Update in Cystic Fibrosis 2009

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The discovery of disease-causing mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene 20 years ago ushered in a revolution in our understanding of the pathophysiology of CF (1). The CFTR protein resides in the apical membrane of epithelial cells and is important in the regulation of airway surface liquid (ASL). Mutations in CFTR result in defective chloride secretion and excessive sodium reabsorption and a depletion of the ASL causing impaired mucociliary clearance (2). Submucosal glands generate most of the airway fluid, and submucosal gland dysfunction is believed to be a primary defect in CF (3). The result is impaired host defenses and chronic bacterial airway infection. Cystic fibrosis is also characterized by an exuberant airway inflammatory response of unknown etiology. This ongoing infection and inflammation leads to persistent symptoms and irreversible lung damage.

BASIC DEFECT AND TRANSLATIONAL THERAPIES

One treatment strategy is to increase the amount of functioning CFTR at the cell surface. The most common CF mutation (F508del) undergoes rapid degradation because of defective folding. Small molecules called "correctors" can promote folding of F508del-CFTR, which retains some functional activity when it reaches the cell surface (4). Thus far, correctors have been poor therapeutic candidates because they promote delivery of only a small fraction of F508del-CFTR to the cell surface. Loo and colleagues (5) evaluated interactions between half-molecules of CFTR and correctors and identified several regions of F508del-CFTR that could be potential targets for more efficient correctors.

Miglustat, an orally available drug currently approved for use in patients with type I Gaucher disease, may have a CFTR corrector effect (6). Oral treatment of miglustat has a high rate of side effects, so Lubamba and colleagues (7) investigated the effects of topical application of miglustat to airway epithelia in mice. They demonstrated clear evidence that local delivery of miglustat counteracts sodium transport even in the absence of CFTR and activates CFTR-dependent chloride transport in F508del-CF mice.

Previous reports have suggested that sarcoendoplasmic reticulum calcium ATPase (SERCA) inhibitors decrease calcium concentrations within the ER and interfere with calcium-dependent chaperone proteins that retain misfolded F508del-CFTR, thereby allowing its escape to cell surface (8). However, Ahmad *et al* (9) demonstrated that SERCA2 is diminished in CF airway epithelium. They also showed that decreasing SERCA2 levels with small interfering RNA (siRNA) actually led to decreased cell survival during oxidative stress. These results demonstrate that decreased SERCA2 may play a significant role in calcium signaling and apoptosis in CF epithelium.

High throughput screening (HTS) has been used to identify compounds with the potential to improve ion transport defects in the CF airway. Van Goor and colleagues (10) demonstrated that VX-770, which was identified by HTS and is now in phase 3 clinical studies, can potentiate chloride transport by both G551D- and F508del-CFTR proteins *in vitro*. Further, VX-770 treatment led to decreased sodium conductance in a G551D/F508del cell line.

Because CF is a disease of chloride transport in respiratory epithelia, alternative chloride channels could partially compensate for the CF defect. Liang and colleagues (11) used HTS to identify spiperone as an activator of an alternative calcium-activated chloride channel in airway epithelium. Spiperone appears to act via a protein tyrosine-coupled phospholipase C-dependent pathway. Lubiprostone has been shown to activate ClC-2 chloride channels in a number of epithelial cell lines so Joo and colleagues (12) examined its effects on tracheal epithelia. Lubiprostone increased the mean secretion rate of airway glands, including those in CF tissues, but the increase was not significant. Nonetheless, the observation suggests the possibility that more potent agents of this type could be beneficial.

Both CFTR and the β2-adrenergic receptor (β2-AR) colocalize at the apical membrane and regulation of CFTR activity may be dependent on activation of β2-AR. Cystic fibrosis airway epithelial cells are not stimulated by a short-acting β2 agonist (e.g., isoproterenol) but Delavoie and colleagues (13) demonstrated that the long-acting β2 agonist, salmeterol, acts as a potent chloride secretagogue through a CFTR-dependent mechanism. The effect was not immediate but occurred after several hours of incubation and was independent of sustained cAMP production. Salmeterol did not alter the level of CFTR mRNA but likely worked by stabilizing the small amount of F508del-CFTR at the cell surface, reducing the internalization of the β2-AR. These findings demonstrate that the long-acting β2-agonist salmeterol can potentially restore chloride secretion and ASL hydration in addition to having a bronchodilatation effect.

The inhibition of sodium reabsorption may prevent airway surface liquid dehydration. Caci and colleagues (14) demonstrated that siRNA transfection can effectively inhibit expression of the amiloride-sensitive epithelial sodium channel (ENaC) and decrease sodium transport in polarized cultures of primary airway cells.

The lack of CFTR function has diverse effects on cellular function beyond ion transport. Altered sphingolipid metabolism in CF epithelium has been proposed as an explanation for increased airway inflammation and defective apoptosis observed in CF airway epithelium. However, there is conflicting evidence regarding the role of ceramide in the CF airways disease. Teichgräber and colleagues (15) demonstrated that ceramide is

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increased in tissues derived from CF murine models and in airway cells obtained from patients with CF. On the other hand, Guilbault and colleagues (16) found decreased levels of ceramide in a different murine CF model. The ceramide level in the serum of patients with CF was also decreased compared with healthy controls. Decreased ceramide levels were associated with derangements in arachidonic (AA) acid and docosahexaenoic acid (DHA) levels in both humans and mice. When treated with the synthetic retinoid fenretinide, the lipid profile of CF mice improved (17). The explanation for these differing results is unclear but may relate to the methods used to measure ceramide and differences in the mice strains and diets used.

Yu and colleagues (18) demonstrated that IB3–1 CF cells and CF knockout mice lack the appropriate increase in acid sphingomyelinase and ceramide in response to *Pseudomonas aeruginosa* exposure. The diminished acid sphingomyelinase response was associated with increased IL-8 levels. A similar inability to increase ceramide levels in response to oxidative stress was observed when CFTR was inhibited in endothelial cells *in vitro* (19).

The role of CFTR in organelle function has been controversial (20). A defect in lysosomal acidification has been proposed as a mechanism for the increased ceramide levels observed in CF cells by some investigators (15). This hypothesis has been challenged by Haggie and Verkman (21) who used fluorescent indicators to study lysosomal pH in both murine and human CF cells. These authors found no lysosomal acidification defect in CF cells, suggesting that other non-CFTR chloride channels modulate lysosomal pH.

Although airway epithelial cells have been the focus of intense investigation, the contribution of other cells to the inflammatory response in the CF airway should not be underestimated. For example, Bruscia and colleagues (22) determined that the response of macrophages derived from CF mice exposed to *P. aeruginosa* and lipopolysaccharide was excessive when compared with macrophages derived from wild-type mice.

The loss of CFTR function leads to a myriad of changes in pulmonary physiology. Hubert and colleagues (23) demonstrated that exhaled nitric oxide levels correlated with airflow limitation in 34 CF patients. Additionally, defective vascular dilatation resulted in wasted ventilation and impaired exercise capacity in these patients. Dufresne and colleagues (24) found that CF patients have increased diaphragm and inspiratory muscle mass compared with healthy controls. Fat-free mass and airway resistance, but not the intensity of systemic inflammation, influenced diaphragm and limb muscle mass the most.

GENE MODIFIERS

There are more than 1,000 identified mutations of the CFTR gene known to be associated with disease, although there is a poor correlation between the genotype and phenotype, especially the severity of lung disease. This observation led to the hypothesis that there are non-CFTR gene variants that affect, or modify, the CF phenotype. Because the sodium hyperabsorption observed in the CF airway occurs through ENaC, Azad and colleagues (25) examined mutations in this channel, finding an increased incidence of several rare polymorphisms in CF patients compared with healthy controls. These findings are intriguing, but a mutation was identified in only a minority of patients, and the overall study population was fairly small. Identifying gene modifiers requires larger populations and replication of associations in multiple independent populations (26).

In addition to investigations of genes involved with the basic defect, there are opportunities to examine genes involved with other aspects of CF pathophysiology, specifically infection and inflammation. Bacteria that enter the airways bind to mucins and the cellular glycocalyx of airway epithelia; genetic differences in the patterns of glycosylation and microbial binding may increase the likelihood of airway infection. Taylor-Cousar and colleagues (27) investigated ABO blood type, which reflects genetic-based differences in glycosylation enzymes as a possible genetic modifier of CF lung disease. However, polymorphisms in the genes encoding ABO blood type were not shown to associate with severity of CF lung disease or age of onset of *P. aeruginosa* infection.

Levy and colleagues (28) evaluated the *IL-1* gene family for polymorphisms associated with severity of disease as *IL-1* plays an important role in inflammatory signaling within the airway. They found two variants of the *IL-1*β gene, a pro-inflammatory cytokine, to be over-represented in patients with more severe disease. Using a genome-wide analysis method, Gu and colleagues (29) identified interferon-related developmental regulator 1 (*IFRD1*) as a potential candidate modifier gene. *IFRD1* has been implicated in cell differentiation and stress responses and has its greatest expression in neutrophils. *IFRD1* has been shown to play a role in neutrophil effector function, but not chemotaxis, in a mouse model, so it might be expected to be associated with impaired neutrophil clearance of bacteria.

The investigation of gene modifiers has expanded beyond their role in severity of lung disease. Bartlett and colleagues (30) identified a candidate gene (*SERPINA1 Z* allele) that was significantly associated with CF liver disease and portal hypertension. The polymorphism is uncommon (~2.2% of patients) but the odds ratio for association with liver disease is relatively high (~5) (30). Blackman and colleagues (31) found that the presence of a variant in *TCF7L2*, previously associated with type 2 diabetes, increased the risk of CF-related diabetes approximately threefold in patients with CF and decreased the mean age of onset by 7 years. These results suggest that CF-related diabetes and type 2 diabetes share at least one common mechanism.

Identification of candidate gene modifiers is a big step forward in our understanding of CF disease. Although the effects of these polymorphisms are not known, such findings could lead to potential therapeutic targets for CF or identification of risk factors early in life.

MICROBIOLOGY

The mechanism of acquisition and maintenance of bacterial infection in the CF airway is unclear. Bragonzi and colleagues (32) demonstrated an alteration in the virulence phenotype of *P. aeruginosa* isolates from the airways of six patients with CF. This "microevolution" led to decreased ability of the serial *P. aeruginosa* isolates to cause acute mortality in murine models but did not alter their ability to produce chronic infection and extensive inflammation.

Bacterial growth in biofilms in the CF airway is associated with decreased susceptibility to antibiotics, even when given in combination (33). The formation of biofilms in culture is dependent on iron, which may be more abundant in the CF airway (34). Therefore, iron chelators are appealing as therapeutic adjuncts for the treatment of endobronchial colonization. O'May and colleagues (35) found that several iron chelators were effective in preventing biofilms *in vitro*, especially when cultures were grown under anaerobic conditions. Moreau-Marquis and colleagues (36) demonstrated that the use of clinically available iron chelators in combination with tobramycin could effectively reduce biofilm formation and lead to enhanced *P. aeruginosa* killing. The combination of deferasirox or deferoxamine with tobramycin could prevent the formation of biofilms by *P. aeruginosa* on cultured CF bronchial epithelial

cells (CFBE41o$^-$) that overexpress F508del-CFTR. These treatments could also disrupt established biofilms, thus leading to greatly enhanced antibiotic susceptibility.

Although *P. aeruginosa* is found in airway secretion in up to 80% of patients with CF (37), other bacteria likely play a role in the pathogenesis of CF airway disease. Evidence is accumulating that anaerobes may play a role in CF airways disease (38). Worlitzsch and colleagues (39) found that one or more obligate anaerobes, which were often resistant to anti-pseudomonal antibiotics, could be detected in sputum samples from 41 of 45 patients with CF aged 6 to 64 years. Analysis of serial cultures suggested that these organisms could persist in the CF airway for at least 11 months.

The reason for persistent airway infection of the CF airway, despite the presence of abundant neutrophils, is unknown. Voglis and colleagues (40) proposed a novel explanation for this paradox. They found that human neutrophil peptides (HNP), which are found in high concentration in the CF airway, inhibited neutrophil phagocytosis of bacteria. This effect was associated with reduced Fcγ RIII expression, disruption of the F-actin filament network, increased intracellular calcium concentration and degranulation. Furthermore, the antibiotic effect of HNP was diminished in the presence of neutrophils. The diminished antibacterial effect of HNP and neutrophils in the CF airway may be explained by their direct interaction.

EARLY INTERVENTION

There is accumulating evidence that CF airways disease begins early in life. Newborn screening has the potential to allow therapy to be instituted before symptoms arise. However, Sly and colleagues (41) found that significant pulmonary pathology may be present at the time of diagnosis by newborn screening. Their comprehensive evaluation of 57 infants (median age 3.6 mo) revealed lower airways bacterial infection in 12 (21.1%) and evidence of neutrophilic inflammation including 44 (77.2%) with detectable IL-8 and 17 (29.8%) with detectable neutrophil elastase. Forty-six (80.7%) children had abnormal CT findings.

Early intervention is of vital importance insofar as some abnormalities may not be reversible. Harrison and colleagues (42) demonstrated that airflow obstruction present at a mean age of 7.4 months was correlated with spirometry abnormalities observed after the age of 6 years. Additionally, Lai and colleagues (43) demonstrated that children who did not recover appropriate nutritional status by the age of 2 years had diminished lung function and more symptoms at 6 years compared with children who had better nutrition at 2 years of age.

P. aeruginosa colonization of the CF airway has previously been associated with increased inflammation, more rapid decline in lung function, and increased mortality (44). Sagel and colleagues (45) demonstrated that the presence of *P. aeruginosa* in the lower airways leads to increased airways inflammation (as measured by bronchoalveolar lavage [BAL]), cytokine and neutrophil profiles, and poorer clinical status. The degree of inflammation was further increased by the presence of *Staphylococcus aureus*. Therefore, strategies aimed at "eradication" of *P. aeruginosa* when it is first detected are very attractive. Douglas and colleagues (46) evaluated results from more than a decade of surveillance BAL cultures obtained from 116 children with CF less than 6 years of age. *P. aeruginosa* was detected in 28.4% of children at a median age of 30.5 months. The *P. aeruginosa* had a mucoid phenotype in 18.2% of the cultures. The good news was that therapy was successful in eradicating the *P. aeruginosa* in 88% of those children who were treated with up to three cycles of antibiotic therapy. Although there has been concern that the aggressive treatment of *P. aeruginosa* early in life may lead to antibiotic resistance, this does not appear to be the case (47).

Clinicians use symptoms (e.g., cough) as indicators of significant lower airways disease. However, the finding that 84.2% of infants (41) and 51.5% of children with *P. aeruginosa* (46) were asymptomatic suggests that this approach may underestimate lung pathology in young children with CF. Further complicating the evaluation of young children with CF is the finding that *P. aeruginosa* can be cultured from the oropharynx of infants without CF (48).

GUIDELINES

Therapeutic progress has been realized in the last two decades with improved health, quality of life, and overall survival (37). However, patients with CF still suffer daily symptoms, frequent exacerbations of pulmonary infection, and an early demise. Therefore, until additional effective therapies can be developed, we need to optimize the use of existing therapies. Approaches include consensus statements describing best practices and, when possible, evidence-based guidelines using systematic methodologies. Newborn screening for CF is now widely accepted as beneficial and is increasingly offered, but there is considerable variation in newborn screening programs for CF. New guidelines describing best practices for newborn screening (49) and how to manage those infants with an equivocal diagnosis (50) represent a big step toward standardization of practice and should prove useful for new programs.

CF is a chronic disease for which chronic therapies are required to slow the progression of disease. There are previously published guidelines for the chronic use of medications (51), and now we have guidelines for airway clearance therapies (52) to maintain lung health. A systematic review of the literature found sufficient evidence to recommend that airway clearance be started early and performed daily. Although no form of therapy has proven superior to others, this does not mean that all will be equally effective in every patient, and a number of factors were provided in the guideline that should guide the prescription for therapy, especially patient preference.

Episodic exacerbations of airways disease occur commonly in patients with CF (37), and we now have guidelines on treatment of the acute pulmonary exacerbation (53). Perhaps the most striking aspect of this systematic review was how little we know about such a common complication of CF. What is clearly needed is a standard definition for a pulmonary exacerbation that can be used to study important questions, such as the optimal duration of antibiotic therapy. This would be the first step toward determining optimal therapeutic approaches to exacerbation therapy. Establishing more sensitive therapeutic endpoints is also key. For example, Klein and colleagues (54) found that ^{18}F-fluorodeoxyglucose-PET/CT imaging may provide a sensitive methodology to monitor the effectiveness of both chronic maintenance and exacerbation therapies.

PATIENT-REPORTED OUTCOMES AND NEW THERAPIES

The development of meaningful patient-reported outcome (PRO) measurements that can be incorporated into clinical care or used as endpoints for clinical trials is underway. Patient-reported outcomes are defined by the Food and Drug Administration as "any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else" (55). The CF Questionnaire Revised (CFQ-R) is a validated PRO measure that has been in use for some time (56). Quittner and colleagues (57) used data from two studies of inhaled aztreonam to de-

termine the minimal clinically important difference in respiratory symptom domain for the CFQ-R. Understanding the relationship between PRO measurements and clinical outcomes is of paramount importance in assuring that measurements are clinically useful. In a study of 223 adults at two CF Centers in the United Kingdom, Abbott and colleagues (58) found that the physical functioning domain of the CF Quality of Life (CFQoL) and the pain domain of the Short Form-36 (SF-36) questionnaires are important predictors of survival. Goss and colleagues (59) are developing a "CF Respiratory Symptom Diary" containing 8 patient-reported symptoms to better define pulmonary exacerbations. Further validation of this instrument is still needed.

Treatment of chronic *P. aeruginosa* infection of the airways with inhaled antibiotics holds great appeal. Retsch-Bogart and colleagues (60) has demonstrated that short-term therapy with inhaled aztreonam is effective in improving lung function. Patients treated with 28 days of inhaled aztreonam (75 mg, t.i.d.) had a 10.3% increase in FEV_1 compared with those patients who received a placebo. This improvement in lung function was associated with a decrease in *P. aeruginosa* in sputum and improved respiratory symptoms as measured by the CFQ-R.

LUNG TRANSPLANTATION

Since the publication of a controversial analysis of lung transplantation outcomes in pediatric patients with CF (61), there has been considerable interest about this procedure in this population. Some have already taken issue with that original paper (62), but there are also new observations that aid our understanding of the role for transplant in patients with CF. A retrospective review of an organ transplant registry identified 1,637 patients with CF who underwent lung transplantation between 1999 and 2007, of whom 245 (15%) were children (63). Their analysis revealed that older patients with CF have improved cumulative survival when compared with younger patients. This is not a surprising finding, as it has been seen in other indications for transplant.

Most analyses of pediatric transplant define pediatric patients as those under 18 years of age. An analysis of school-age (6–10 yr) CF patients with lung transplants showed no significant difference in the 1-, 3-, and 5-year rates of overall survival following lung transplantation, compared with children without CF of similar sex and age (64). These findings are encouraging, in that our pediatric patients with CF who have advanced stage disease are not at a greater disadvantage than children with other lung diseases.

But lung transplantation is not a cure, and this option remains a difficult choice for patients. Vandemheen and colleagues (65) developed an evidence-based decision aid for patients with CF and severe lung disease. A randomized controlled clinical trial was then performed to determine whether this decision aid improved the quality of decision-making for patients when considering referral for lung transplantation in comparison with usual care. They found that use of the decision aid improved realistic expectations of lung transplantation. If incorporated into general practice, this decision aid might better prepare patients for the post-transplantation experience.

CONCLUSION

Our increased understanding of how mutations in CFTR disrupt cellular function and the identification of genes that modify the phenotype (or severity of disease) have led to a new era of potential therapeutics that may increase the survival and quality of life of patients with CF However, until new and more effective drugs become available, the appropriate application of currently available therapies is key to improving the lives of our patients. Early intervention and application of best practices, to optimize nutrition and lung function and prevent airway colonization by *P. aeruginosa*, are crucially important.

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