The Challenges and Promises of New Therapies for Cystic Fibrosis

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 mucosal 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 either 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, Ivcaftor (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

G. Pier is at the Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

CORRESPONDENCE
G.B. Pier: gpier@rics.bwh.harvard.edu

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disease; this strategy was also used to create transgenic CF mice with normal GI tract function (Zhou et al., 1994).

In 2008, Quinton proposed (Quinton, 2008) that the highly compacted mucins in intracellular granules are held together by Ca^{2+} and H^+ cations, and that removal of these cations by bicarbonate is critical for mucin unfolding and expansion. Accordingly a bicarbonate transport defect such as that in CF would result in a HCO_3^- anion-poor extracellular milieu that could not remove the Ca^{2+} cations and would leave the mucins compacted, not readily soluble, and thus poorly transportable, as shown in the mouse intestine (Garcia et al., 2009). In this issue of *The Journal of Experimental Medicine*, Gustafsson et al. confirm that bicarbonate plays a crucial role in increasing local pH and removing Ca^{2+} cations to facilitate unpacking of mucins secreted from goblet cells. They demonstrate that adding bicarbonate to CF mouse intestinal mucus led to normal mucin unfolding and function.

Mucociliary transport and chronic lung infection in CF

Can we exploit this knowledge of the importance of CFTR-secreted bicarbonate to treat other disease manifestations of CF? This depends, in part, on the degree to which poor mucus transport plays a role in chronic infection and inflammation in the lungs of CF patients. Many investigators believe that defective mucus transport does contribute to CF lung disease (Clunes and Boucher, 2007), and numerous therapies directed at enhancing mucus transport have been developed or are under investigation. But there remains a fundamental problem with this hypothesis; it fails to explain the observation that infection with *Pseudomonas aeruginosa*, more specifically the mucoid phenotype of *P. aeruginosa* that emerges in the CF lung, is the predominant cause of pulmonary decline in CF patients. Although a progression of pathogens, notably nontypable *Hemophilus influenzae* and *Staphylococcus aureus*, have been seen in early CF lung disease for years, the vast majority of papers examining correlates of lung function decline in CF find *P. aeruginosa* infection to be the primary factor (Mott et al., 2012). Some papers have associated 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 infections occur in addition to preexisting *P. aeruginosa* infection. More recent high-throughput sequencing techniques revealed microbial DNA in lung secretions 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 by 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 mucociliary clearance does not underlie many of the manifestations of mucoid *P. aeruginosa* infection in CF, enhancing microbial clearance by promoting mucociliary transport has potential. The success of hypertonic saline aerosolization in improving the lung function in some but not all groups of CF patients supports the utility of developing strategies to enhance mucociliary transport; however, recent investigations into how hypertonic saline inhalation therapy works suggests it also has antiinflammatory 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* infection 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 recruitment of polymorphonuclear neutrophils (PMN) to the lung, where they phagocytose and kill *P. aeruginosa*. Binding 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 emerge as the main pathogen in CF do not bind CFTR because of alterations in the LPS outer core structure and overproduction 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 infectious dose of *P. aeruginosa* applied to the nares plunges from $\geq 10^7$ 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

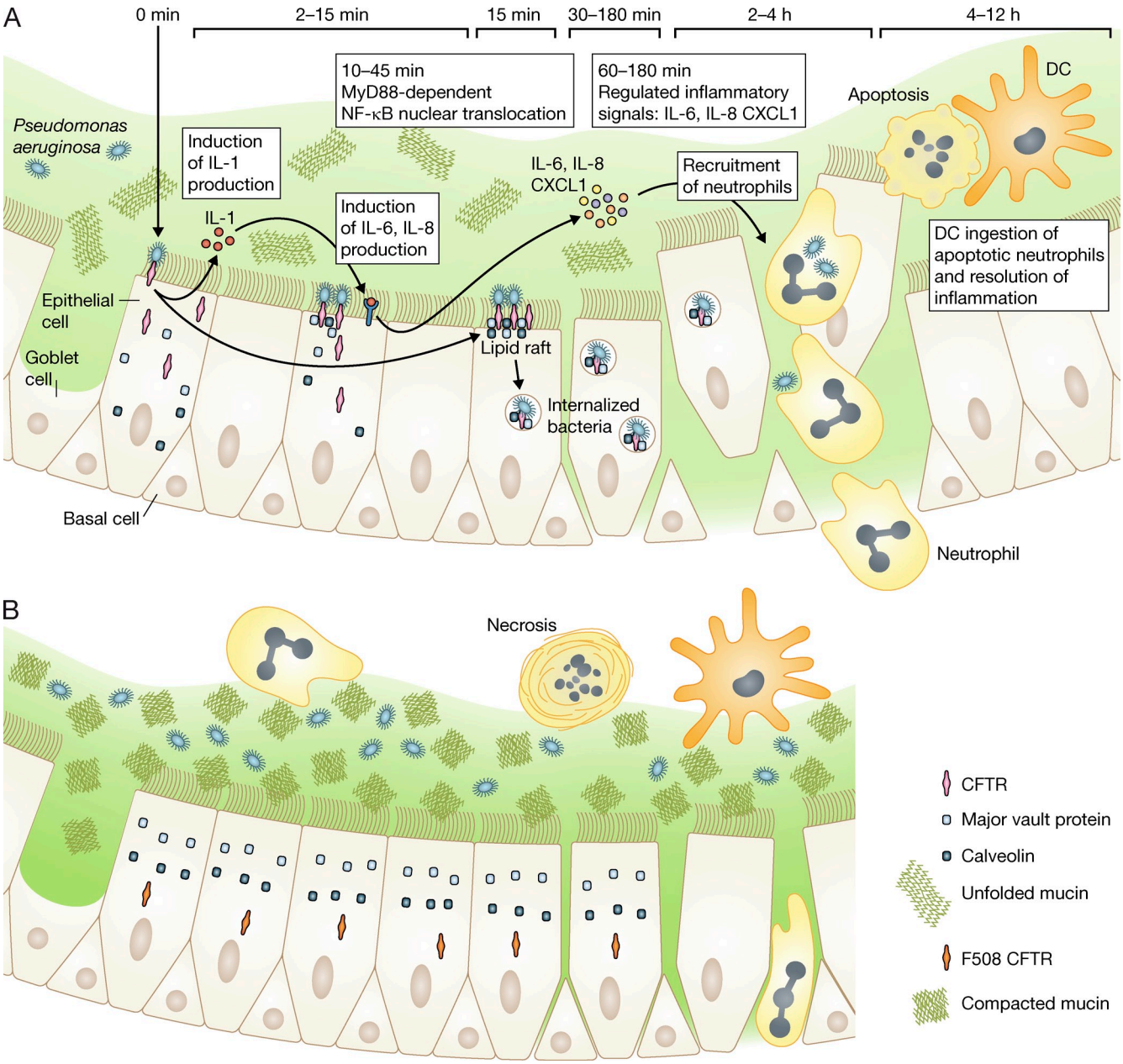

Figure 1. Protection versus susceptibility to *P. aeruginosa* infection in lungs expressing wild-type or mutant CFTR. (A) Proposed factors responding to *P. aeruginosa* in the airway of humans with intact, wild-type CFTR. Some of the bacteria in normal mucus with properly unfolded mucins bind to CFTR 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 leads 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 (Kocharova 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 antibiotics 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 desirous 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.

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