.

Cystic Fibrosis Transmembrane Regulator Pharmacotherapy

Another treatment alternative is drugs that affect intracellular function, including the trafficking, expression, or functioning of CFTR. CFTR gene mutations have different functional consequences, so CFTR pharmacotherapy will not work for all patients, but, rather, must target the distinct classes of mutation and therefore limited patient groups. Promising compounds are in development for several intracellular processes.

Class I mutations are the "stop mutations" that decrease or eliminate the production of certain proteins. Aminoglycosides induce read-through of premature stop codons, which can result in formation of a full-length CFTR protein with functional activity, and topical application of aminoglycoside to the nasal epithelium in CF patients improves CFTR functioning.[42] However, the concentration required to achieve an effect on chloride transport is high and would not be suitable for clinical use because of the adverse effects of aminoglycosides. PTC 124 [premature termination codon] is a derivative that lacks antibiotic function and toxicity, and it has shown some promise in clinical trials.[43,44] Treatment response differed among patients with different genotypes, which exemplifies that even CFTR class-specific treatment may not necessarily work for all patients with a given mutation class. Recent evidence suggests that an in vitro assay might help predict the in vivo efficacy of PTC 124.[45] Though clinical efficacy still needs to be demonstrated in a placebo-controlled trial, the preliminary research illustrates that CFTR-specific therapy may become feasible in the near future. Class I mutations are highly prevalent in Israel, whereas only about 5% of the CF population in other countries carry these stop mutations, so this treatment will be an option for only a small fraction of the CF population.

Class II mutations include the most common mutation, DF508, in which CFTR trafficking is impaired due to proteosomic degradation of misfolded CFTR. Since this misfolded CFTR does have a chloride-conducting function, compounds that reduce degradation and increase the trafficking of CFTR to the cell membrane are a potential treatment option.[46] High-throughput screening assays have been used to screen libraries of known drugs, and some drugs (called correctors) have shown promise in improving CFTR processing. In addition, screening of chemical libraries and modifications to optimize compounds have resulted in new drugs that are now in later stages of development.[47] Because intracellular degradation of misfolded protein is an important intracellular control mechanism, and interference with this pathway may have adverse effects, great care is required to find the right balance between efficacy and safety, but some compounds look promising in preclinical studies and are expected to enter clinical trials soon.[48]

Though class III mutations, which reduce the opening probability of the CFTR channel, are relatively rare, studies of compounds that activate CFTR (potentiators) may have a role beyond the class III patient group. VX770 is a potentiator with a good efficacy and safety profile, and is currently in clinical trials with CF patients who carry the G551D mutation. Preliminary results look very promising; orally administered VX770 improved CFTR function in nasal-potential-difference measurements and markedly reduced the sweat chloride concentration.[49] If those findings are confirmed, VX770 may also be a treatment option in other patients, such as those with class II mutations, when combined with a corrector compound, which would bring CFTR to the cell membrane, where the potentiator could activate it.

Stimulation of Alternative Chloride Channels

Chloride secretion in airway epithelial cells is not limited to CFTR; a calcium-dependent chloride channel also secretes chloride in epithelial cells,[50] and increasing the activity of that chloride channel might compensate for the lack of CFTR-mediated chloride secretion. CFTR-deficient mice do not develop the typical lung disease seen in patients, which is probably at least partially attributable to up-regulation of the alternative chloride channels.[51] Activation of this channel can occur either directly or through the purinergic P2Y receptor. Two agents that stimulate that chloride-secretion pathway (denufosol and lancovutide [Moli1901]) are in clinical trials.

Denufosol. ATP plays a role in increasing airway surface liquid, and is induced by movement and is functional in both healthy and CF epithelium. ATP and other purines bind to the P2Y receptor and induce intracellular calcium release. About a decade ago, uridine-5′ triphosphate was tested as an agent to promote mucociliary clearance, and it had some positive effects, but its short half life limited its clinical value.[52] Denufosol has greater stability, longer activity in vitro, and increases mucociliary clearance in healthy subjects.[53] In patients with CF, 3 placebo-controlled phase-2 studies found a significant difference in FEV_1 between the denufosol-treated and the control patients.[54] The first phase-3 study (the Transport of Ions to Generate Epithelial Rehydration study [TIGER1]) recently completed the 6-month placebo-controlled phase, and is continuing in the open-label 6-month extension phase. The results look promising for the primary outcome measurement, FEV_1, and a second placebo-controlled study (TIGER2) is underway. Both studies focus on patients with relatively mild CF, because the concept of an inhaled agent to stimulate chloride secretion is most compelling as an early intervention strategy in patients with milder disease, in whom lung aerosol deposition is likely to be better.

Lancovutide. Lancovutide (Moli1901) was initially developed as an antibiotic, but was also found to increase the intracellular calcium level and activate the alternative chloride channel. It does not bind to a receptor, but directly interacts with phospholipids in plasma and organelle membranes. Its mechanism of action is unclear, but is does result in intracellular calcium release and activation of chloride transport. In a proof-of-concept study, lancovutide increased chloride conductance, measured via the nasal potential difference, when applied topically to nasal epithelium in patients with CF.[55] A phase-2 placebo-controlled double-blind single-center study on the safety and tolerability of aerosol lancovutide, in 24 patients with CF, found safety and benefit to pulmonary function.[56] A subsequent 4-week study supported those initial findings, and a larger study powered to assess efficacy is under way in Europe.

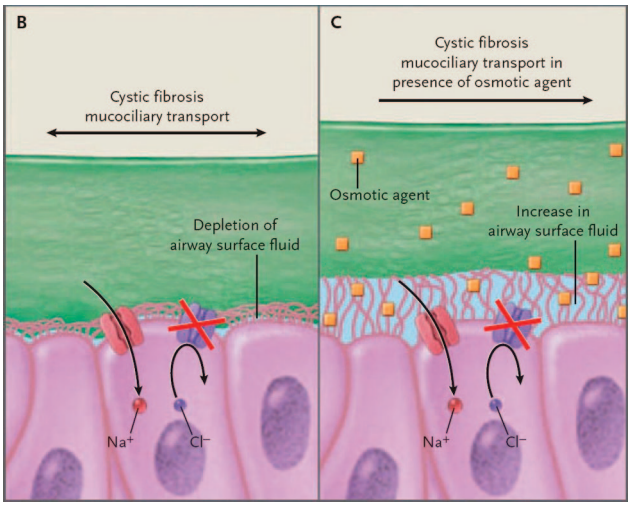

Fig. 4. Proposed mechanism of action of osmotic agents such as hypertonic saline in cystic fibrosis. (From Reference 62, with permission.)

Inhibition of Sodium Absorption

An alternative approach to CFTR pharmacotherapy is to inhibit sodium absorption through the epithelial sodium channel. This approach is supported by evidence that mice that overexpress the β subunit of the epithelial sodium channel and therefore have sodium hyperabsorption do have signs of CF lung disease, such as mucus plugging and neutrophilic inflammation.[57] In clinical trials, amiloride (an epithelial sodium channel blocker with a short half life) not only had no clinical benefit, it showed a trend toward poorer lung function in the treated patients.[58] Interestingly, studies with the epithelial sodium channel mouse model found that amiloride lacked efficacy when administered after airway disease had developed, whereas early administration prevented the development of lung disease.[59] So far, in vivo data from humans are lacking to support this concept.[60] Amiloride has a short half life, and epithelial-sodium-channel blockers with a longer half life may be a better option; at least one compound is being developed and will soon enter clinical trials.[61]

Airway Rehydration

Inadequate airway surface liquid is thought to be an important factor in the development of CF lung disease, so one treatment approach is to increase the airway fluid layer with an inhaled osmotic agent (Fig. 4).[62] Hypertonic saline was initially used as an irritant to obtain sputum samples in patients with airway diseases, but

studies also found positive effect on mucociliary transport and lung function,[63] which was thought to be largely due to the acute effects of inducing cough and hydrating the mucus, but recent evidence suggests that hypertonic saline also increases the depth of the airway surface liquid in CF.[64] A multicenter trial in Australia found a relatively modest improvement in lung function but a more remarkable reduction in pulmonary exacerbations in the treated patients.[65] Inhaled powdered mannitol is being tested as an alternative to hypertonic saline, and a phase-2 study found benefit to lung function.[66] A phase-3 study is in preparation.

So far the evidence for osmotic therapy is limited to patients with established lung disease, but if the proposed mechanism of action is confirmed, hypertonic saline might be a good early intervention. One study found that infants tolerated hypertonic saline, and a large trial with infants and young children is underway.[67] Studies with very young patients pose challenges as to the outcomes selected, but are highly relevant because early airway-hydration therapy might prevent lung damage and therefore have important long-term benefits.

Summary

Exciting new treatments are being developed that have the potential to treat the causes, rather than just the symptoms, of CF lung disease. There is continuing progress on treating the downstream aspects of CF, such as sputum retention, airway infection, and inflammation, but our improving understanding of the underlying pathophysiology will help us target the early abnormalities in CF, and early results from studies of several compounds look promising. Treating the early and root causes of CF will improve outcomes and hopefully also reduce the substantial burdens of treatment. It would be ideal to develop a "single hit" cure and thus obviate other treatments, but until that cure is found, we continue to seek ways to suppress the progression of the disease process.

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Discussion

Rubin: Felix, that was wonderful, to be able to bring that all together so quickly. I'm not even going to get into phlegm and mucus and stuff until much later. I've been trying to figure out for the longest time what to call this stuff in the airways: spucus or mucum. I think we've come up with a reasonable solution. A couple of things—it's still not clear what is the pathogenesis of CF lung disease. Mucociliary clearance is completely abolished in patients with primary ciliary dyskinesia. These patients have bad ear disease, they have runny noses, but very little in the way of lung disease when compared with patients with CF. Mucociliary clearance in large airways is preserved in patients with CF. So that doesn't seem to be the most important thing going on in the airway.

Ratjen: We can learn quite a bit from primary ciliary dyskinesia, and we haven't yet studied what is specific about CF. If we look at the rate of decline and lung function, even though this has substantially improvedin CF, it's so much more than in primary ciliary dyskinesia. The Chapel Hill group[1] believes that if you have preserved airway surface liquid, then cough clearance is maintained, and that cough clearance is more efficient than what we see in CF. But we haven't looked in detail at some of the other aspects such as the inflammatory response in primary ciliary dyskinesia versus CF. Andy Bush looked at it cross-sectionally in a group of patients and didn't see much difference between the 2 diseases.[2] If we look at this more closely to figure out what is specific to CF, it may help us to learn what is related to mucociliary clearance and what is related to the CFTR problem.

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Rosenblatt: I thought it was interesting that you referred to infection in the mucus in the paper published in *The Lancet*, whereas in the mouse model they found inflammation but not infection. This seems to be counterintuitive in the context of these models. What happens to the mice later on? Do they develop lung disease and destruction without infection, or do they develop infection? How does that relate to CF?

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Ratjen: An upcoming report in *American Journal of Respiratory and Critical Care Medicine* is going to address long-term follow-up of those mice. Andy Bush and I wrote an editorial[1] on that, because the mice don't behave like CF in the longer term, but, rather, more like COPD [chronic obstructive pulmonary disease], which is interesting because some data suggest that even COPD may have a CFTR-related problem, because if you look at ion transport in smokers, they certainly have some abnormalities that are similar to CF.[2]

But we have to understand that all of these models are incomplete models of the phenotype that we observe in patients. They will be useful to study some, but not all, aspects of the disease. And that's certainly something we have struggled with in the past. And because mice differ from humans in many respects, including their absence of submucosal glands, there are lots of differences that make them incomplete models of the human phenotypes. There's some hope that the pig model developed by the group in Iowa may be better. A ferret model is also in the pipeline, but the pig model is further along.

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Marshall: Regarding the ISIS [Infant Study of Inhaled Saline] trial,[1] you mentioned that pulmonary exacerbation was the primary end point. How did you develop that as an end point for that population, and how will you use it?

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Ratjen: Thank you for that challenging question. It isn't easy. You could have a whole talk on the validity of the various definitions of pulmonary exacerbation. The bottom line is that most of them are not well validated. We took advantage of the ongoing EPIC [Early Pseudomonas Infection Control] trial,[1] and we used the observational part of EPIC, which was studying a large population of CF patients and offered a good opportunity to study the exacerbation rate.

We know that many of these young children with CF will have viral infections and we do not necessarily expect an intervention such as hypertonic saline to prevent that, though it might alleviate it. We used those numbers to make a very conservative estimate of our effect size. That's a strength of these observational studies—that they can help us develop outcome measures. And if we use definitions that have been built in and that are specific for patients, that hope to

be more specific for patients with moderate disease, such as the one used in the EPIC trial, then that may be very useful for these early intervention trials.

What we struggle with in many of the studies now is that if we use the initial criteria of pulmonary exacerbation, such as the Fuchs criteria that were used in the DNAase trials,[2] then at least in pediatrics we see very few patients who develop pulmonary exacerbation that require intravenous antibiotics. So these outcome measures may be less useful now, so it's important that we develop these new definitions.

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Lester: I love the use of hypertonic saline in my practice, but of our pediatric patients under 6 years old, I don't think we have any on hypertonic saline.

Ratjen: Great, so they can all join our study.

Lester: Is the study going to help us know when to start hypertonic saline? We don't know what age to start it. You mentioned getting it to the smaller airways. What device do you use to deliver it?

Ratjen: We will use the Pari LC Sprint, which provides a somewhat smaller aerosol particle size, but ideally we want an even smaller particle size. There's also a balance in terms of tolerability and what you can do. We did our safety studies with a certain device and we're going to stick with that device in the efficacy trial. And our hope is that we'll finally get some data on that young age group; if we do, it may be the first time we'll have some proof of efficacy for any kind of treatment that we use in that age group. We struggle with many of the medications, including Pulmozyme, because of a lack of good clinical studies with these young individuals.

Lester: Did you use 7% in that study?

Ratjen: Yes we did use 7%, because in various studies, including one in Australia,[1] which used various concentrations up to 12%, there was certainly a dose-dependent increase in mucociliary clearance up to 7%, whereas there wasn't much increase past 12% but there were more adverse effects. And we found that 7% can be safely administered to infants. So we're pretty confident that 7% is the best concentration.

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1. Robinson M, Hemming AL, Regnis JA, Wong AG, Bailey DL, Bautovich GJ, et al. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. Thorax 1997;52(10):900-903.

Lester: You talked about lessening the treatment burden. When I suggest starting hypertonic saline, I do it cautiously because it will add 15 minutes to their therapy routine.

Ratjen: Right, but, hopefully, if we can show that this is a useful early intervention, then we would use it before the patient is on 5 other medications already. It will be the first thing they start, and, hopefully, it will prevent enough lung disease that they don't have to use all the other medications. Ultimately, that's what we struggle with. We get more and more drugs that we can use—hypertonic saline, DNAse [recombinant human deoxyribonoclease], inhaled antibiotics, and—if denufosol pans out—we'll have denufosol 3 times daily, and other medications. It's just going to be very complicated to convince our patients to do all of that unless we can show that earlier treatments will have long-term benefit.

One of the things in which we seem to be successful is in pseudomonas eradication. The recent data from the European ELITE [Early Inhaled Tobramycin for Eradication] trial[1] makes it appear that a relatively short treatment period can be advantageous. This may alleviate the treatment burden, because if we have less pseudomonas-positive patients, then we'll have fewer patients who need to be on regular inhaled antibiotics, which eliminates at least one treatment.

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Newton: How many times a day do you recommend for hypertonic saline?

Ratjen: For this study we're going to use it twice daily, but there's certainly an argument that the more frequently you use it, the better. People use it up to 4 times daily. Interestingly, in cell culture models done in Chapel Hill,[1] the hypertonic saline had a long-lasting effect on airway surface liquid and mucociliary clearance, which was not necessarily expected, because we have the cycle of sodium hyper-absorption, and people expected that this would be very short lived, but that doesn't necessarily seem to be the case. The ideal frequency is not yet known.

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fibrosis with hypertonic saline. N Engl J Med 2006;354(3):241-250.

Rosenblatt: Felix, if the sodium channel is open and we put hypertonic saline in the airway, would you not expect more sodium to be absorbed? Clinically, we do not see this paradoxical effect.

Ratjen: I don't have a good explanation for that, and those experiment results are confusing. You would expect that if you put sodium on top of respiratory epithelial cells, that because of the up-regulation of the epithelial sodium channel, it would be taken up very quickly and the effects would be very short-lived. But that was not the case, and the reason is unclear. That certainly needs to be worked out, because it may tell us about how we can interact with the sodium channel, for which we currently don't have very effective treatments, and that could inhibit the sodium hyper-absorption.

Rosenblatt: Jaques et al studied inhaled mannitol in CF.[1] Does mannitol have a different effect on the sodium channel absorption? Have there been any comparisons of mannitol and hypertonic saline?

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1. Jaques A, Daviskas E, Turton JA, McKay K, Cooper P, Stirling RG, et al. Inhaled mannitol improves lung function in cystic fibrosis. Chest 2008;133(6):1388-1396.

Ratjen: I don't know about any data on that. Some cell experiments were done with mannitol, but I don't think they were conclusive.

Geller: You've done some work with glutathione[1] and nitric oxide,[2] which concerns the intermediate stage in CF disease, where we're looking at inflammation and antioxidants. Can you comment on that?

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Ratjen: There certainly are a number of abnormalities in CF, and glutathione may be related to the underlying defect, because there are sufficient data to believe that CFTR also acts as a transporter for glutathione. We know that CF airways are deficient in nitric oxide, and that's thought to be important for bacteria-killing and other organisms such as *S. aureus* and *Pseudomonas*. We are interested in trying to augment nitric-oxide production, and we've done some studies[1] with L-arginine, which is a simple amino acid that you can administer via inhalation. In a single-dose study we found that it improved nitric-oxide production and lung function. We are about to finish a 2-week study of L-arginine in CF patients, but I can't give you any data right now, because it's still blinded, but it's going to be very interesting.

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