The Challenges and Promises of New Therapies for Cystic Fibrosis

Citation

Published Version
doi:10.1084/jem.20121248

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:10612871

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
The challenges and promises of new therapies for cystic fibrosis

Gerald B. Pier

Therapeutic intervention in cystic fibrosis (CF) remains a challenge, partly because of the number of organs and tissues affected by the lack of a functional cystic fibrosis transmembrane conductance regulator (CFTR) protein. CF was originally regarded primarily as a gastrointestinal (GI) disease because of the failure to thrive and early death from malnutrition in infants with CF. However, successful interventions for the GI manifestations of CF have left chronic lung infections as the primary cause of morbidity and mortality. Despite a complex microbiology within the CF lung, one pathogen, Pseudomonas aeruginosa, remains the critical determinant of pulmonary pathology. Treatment and management of this infection and its associated symptoms are the major targets of extant and developing CF therapies. Understanding the multitude of effects of CFTR on mucus physiology and susceptibility and progression of chronic lung disease, and how host immune responses fail to adequately control lung infection, will be essential for the development of improved therapies for CF.

Many of the basic genetic, physiological, and clinical consequences associated with CF have been studied in great detail, making CF one of the most investigated diseases in modern medicine (Griesenbach and Alton, 2011; Cohen and Prince, 2012). Discovery of the CFTR gene in 1989 was expected to lead to breakthroughs and new therapies. However, 23 yr later one can look at these new therapies with great enthusiasm for what has been developed or with disappointment in the small number of truly new drugs. New formulations of older drugs, including aerosolized antibiotics for lung infection, and improvements in clinical management of symptoms have had a major impact on disease progression (Royce and Carl, 2011). Treatments such as hypertonic saline, ibuprofen, and several vitamin and pancreatic supplements have also shown benefits in CF clinical trials. Pulmozyme, which is human DNase aerosolized into the lungs to break up DNA associated with the sticky lung secretions in infected CF patients, was approved in 1993 based on clinical observations of the composition of CF mucus. However, only one truly new drug has been approved for CF patients, and its development was based on knowledge gained from the discovery of the CFTR gene and studies of CFTR protein function. This drug, Ivacaftor (Ramsey et al., 2011), improves lung function in the 4–5% of CF patients who bear a specific CFTR mutation, G551D. The G551D channel is present in the plasma membrane but has poor functionality. Numerous other drugs and therapies are in various stages of development (http://www.cff.org/research/DrugDevelopmentPipeline/), leading to hope for more improvements in the quality of life for CF patients (Cuthbert, 2011); however, even among these drug candidates only a minority are directed toward modifying the mutant CFTR gene or modulating protein function.

CFTR functions in disease: the role of bicarbonate

Does this situation reflect the overall difficulty of modern drug development wherein development and approval of a new drug may take two decades or longer? Or does it reflect the complexity of CFTR function and subsequent disease manifestations (Cuthbert, 2011)? Likely both. Primarily studied and defined as a chloride ion channel-regulating mucosal fluid composition, CFTR can also transport bicarbonate and can regulate the epithelial sodium channel ENaC, the outwardly rectifying chloride channel ORCC, and two inwardly rectifying K+ channels (ROMK1 and ROMK2). CFTR also transports ATP and glutathione, and may regulate the pH of intracellular organelles. The importance and impact of these various CFTR functions on CF pathogenesis are controversial.

However, it seems that the bicarbonate transport function of CFTR is central to one set of manifestations of CF: the thick mucus secretions in the GI tract and lung and the impacted ducts in the pancreas. GI problems are still a fundamental aspect of CF, although medical management via pancreatic enzymes and nutritional supplements has dealt with this problem relatively effectively. Many, but not all, mouse models of CF mimic the GI pathology seen in untreated human CF disease (Guilbault et al., 2007), and CF mice must often be maintained on laxatives and liquid diets. CF pigs (Ostedgaard et al., 2011) and ferrets (Sun et al., 2010) also show GI disease, which manifests as meconium ileus at birth and requires proper management. Cloning a wild-type Cftr gene in front of an intestinal-specific promoter led to proper synthesis of CFTR in the ferret GI tract and alleviated the GI manifestations of the
The challenge of treating cystic fibrosis

3

7

+ 3

60x58

papers examining correlates of lung

Hemophilus influenzae

sion of pathogens, notably nontypable

cline in CF patients. Although a progres

dominant cause of pulmonary de

Pseudomonas aeruginosa

that emerges in the CF lung, is the

P. aeruginosa

function decline in CF find P. aeruginosa

infection to be the primary factor (Mott

et al., 2012). Some papers have associ-

ated lung function decline with infection

by methicillin-resistant S. aureus and

Streptococcus milleri (Cohen and Prince,

2012); and rapid declines in CF patients’

conditions have also been associated with

Burkholderia infections (Courtney et al.,

2007). However, most of these later in-

fections occur in addition to preexisting

P. aeruginosa infection. More recent

high-throughput sequencing techniques

revealed microbial DNA in lung secre-

tions of CF patients (Zemanick et al.,

2011); however, the actual impact of

these diverse microbial communities

on airway disease is mostly speculative.

Overall, it is still mucoid P. aeruginosa

that drives lung function decline in CF, and

how this specificity is accounted for

defects in lung mucociliary transport

is unexplained.

Would aerosolized bicarbonate have

a therapeutic role in CF lung disease by

allowing proper unfolding of airway

mucins? Perhaps. Even if defective mu-

cociliary clearance does not underlie

many of the manifestations of mucoid

P. aeruginosa infection in CF, enhancing

microbial clearance by promoting mu-

cociliary transport has potential. The

success of hypertonic saline aerosoliza-

tion in improving the lung function in

some but not all groups of CF patients

supports the utility of developing strate-

gies to enhance mucociliary transport;

however, recent investigations into

how hypertonic saline inhalation therapy

works suggests it also has antiinflam-

matory and antimicrobial effects that

could contribute to the benefit of this

therapy (Reeves et al., 2012). Overall, we

don’t know how much defective mucociliary

transport contributes to initiation or

progression of chronic P. aeruginosa in-

fection in CF, but as long as there is a

safe means to aerosolize bicarbonate,

there seems to be no reason not to try

this strategy.

Establishment and progression

of chronic lung infection

Many studies implicate immune system

dysfunction in driving the progression of

lung disease in CF (Cohen and Prince,

2012; Ratner and Mueller, 2012). Few

provide an explanation for the highly

specific association between CF and

P. aeruginosa infection. My group over

the past 15 yr has provided evidence that

CFTR itself is a receptor for P. aeruginosa

and that binding of this organism to

CFTR activates host defenses needed to

clear the organism from the lung

(Campodónico et al., 2008; Fig. 1). The

key component here is the recruit-

ment of polymorphonuclear neutro-

phils (PMN) to the lung, where they

phagocytose and kill P. aeruginosa. Bind-

ing of the outer LPS core of nonmucoid

P. aeruginosa to the first extracellular loop

of CFTR, initiates formation of lipid rafts

incorporating molecules such as caveolin

and major vault protein; lung epithelial

cells then internalize the bacteria and

release IL–1. This IL–1 signals through

the IL–1 receptor and MyD88 adaptor

protein, ultimately leading to NF–κB

nuclear translocation and synthesis of

cytokines (e.g., IL–6, IL–8, CCL1) that

recruit PMNs (Fig. 1; Reiniger et al.,

2007). In individuals with WT CFTR this

process effectively controls P. aeruginosa

lung infection. It is noteworthy that

the mucoid, LPS rough P. aeruginosa that

erase as the main pathogen in CF do not

bind CFTR because of alterations in

the LPS outer core structure and over-

production of alginate (Massengale et al.,

2000). However, even in the presence of

WT CFTR, in the absence of rapid and

effective PMN lung recruitment (e.g.,

in neutropenic mice or MyD88-deficient

mice; Koh et al., 2009), the lethal infec-

tious dose of P. aeruginosa applied to the

nares plunges from ≥10⁷ CFU to <60

CFU, and for some strains as few as 10

CFU is a lethal dose. Neutropenic and

MyD88-deficient humans are at high risk

for P. aeruginosa infections (von Bernuth

et al., 2008; Kerr and Snelling, 2009).

An early failure to clear P. aeruginosa may

then allow bacterial attachment to and

entry into stagnant CF mucus; this may

be the next key step in establishment of

chronic P. aeruginosa lung infection in CF,

and the place where mucolytic agents or

inhaled bicarbonate might be effective.

Once infection is established and

bacterial levels increase, P. aeruginosa

must evade adaptive immunity. Within the
Figure 1. Protection versus susceptibility to *P. aeruginosa* infection in lungs expressing wild-type or mutant CFTR. (A) Proposed factors responding to *P. aeruginosa* in the airway of humans with intact, wild-type CFTR. Some of the bacteria in normal mucus with properly unfolded mucins bind to CFTR in the plasma membrane, initiating rapid (2–15 min) IL-1 release; the resulting IL-1 triggers autocrine or paracrine signaling through the IL-1 receptor. Bacteria binding to CFTR also initiates formation of lipid rafts and recruitment of caveolin, major vault protein (MVP), and other proteins to the rafts; this is followed by bacterial internalization. These processes lead to MyD88-dependent activation and nuclear translocation of NF-κB and regulated inflammatory responses involving production of IL-6 and IL-8 and increases in ICAM-1 and Gro-1 (CXCL1), all of which participate in recruitment of polymorphonuclear neutrophils (PMN) to the airway mucosa. The remaining, viable *P. aeruginosa* are phagocytosed and killed, and those entrapped within epithelial cells are carried out in the mucus. (B) On the CF airway surface, lack of functional CFTR, such as the ΔF508 variant that is unable to make it to the plasma membrane, leaves the *P. aeruginosa* bacteria trapped in the mucus, which is dehydrated and more viscous because of the compacted mucins released from the secretory granules of goblet cells. PMN recruitment does occur, but in a dysregulated, uncoordinated, and slower fashion, and when the PMN do arrive their ability to phagocytose the *P. aeruginosa* cells trapped within the airway mucus is poor. The frustrated phagocytosis can lead to release of granule contents containing toxic factors. The PMN undergo necrosis instead of apoptosis, and this results in a failure to clear bacteria and resolve inflammation. The chronic infection is perpetuated by an ineffective adaptive immune response, allowing the progression of chronic infection, inflammation, and destruction of lung tissue.
CF lung major changes in the phenotype of *P. aeruginosa* occur. These result in the overproduction of the cell surface polysaccharide alginate, loss of the LPS O antigen (Campodónico et al., 2008), and accumulation of DNA mutations caused by mutations affecting the DNA repair enzyme MutS (Oliver and Mena, 2010). Thus, the organism presents an ever-changing set of antigens for adaptive host immune responses. As CF patients do not have known defects in adaptive immunity, why can’t they mount an effective immune response to clear *P. aeruginosa*? Some do, as it was shown almost 25 yr ago that a small subset of CF patients >12 yr old remained uninfected and had serum antibodies that recognize alginate and facilitate opsonic killing (Pier et al., 1987). This antibody is not found in other CF patients or even in healthy humans, most of whom produce natural nonopsonic/nonprotective antibodies to alginate. Engineering alginate to induce opsonic antibody production has been a major challenge and, to date, not highly successful, thus preventing pursuit of alginate vaccines in CF patients. A fully human IgG1 mAb to alginate, which is opsonic and protective in animals, has been made (Pier et al., 2004) but has not yet progressed to clinical trials.

Other potential conserved antigens of *P. aeruginosa* that could be targeted for vaccination or passive mAb therapy include Oprs, the LPS core, and two other surface polysaccharides known as Pel and Psl. These latter two antigens, along with alginate and DNA, participate in biofilm formation by *P. aeruginosa*. Psl is also involved in attachment of *P. aeruginosa* to host cells (Ghafoor et al., 2011). The structure of the Psl polysaccharide was previously described (Kochava et al., 1988) as a neutral polysaccharide present in the LPS of different strains of *P. aeruginosa*. This structure was confirmed as the Psl polysaccharide by Byrd et al. (2009). No definitive chemical linkage of Psl to the LPS has been established, and its role in immunity has not been explored. In this issue of *The Journal of Experimental Medicine*, in a robust display of modern technology, DiGiandomenico et al. screened single-chain variable fragment antibody-producing phage libraries expressing antibody V regions genes isolated from both healthy humans and those recovering from *P. aeruginosa* infection. They identified several antibodies that bound to distinct epitopes on *P. aeruginosa* Psl and, when one of these was engineered into a human IgG1 expression vector, it bound to many strains of *P. aeruginosa*, mediated opsonic killing and showed protective efficacy in several animal models of infection. The potential of the mAb as an immunotherapeutic is thus strong, but important questions remain, particularly for testing in CF patients. As DiGiandomenico et al. (2012) found that the Psl polysaccharide was not essential for virulence in animal models, the microbe may escape immune defenses targeted to Psl. Alginate, also produced by most strains of *P. aeruginosa*, is essential for chronic but not acute *P. aeruginosa* infection (Campodónico et al., 2008). Another question to be asked regarding the mAb to Psl is whether or not infected CF patients have opsonic antibody to Psl, and whether disease progresses regardless of the presence of the antibody. Also of importance is whether the effects of the mAb to Psl are manifest when *P. aeruginosa* is growing in a microcolony within the CF lung. If not, the mAb to Psl might be more useful for preventing infection or controlling it at an early stage of CF, as is now being done by early administration of antibiotics. Efficacy and cost considerations will drive the decision as to whether routine antibody administration to CF patients is warranted. Currently, significant issues related to compliance and safety impact the efficacy and use of at-home inhaled antibodies in CF, although this might be improved by newer dry-powder formulations that can be administered in a short time period. However, if a mAb is at least as efficacious and cost-competitive as inhaled antibiotics, clinician–observed administration of this mAb every 3–6 mo could be an important advance in the immunotherapy of CF patients. Finally, an effective vaccine capable of inducing antibodies to Psl, alginate and/or similar antigens would be the most desirable outcome of all for preventing *P. aeruginosa* infection.

G. Pier is an inventor of a monoclonal antibody directed to the alginate antigen of *P. aeruginosa*. The antibody has been licensed by Brigham and Women’s Hospital (BWH) to Aridis Pharmaceuticals. As an inventor, G. Pier receives a share of licensing-related income (royalties and fees) through BWH. G. Pier’s interests were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

REFERENCES


