Update in Cystic Fibrosis 2006

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Cystic fibrosis (CF) is a complex inherited condition resulting from abnormalities in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR, a membrane glycoprotein present in certain epithelia, contributes to regulation of ion flux across the cell surface in a number of ways, including direct chloride channel activity. The clinical picture of CF includes variable, but often severe, injury to the primary organs involved (exocrine pancreas, lung, sinus, liver, intestine) as well as a staggering array of secondary complications (including polymicrobial infection, malnutrition in many forms, hypoelectrolytemia, diabetes, vasculitis, nasal polyps, and pulmonary hypertension). Chronic, progressive lung disease results in most of the morbidity and mortality in CF and is therefore the main focus of clinical care and research.

Care and research in CF have developed in parallel since the first clear definition of this condition in the 1930s (1). The curious elevation of sweat electrolytes seen in CF, first described in the 1950s, resulted in a robust diagnostic test. Comprehensive care centers soon followed and have evolved to include newer quality improvement and evidence-based approaches. The discovery and characterization of the abnormal gene in 1989 opened up many paths to research in model systems and in patients. It is now appreciated that CF airway disease involves intricate interrelationships among airway surface liquid, mucus clearance, infection, inflammation, repair, and fibrosis.

CELL BIOLOGY

Intracellular Trafficking

Intracellular trafficking of wild-type and mutant CFTR has been the subject of dozens of reports in the past year. This is because the most common mutation in CF, ΔF508, results in almost complete degradation of CFTR during endoplasmic reticulum processing. Treatment of this mutation is a central goal of research in CF because approximately 90% of all individuals in the United States with CF have at least one copy of the ΔF508 mutation. Wang and colleagues, through an elegant proteomic approach, described the CFTR interactome, the complement of intracellular proteins that interact with CFTR (2). More than 200 proteins were identified, providing a foundation for more detailed study of individual protein complexes. Using small interfering (si)RNA, they further showed a special role for Aha1, part of the heat shock protein-90 complex, in premature degradation of the CFTR. Other therapeutic targets have emerged from studies using a variety of approaches (3).

GENETICS

Modifier Genes

Clinical course in CF is remarkably variable, even when controlling for CF genotype. It is still unclear how much of this variability can be explained by environment, how much by genetics, and how much by the interaction of environment and genetics. Identification of modifier genes could provide clues to pathogenesis as well as to new treatments. One approach to identifying modifier genes is to target a specific gene that is important in a presumed pathway of injury. McKone and coworkers examined in patients with CF a gene that is key in oxidant injury, glutamate-cysteine ligase (4). This study made use of several centers and very careful modeling of lung function to demonstrate an association of variations in this gene with lung function among patients with mild CFTR genotypes. This modifier gene explained only 4% of the variation in FEV_1. However, these results provide impetus for additional studies of interventions that improve oxidant–antioxidant balance in CF.

Intestinal disease in CF is another major component of morbidity and occasionally mortality. Blackman and coworkers, in a large twin study, confirmed that meconium ileus, an intestinal obstruction at birth occurring in 20% of newborns with CF, is indeed heritable, implying the existence of modifier genes (5). They further identified three regions of the genome, through use of whole genome scanning, that potentially could modify the occurrence of meconium ileus. Interestingly, they could not confirm linkage to a region on chromosome 19 that had been previously linked to meconium ileus.

PATHOPHYSIOLOGY

Small Airways

The small airways are involved early in CF pathogenesis but little is known about the physiology of ion and fluid flux at this level. Blouquit and coworkers examined airway surface liquid height and bioelectric properties of bronchial and bronchiolar epithelial cells from individuals with and without CF in Ussing chamber studies (6). The same abnormalities present in CF bronchial tissue, including decreased airway surface liquid height and lack of response to forskolin, were present in CF bronchiolar tissue. In addition, CFTR activity seemed to produce greater effects in normal bronchiolar tissue than in normal bronchial tissue. This work demonstrated that CFTR-related physiologic abnormalities are present in small airways in CF and also showed that small airway epithelial cells can be studied in CF.

CFTR and Cigarette Smoke

An intriguing article by Cantin and associates links cigarette smoking to CFTR dysfunction in normal individuals (7). They

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first demonstrated that cigarette exposure decreased CFTR expression and function in well-characterized cell lines. They then found that smokers exhibited blunted nasal potential differential responses similar to those that can be seen in CF. These observations raise the possibility that chronic bronchitis or other smoking-related respiratory conditions could be explained in part by decreased CFTR activity. They also underscore the importance of smoking prevention in individuals with CF.

Airway Morphology and Mucous Elements

Histologic studies have shown that several levels of the airway are involved in CF. The epithelium may show varying degrees of denudation. The submucosa is often thickened with prominent glandular elements and there are inflammatory infiltrates around cartilaginous regions. The serous elements of submucosal glands express CFTR in high concentration, yet there are few data on mucous cell and submucosal glands in patients without severe disease. Hays and Fahy performed bronchial biopsies to better characterize mucous cells and submucosal glands in CF (8). They found that goblet cell size but not number was increased in the CF airway. Similarly, submucosal gland size was also remarkably increased but their number was not different from that found in normal airways. No difference in the proportion of gel-forming mucins was seen in epithelium or submucosa from normal subjects, suggesting that mucous cell to serous cell transdifferentiation does not occur. Finally, glandular changes associated with CF do not seem to be related to neutrophil-derived inflammation as there were few neutrophils in the submucosa. Taken together, these findings raise intriguing questions about the mucus-producing elements in CF, goblet cells and submucosal glands.

Repair and Regeneration

The use of stem cells to replace airway epithelium deficient in CFTR has also received attention recently. Loi and coworkers treated Cftr knockout mice with adult bone marrow–derived cells containing normal CFTR (9). After naphthalene injury to increase airway cell recruitment, chimeric cells were evident in the airway in very small numbers. CFTR protein was also detected, but only in 1 cell out of 10,000. This study demonstrated the feasibility of replacement of airway epithelia by exogenous stem cell treatment, but ways to increase expression are clearly needed.

INFECTION

Animal Models

The murine models of CF capture some of the features of the human disease but as of yet do not fully represent the lung involvement. Van Heeckeren and coworkers studied the response to acute *Pseudomonas aeruginosa* lung infection in CF and control mice (10). They found that CF mice experienced higher mortality, greater weight loss, and higher levels of inflammation (neutrophils and mediators) compared with control mice. Inflammation was prolonged in CF mice compared with control mice. Chronic infection could not be established in CF or wild-type mice. This study suggests that lack of CFTR activity alone can lead to exaggerated inflammation in mice. In humans, other factors or perhaps a different role for the CFTR might be needed to permit the establishment of permanent infection.

Biofilms

Bacterial biofilms are increasingly seen as important in CF pathogenesis and almost certainly contribute to the inexorable spread of infection. In addition, biofilms present strong interference with antibiotic activity. Although there has been a good deal of biofilm research in *P. aeruginosa*, nontypeable *Haemophilus influenzae* (NTHi) is the initial organism usually retrieved from the CF airway in infants and young children. Starner and coworkers provide evidence that NTHi can form biofilms *in vivo* and *in vitro*. In addition, through coculture with airway epithelium, they found that the biofilm form of NTHi stimulated an inflammatory response (11). This work raises questions as to the early treatment of CF; in particular, whether NTHi should be treated in addition to *Staphylococcus aureus* and *P. aeruginosa.*

Burkholderia dolosa

It has been known for several decades that *Burkholderia cepacia* complex bacteria can be associated with poor outcome in CF. A newly characterized member of this family, *Burkholderia dolosa* (genomovar VI), has been found to accelerate the decline in lung function and significantly increase short-term risk of death in CF (12). *B. dolosa* appears to carry an epidemic risk as well. The devastating impact of this bacterium in one CF clinic has sparked a renewal of the debate about whether each patient with CF should be seen with full infection control precautions.

Fungal Colonization

Fungal colonization of the airway is very common in CF. Approximately 5 to 10% of patients have full allergic bronchopulmonary aspergillosis (ABPA), which carries significant morbidity. Improved diagnostic and monitoring techniques in patients with ABPA are needed. On the basis of murine studies, Hartl and colleagues examined circulating levels of thymus and activation regulated chemokine (TARC) and other candidate cytokines in subjects with CF and a variety of control subjects (13). They found that TARC levels hold great promise in distinguishing patients with ABPA from patients colonized with aspergillus and also in predicting ABPA exacerbations. ABPA was shown to be a risk factor for increased lung function decline, especially involving the small airways, in another study (14). Finally, Shoseyov and coworkers raise the possibility that aspergillus may be a cause of endobronchitis in CF in the absence of ABPA. This opens the door for new considerations of antifungal treatment (15).

Nitric Oxide in CF

Nitric oxide is being investigated in CF in a number of contexts because it has effects on airway smooth muscle as well as antiinfective and antiinflammatory activity. Yoon and coworkers reported that acidified nitrite derivatives can kill *P. aeruginosa* under anaerobic conditions that could be encountered in the CF airway (16). This study supports further investigation into effects of nitric oxide in CF-associated infection. Grasemann and coworkers examined nebulized L-arginine, the precursor of nitric oxide formation, as a treatment in CF (17). In this well-controlled study, acute administration of nebulized L-arginine improved lung function and oxygen saturation, paving the way for larger trials of longer duration.

INFLAMMATION

The role of inflammation in CF airway disease is an area of concentrated research. Neutrophils and their products are believed to be of key importance in CF. A potential role of platelets in CF lung disease has recently been proposed (18). Platelets are dysfunctional in most patients with CF and may contribute to inflammation through nitric oxide– and lipid mediator–related effects.

Assessment of Inflammation through Imaging

It is clear that neutrophil-dominated inflammation has the potential to mediate bronchiectasis and fibrosis encountered in the CF

lung. However, there have been no accepted ways of quantifying inflammation in the CF lung or, going the next step, to determine regional differences in inflammation. Chen and coworkers proposed the use of positron emission tomography to quantify pulmonary inflammation in CF (19). This scanning technique, which makes use of labeled fluorodeoxyglucose uptake in the lung, essentially gives a picture of neutrophil activity in the lung. Through this technique, they found that patients with CF had increased uptake compared with that in control subjects. Within the CF group, patients with increased decline in lung function had further increased uptake. Results from positron emission tomography scanning could be correlated with bronchoalveolar lavage neutrophil counts. This imaging technique holds promise as a way to identify patients with increased inflammatory burdens, which may guide therapy and aid with stratification for treatment trials.

CLINICAL INVESTIGATIONS AND TRIALS

Hypertonic Saline

Two recent landmark trials found benefit of hypertonic saline inhalation in CF. In a large multicenter trial, Elkins and coworkers treated patients with CF who were older than 6 years with 7% hypertonic saline or normal saline twice a day for 48 weeks (20). The group receiving hypertonic saline had strikingly fewer exacerbations and better overall lung function. Donaldson and coworkers studied administration of hypertonic saline four times a day with or without amiloride over a 2-week period (21). They reported improvement in lung function and mucociliary clearance in the group treated with hypertonic saline. Taken together, these studies argue strongly for a role for hypertonic saline in regular treatment of CF.

Inhaled Corticosteroids

As new treatments are added to our therapeutic armamentarium, it is important to reevaluate existing treatments. In particular, treatments that are not supported by controlled trials should be reexamined. In a very important step in this direction, Balfour-Lynn and associates performed a multicenter randomized controlled trial of withdrawal of inhaled fluticasone in CF (22). They found that discontinuation of inhaled fluticasone had no effect. It therefore appears that it is safe to withdraw this treatment, resulting in several advantages, including reduced drug burden, fewer adverse effects, and potential financial savings.

Azithromycin

Chronic administration of azithromycin has been shown to be of benefit in patients with CF and *Pseudomonas* colonization. Clement and coworkers demonstrated benefit of chronic azithromycin in children older than 6 years without *Pseudomonas* in a multicenter trial (23). This study raises the possibility that azithromycin should be used more regularly in almost all children with CF.

Lung Function

We need better characterization of lung function in patients with CF to improve investigation of the impact of genetic, environmental, and treatment approaches on outcome. Large multicenter studies can be used toward this end. Schluchter and coworkers provided a careful analysis of different approaches to classify pulmonary disease at varying ages (24). This study has utility for future large trials and also has clinical implications because of the clear predictive value of FEV_1 at age 20 years. Factors relating to risk of death while awaiting transplant have also been recently studied (25).

Nutritional and Metabolic Studies in CF during 2006

A recent multicenter trial of human recombinant growth hormone showed definite benefit in terms of height and weight but not in predicted lung function in prepubertal children with CF (26). It is known, however, that outcome is related to height in CF, suggesting that growth hormone may have a role in treatment of some school-age children with CF.

Risk of osteoporosis is increased in CF, but the mechanisms are only incompletely understood. Shead and colleagues found that circulating osteoclastic precursors in patients with CF were elevated during an acute pulmonary exacerbation (27). This suggests that early and aggressive treatments of exacerbations may also contribute to improved bone health in CF. A multicenter trial of a newly developed recombinant pancreatic enzyme was successful as replacement therapy in patients with CF and pancreatic insufficiency (28). Results from a single-center study of oral supplementation of N-acetylcysteine, an antioxidant, were also encouraging in terms of improvement in airway inflammation (29).

Participation in Clinical Trials

Advances in CF care will involve enrollment of patients into future randomized treatment trials. It is important to understand whether the patients who participate in clinical trials are representative of the population and whether outcome is different in those who participate compared with those who do not participate. Goss and colleagues examined the U.S. Cystic Fibrosis Foundation patient registry and found that those who participate in clinical trials were balanced with respect to sex but were more likely to have private insurance (30). In addition, despite starting from a lower level of lung function, their decline in lung function was less than in patients not participating in clinical trials. This study has immediate implications for how we recruit patients into clinical trials and it is somewhat reassuring that the participants in these trials did not have a worse outcome. In addition, as clinical trials accumulate in CF, there is improving information on baseline abnormalities, which are important to track in such studies (31).

Diagnosis

Patients without the full syndrome of CF but with mutations in CFTR come to medical attention in a variety of ways, including recurrent pancreatitis, elevated trypsinogen levels after newborn screening, and, especially, congenital bilateral absence of the vas deferens (CBAVD) after male infertility evaluations. These patients often undergo evaluation by nasal potential difference and sweat electrolyte testing. Wilschanski and coworkers provided a thorough examination of control individuals, obligate heterozygotes, patients with CBAVD, and pancreatic-sufficient and pancreatic-insufficient patients with CF (32). They found important correlations with number and severity of CF mutations. Beyond that, however, they provide presumptive evidence of change in nasal potential difference and sweat electrolytes that might indicate improvement with new treatments. This is because it is believed that different CF mutations correspond to different levels of CFTR activity. Thus, new treatments could be defined in terms of change in nasal potential difference or sweat chloride.

Newborn Screening for CF

The American Academy of Pediatrics Committee on Newborn Screening outlined requirements for newborn screening programs for CF in 2006 (33). These recommendations came at a time when the number of states that are screening is rapidly increasing. This movement to early diagnosis through newborn

screening will allow treatment trials of very early intervention in CF. Along these lines, Sontag and coworkers presented data on decline in circulating trypsinogen levels in several hundred infants with CF identified through newborn screening (34). They found that decline in trypsinogen is heritable. In addition, decline in trypsinogen provides a biomarker for treatment trials of agents aimed at slowing exocrine pancreatic injury. It is expected that treatment trials in infancy will increase over the next few years.

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