Pulmonary, Sleep, and Critical Care Updates

Update in Cystic Fibrosis 2012

Christopher H. Goss^{1,2} and Felix Ratjen³

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Washington, Seattle, Washington; ²Division of Pulmonary Medicine, Department of Pediatrics, Seattle Children's Hospital, Seattle, Washington; and ³Division of Respiratory Medicine, Department of Pediatrics, and Program in Physiology and Experimental Medicine, SickKids Research Institute, Hospital for Sick Children, and University of Toronto, Toronto, Ontario, Canada

Although cystic fibrosis (CF) remains a life-limiting disease, we have witnessed major advances in the care and clinical outcome of persons with CF (1). Animal models are shaping our understanding of the early pathophysiological changes in the CF airway (2), but significant challenges persist in understanding key components of airway infection, airway inflammation, as well as how to translate this knowledge into treatment of persons with this lung disease. We highlight some of the important scientific developments in CF during 2012, focusing on infection, airway inflammation, novel treatments, and issues surrounding lung transplantation.

PATHOPHYSIOLOGY

Although it is unclear whether sodium hyperabsorption is a primary or secondary event in the CF airway, most data would suggest that dysfunction of the cystic fibrosis transmembrane conductance receptor (CFTR) protein changes the balance of chloride secretion and sodium absorption to an absorptive state that leads to airway surface dehydration. Airway surface liquid depletion is thought to impair mucociliary clearance and reduced ability to clear bacteria from the lower airways. However, CFTR has other functions beside chloride transport, and data using a pig model of CF have raised a question concerning whether airway surface liquid depletion alone explains bacterial persistence in the airways. In a series of experiments, Pezzulo and colleagues demonstrated that reduced bicarbonate secretion through CFTR lowers pH in CF airways compared with wild type and that this alteration in pH reduces the epithelium's ability to kill bacteria placed on the airway surface; a process that could be reversed by increasing surface pH (2). This raises a question concerning whether inhalation with bicarbonate could potentially be used as an early intervention strategy to improve bacterial clearance in CF.

One of the roadblocks to study response to disease-modifying interventions in CF is the lack of valid patient-derived model systems. At present, the major source of bronchial epithelial cells is from CF lungs explanted during lung transplantation. These cells are not renewable and the supply is rather limited. Two new stem cell-based approaches were successfully pursued in 2012: In the

first study transformation of induced pluripotent cells into airway epithelium could be successfully demonstrated (3). Wong and colleagues used skin-derived stem cells from patients with CF and not only could demonstrate the generation of airway epithelial cells with ciliated morphology, but could also show that correctors of CFTR function could be successfully tested in these transformed cells (4). This opens the possibility to generate a renewable source of CF airway epithelial cells for *in vitro* studies. In addition, patient-derived tissue could potentially allow individualized testing of drugs in patients with CF; which ultimately could be the most suitable approach to capture variability in treatment response.

CYSTIC FIBROSIS MICROBIOLOGY

There is growing evidence that the microbiological ecology of the CF lung is rather complex and we are just at the beginning of understanding the relevance of individual bacteria detected in the CF airway microbiome (5). However, the role of *Pseudomonas aeruginosa* in CF lung disease is well established, as chronic infection negatively affects clinical outcome in patients with CF. This is further supported by data demonstrating that sputum concentrations of phenazines, small molecules produced by *P. aeruginosa*, are correlated to lung function decline (6). Similar to *P. aeruginosa*, chronic infection with methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with steeper lung function decline and increased mortality in patients with CF (7, 8). The role in disease progression of a number of other pathogens, notably *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*, is evolving and poses significant clinical challenges. Current maintenance therapy of inhaled antibiotics is directed toward *P. aeruginosa*, but broader spectrum antibiotics could potentially be more beneficial by targeting other bacteria as well. One study assessed the effect of a combination of fosfomycin, an antipseudomonal antibiotic, with *in vitro* activity against staphylococci including MRSA as well as anaerobic bacteria, and tobramycin (fosfomycin/tobramycin for inhalation; FTI) in adult patients with CF (9). Lung function improved in both treatment groups during the run-in phase, in which all patients received inhaled aztreonam. Patients subsequently switched to a 28-day course of FTI maintained lung function whereas it decreased to baseline in the placebo group. However, it remains unclear whether this treatment effect could have been achieved with fosfomycin alone as the tobramycin doses in this study (20 and 40 mg) are well below the concentrations that have been used in previous studies of inhaled tobramycin. Interestingly, one-quarter of the patients were coinfecting with MRSA and FTI resulted in a remarkable reduction in sputum density of MRSA in these patients. This suggests that fosfomycin could potentially be a useful antibiotic in patients with CF with chronic MRSA infection, for whom currently treatment options are rather limited.

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Correspondence and requests for reprints should be addressed to Christopher H. Goss, M.D., M.S., University of Washington Medical Center, Campus Box 356522, 1959 N.E. Pacific, Seattle, WA 98195. E-mail: goss@u.washington.edu

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Although the role for many bacteria found in CF sputum is not well defined, this is even more the case for fungi such as *Aspergillus fumigatus*. The rate of sputum positivity in patients with CF is high, but only a fraction of patients develop the well-established clinical manifestation of allergic bronchopulmonary aspergillosis (ABPA). Whether the detection of *A. fumigatus* in CF airways is clinically relevant in patients without ABPA is less clear, although pulmonary exacerbation rates are higher in patients chronically positive for *A. fumigatus* (10). Studies provide further support for the concept that *A. fumigatus* may not be an innocent bystander in CF lung disease. Chaudhary and colleagues used a combination of *in vitro* and *in vivo* studies to demonstrate that *A. fumigatus* uptake by CFTR-deficient cells is reduced, but that epithelial cell apoptosis after exposure to *A. fumigatus* is increased (11). This raises a question concerning whether CFTR plays a role in intracellular uptake of *A. fumigatus* as previously demonstrated for *P. aeruginosa* (12). Responses in inflammatory mediators were not consistent, but overall supported an exaggerated inflammatory response to *A. fumigatus*, a finding that was supported by higher inflammatory cell burden in *cftr*-deficient mice after *A. fumigatus* exposure (10). Interestingly, the cellular response as well as the cytokine profile would support stimulation of a helper T-cell type 2 (Th2) response, which differs from the neutrophil-dominated response triggered by both bacteria and viruses in CF.

Vitamin D deficiency has been implicated as a risk factor for ABPA and deletion of the vitamin D receptor increases Th2 cytokines and airway inflammation in animal models. Coughlan and colleagues explored the role of *A. fumigatus* on vitamin D receptor expression as well as the effect of azole treatment both *in vitro* and in a prospective cohort study in patients with CF (13). Gliotoxin produced by *A. fumigatus* was found to down-regulate vitamin D receptor expression, a process that could not be counteracted by vitamin D. Using bronchoalveolar lavage in a small sample of patients with CF, the authors confirmed that Th2 cytokines are increased in patients with *A. fumigatus*-positive CF; treatment with itraconazole decreased airway inflammation and was also associated with improved air trapping (13). However, the clinical part of the study needs to be interpreted with caution as it lacked a control group and a placebo-controlled pilot study did not demonstrate any effects of itraconazole treatment on clinical outcomes (14). Nevertheless, these reports highlight the need to further understand the role of *A. fumigatus* in CF lung disease and to design sufficiently powered interventional studies to define the patient population that could potentially benefit from antifungal therapy.

AIRWAY INFLAMMATION IN CF

Although infection is considered the major trigger of airway inflammation, the response to any proinflammatory stimuli is altered in CF and neutrophil-dominated inflammation persists in the majority of patients. Airway inflammation is thought to be an independent risk factor for lung function decline and biomarkers of inflammation could potentially be used to define an at-risk population (15). Previous cross-sectional studies have provided evidence that sputum neutrophil elastase (NE) levels are higher in patients with severe lung disease (16) and elastase burden in bronchoalveolar lavage is correlated to lower lung function and bronchiectasis in infants with CF (17). In a longitudinal cohort study Sagel and colleagues demonstrated that elevated neutrophil elastase concentrations in sputum predict subsequent lung function decline and that longitudinal changes in NE are correlated to evolution of lung function (18). This study not only highlights the importance of NE as a biomarker of lung damage, but also supports the concept that inhibition of

NE is a potentially viable treatment option that should be further explored in future interventional trials.

As inflammation contributes to lung damage and disease severity varies widely within patients carrying the same CFTR genotype, genes affecting key components of the inflammatory response may modify disease severity in patients with CF. One of these proposed genetic modifiers is the gene encoding the macrophage migration inhibitor factor (MIF). Although previous studies described an association between a repeat polymorphism in the MIF gene and earlier acquisition of *P. aeruginosa* (19), the same group has now demonstrated that higher repeat numbers in this gene are linked to higher MIF production and more rapid decline of lung function in patients with CF (20). In a series of animal experiments the authors could also provide evidence that MIF deficiency or inhibition attenuated inflammation and disease severity in *P. aeruginosa*-infected mice. Using a small molecule to inhibit the tautomerase-regulating MIF also reduced MIF production in mononuclear cells from patients with CF (20). Although drugs inhibiting MIF tautomerase are currently not available, these elegant experiments have defined a potential new target for antiinflammatory therapy in CF.

NEW THERAPIES

Advances in our understanding of the pathophysiology of CF and from *in vitro* studies have led to an increasing array of therapies available for the treatment of patients with CF. One common approach to treatment has been to enhance defective mucociliary clearance in the CF airways rehydrating the mucus and airway lining fluid in patients with CF. The first of such therapies to gain widespread use has been hypertonic saline (7% NaCl) solution (21). Additional work has suggested that hypertonic saline use in CF could also mitigate inflammatory processes in the CF lung by reducing sputum IL-8 levels (22). Despite compelling evidence that hypertonic saline has clinical benefit in persons with CF over age 6 years, the Infant Study of Inhaled Saline in Cystic Fibrosis (ISIS), a multicenter, randomized, double-blind, placebo-controlled trial assessing the impact of hypertonic saline compared with normal saline (0.9% NaCl) on infants with CF ages 4–60 months, failed to demonstrate a reduction in pulmonary exacerbation rates, the primary end point of the trial (23). In contrast, in a substudy evaluating infant pulmonary function testing, forced expiratory volume at 0.5 second (FEV_{0.5}), a measure comparable to FEV₁ in older subjects, was significantly higher at study completion in infants treated with hypertonic saline. This study points out some of the major challenges to demonstrating efficacy in children with CF in the setting of very early disease. Advancing our abilities to assess early lung disease using other physiological measures such as the lung clearance index or structural injury via imaging techniques will be key in assessing efficacy of therapies early in the disease (24).

An alternative approach to improving clearance of mucus from the CF airway is to administer a nonabsorbable sugar to enhance an osmotic gradient to hydrate the mucus and pericellular lining fluid and thus increase mucociliary clearance. One of the significant successes in 2012 was the assessment of inhaled mannitol given as a dry powder inhaler. Aitken and colleagues performed a phase 3 multicenter, randomized, double-blind, placebo-controlled trial comparing 400 mg of dry powder mannitol twice per day with 50 mg of dry powder mannitol twice per day for 26 weeks, followed by 26 weeks of open-label treatment (25). The study narrowly missed a statistically significant increase in their primary end point (absolute change in FEV₁ in milliliters), but lung function improvements were also seen in the control group that received a low dose of mannitol, which also may

have some efficacy. The study did demonstrate statistically significant improvements in relative FEV₁ and absolute and relative FEV₁% of predicted. The safety profile of the 400-mg dose appeared similar to that of the 50-mg dose with no evidence of a difference between the treatment arms in *Pseudomonas aeruginosa* density in the sputum. However, the treatment was associated with a higher rate of hemoptysis and a higher rate of treatment-related adverse events leading to treatment withdrawal. A parallel phase 3 clinical trial conducted in Australia, New Zealand, and Europe demonstrated improved FEV₁ and reduced pulmonary exacerbation rate in the treatment arm (26). Taken together, as noted in the accompanying editorial (27), this therapy appears to be efficacious in CF at improving lung function. The agent is currently available in Europe for persons over 18 years of age and in Australia and New Zealand.

Although much attention in CF therapies has centered around the advances in care provided by the CFTR potentiator ivacaftor to individuals with CF with the CFTR gating mutation G551D (28), ultimate success for controlling CF lung disease in patients with other CFTR mutations will likely require a multitude of approaches to reverse the primary gene defect. Gene therapy continues to hold some promise, but has a number of key hurdles to overcome to become a therapeutic option. Challenges include low rates of gene transfer, toxicity, and development of immune response to vectors that require repeated administration. Griesenbach and colleagues presented data on a novel lentiviral vector (simian immunodeficiency virus [SIV]) pseudotyped with the Sendai virus envelope proteins F and HN (29). Gene expression was assessed with luciferase expression, quantified by *in vivo* bioluminescence imaging. After only a single induction by nasal sniffing in a mouse model, long-term gene expression was found up to 22 months; all of the vector-transduced mice had detectable luciferase expression at all time points. The authors could also demonstrate that the vector needed only brief contact with the epithelium to achieve transduction. Daily and monthly administration of the vector was feasible and the toxicity profile was good in the mouse model employed. In support of the data in mice, the vector showed lasting gene expression in three different *ex vivo* human models (differentiated human air-liquid interface cultures; primary human airway epithelial cells; and precision-cut human lung slices). Although these initial results are exciting, the ultimate challenge is to prove that the vector can be successfully administered to humans with CF. The lungs of patients with CF remain an inhospitable place with high levels of proteases, thick tenacious mucus, and large areas of poorly ventilated lung; especially in patients with established lung disease.

In addition to new therapies in CF clinical trials, we have seen important advances in clinical outcome assessments. Quinton and colleagues have demonstrated that β -adrenergically stimulated sweat secretion measured with a highly sensitive evaporimeter is an alternative method of assessing CFTR function (30). Sweat secretion rates could discriminate between patients with CF, healthy heterozygous carriers, and noncarriers of CFTR mutations; the classic sweat chloride test using quantitative pilocarpine iontophoresis is unable to discriminate between healthy carriers and noncarriers. Given the findings of investigations of the CFTR potentiator ivacaftor for persons with CF with the CFTR gating mutation G551D (28), measuring CFTR activity *in vivo* is essential to evaluate future CFTR-directed therapeutics.

LUNG TRANSPLANTATION

When therapies directed at improving or stabilizing lung function fail, persons with CF have the option to move forward with

double-lung transplantation. One of the key clinical questions that confront CF providers is when to refer a patient with CF for lung transplantation. The most accepted point of referral is when the patient's FEV₁ is no greater than 30% of predicted or with the development of hypercarbia (31, 32). Advances have added key assessments that could improve prognostication in severe CF and have implications for the timing of lung transplantation. Loeve and colleagues took advantage of existing data and evaluated the predictive ability of scored computed tomography (CT) scans of the lungs of patients referred for lung transplantation, a test performed in all subjects who undergo a referral for lung transplantation (33). The study involved blinded scoring by one observer of CT scans from 411 subjects who had been listed for lung transplantation on the basis of the Severe Advanced Lung Disease (SALD) four-category scoring system. Elements of the SALD were correlated with lung function and more importantly were associated with survival independent of the lung allocation score. Interestingly, large areas of the lungs showed nonstructural changes such as air trapping and thus potentially reversible alterations, raising a question concerning whether more effective therapy could potentially improve lung function and clinical status in these patients who to date have been considered to have irreversible lung disease.

A complementary study by Vilozni and colleagues took a new look at an old technique—spirometry (34). They studied over an 8-year period the relationship of the ratio of low flow to lung volume (forced expiratory flow between 25 and 75% of vital capacity and forced vital capacity [FEF₂₅₋₇₅/FVC]) on outcome in 93 patients with CF. The patients were distributed into three groups (FEV₁ > 80% predicted, FEV₁ $\leq$ 80% predicted, and those waitlisted for lung transplantation). Serial FEF₂₅₋₇₅/FVC measurements demonstrated a striking decline greater than ± 2 z scores 4 years before transplantation, differing significantly from the gradual decline in all other indices such as FEV₁/FVC. Those subjects with a bronchodilator response or growing *Mycobacterium abscessus* also were noted to have a significantly greater decline in FEF₂₅₋₇₅/FVC over time. The sample size of the study was relatively small (particularly the subgroup of interest) and represented only one CF center, leading one to interpret the results with caution. However, given the ready availability of this index, the FEF₂₅₋₇₅/FVC ratio should be further assessed as a tool to better prognosticate lung function decline in CF (35).

When to refer patients to lung transplant represents just part of the assessment; it is also important to address what happens after referral. Quon and colleagues conducted a provocative analysis of data from the U.S. Cystic Fibrosis Foundation Patient Registry (36). The authors assessed whether socioeconomic status impacted the chances of listing for lung transplantation after referral. They found that the odds of not being accepted on the transplant list was 1.56-fold higher for patients with CF ensured with Medicaid compared with non-Medicaid-ensured patients after adjusting for markers of disease severity, relative contraindications for transplantation, and demographic characteristics. This association was independent of other indicators of socioeconomic status (race, education, median household income) and driving distance to the closest transplant center. Having less than a high school education and living in a ZIP code with a median household income less than 200% of the Federal Poverty Level were also associated with higher odds of not being listed after referral. As noted in the accompanying editorial, although the authors may have identified a disparity in access to a treatment option, lung transplantation is an unusual treatment that dictates that a center distribute the limited organs to achieve the greatest good (37). Characteristics associated with low socioeconomic status could potentially also

be associated with poor post-lung transplantation survival. Understanding the mechanisms leading to the differences noted by Quon and colleagues will be key to assessing the means of reducing disparities.

CONCLUSIONS

The CF community has seen tremendous successes in 2012. Survival continues to improve and, for the first time, an agent that activates mutant CFTR (the potentiator ivacaftor) was approved by the U.S. Food and Drug Administration. This advance was a major breakthrough in drug development for CF and represents a paradigm for drug development in the future for other CF mutations and other rare disorders. CF drug development is quickly moving toward gene-specific therapy and personalized medicine (38). A significant number of compounds are already in CF clinical trials employing similar approaches to drug development as for ivacaftor. Despite these advances and excitement, clinicians will need to continue to focus on ensuring that patients are both prescribed indicated therapies and achieve adequate adherence to those therapies by understanding the barriers to high adherence (39).

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