Cystic fibrosis: Bench to bedside 2003

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In the 17 years since the cystic fibrosis transmembrane ion regulator (CFTR) gene and protein were first identified at the Hospital for Sick Children in Toronto, Ontario (1), much has been learned about the primary defect in cystic fibrosis (CF) and how this relates to some of the clinical manifestations of this disease. The CFTR protein regulates airway chloride transport, and is also a regulator of sodium and water transport across the epithelium (2). In addition, it appears to play a role in the regulation of protein assembly and degradation within the cell (3). The complex regulatory network attributed to CFTR has made it difficult to link abnormalities of the gene and protein to the chronic airway infection and inflammation that are hallmarks of this disease. Thus, although the 'cure' for CF remains tantalizingly out of reach, new knowledge of disease pathogenesis and CFTR function has led to the development of novel therapies that have promise in controlling the relentless progress of lung disease (4).

We have also learned that mild CFTR mutations can lead to a number of disorders now referred to as nonclassical CF (5). Some of these patients have chronic sinusitis and a later onset of chronic airway infection (6). Because of preserved exocrine pancreatic function, some patients develop pancreatitis in early adulthood (7). Sweat chloride values, usually in the range of 60 to 90 mmol/L, often are normal (8). Men with specific, mild CFTR defects can have no lung disease or pancreatic malabsorption, but only infertility with agenesis of the vas deferens and obstructive azoospermia (9). Because preservation of even a small amount of CFTR function appears to ameliorate lung disease, strategies have been proposed to augment abnormal CFTR function and enhance ion transport (10).

This manuscript reviews some of the recently developed therapies for managing infection and inflammation in the CF airway, as well as progress toward correcting the abnormal chloride transport associated with CFTR protein malfunction, gene activation and gene replacement therapy, and finally the role of lung transplantation in end-stage CF lung disease.

INFECTION AND INFLAMMATION

Although it is not presently obvious how CFTR protein dysfunction leads to chronic airway infection and inflammation, it is apparent that CF airway disease is associated with neutrophil recruitment, activation and necrosis with spilling of large amounts intracellular DNA and filamentous actin (F-actin) into the airway, and the proliferation of proinflammatory chemokines and cytokines, proteases and reactive oxygen species (11).

Increases in CF life expectancy have largely been attributed to better control of airway infection (3,11). The CF airway is sterile at birth, but soon thereafter, intermittent colonization by *Staphylococcus*, *Haemophilus* and *Pseudomonas* species leads to chronic infection and inflammation, and formation of bacterial biofilm in the airway. Strategies to prevent or eradicate early airway infection with *Pseudomonas* include cohorting of patients to keep those infected with *Pseudomonas* away from those who have not yet been infected; aggressive, early treatment with intravenous and inhaled antibiotics to eradicate *Pseudomonas*, followed by periodic 'tune-ups' using antibiotics; and continuous use of inhaled anti-*Pseudomonas* species antibiotics in an effort to prevent chronic infection. These strategies have been well studied at the CF Center in Copenhagen, Denmark (12,13). At the first isolation of *Pseudomonas*, patients are treated with oral ciprofloxacin and inhaled colistin, and they receive intravenous antibiotics for a fortnight every four months to prevent reinfection. This has led to a significant reduction in chronic *Pseudomonas* infection, and better pulmonary function and nutrition. Elements of this aggressive series of interventions have been adopted by a number of centres throughout North America as part of prospective studies.

Another means of combating airway infection is the use of inhaled antibiotics such as high dose, inhaled tobramycin (TOBI, Chiron Corporation, USA) and inhaled colistin. In patients with established *Pseudomonas* infection, inhalation of tobramycin improves pulmonary function, reduces the number of hospital days and decreases sputum bacterial density (14). There are now data that suggest that early use of inhaled tobramycin may prevent the establishment of chronic *Pseudomonas* infection in infants and young children with CF.

In the environment, *Pseudomonas* species generally lives as a planktonic organism, but in the CF airway, this organism undergoes a transformation, leading to infection with large and well-organized colonies of bacteria embedded in an

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exopolysaccharide matrix called biofilm. Not only are biofilms more difficult to eradicate, but they lead to an intense inflammatory response, and because of alterations of bacterial metabolism and difficulties in penetrating the biofilm, antimicrobial resistance develops. It is thought that reducing pulmonary inflammation prevents the development of biofilm, although some immunomodulatory agents such as the 14- and 15-member macrolide antibiotics may have a direct effect on biofilm formation (15).

Chronic infection also leads to disregulation of airway inflammation, although there are data that suggest that abnormal inflammation and airway tissue destruction precedes chronic infection (16). CF airway inflammation is characterized by an abundance of activated neutrophils, high levels of interleukin-8, tumour necrosis factor-alpha and other proinflammatory cytokines, and the production of oxygen radicals and secretory phospholipases that damage the epithelium and disrupt mucociliary clearance. Neutrophil necrosis spills highly polymerized DNA and F-actin into airway secretions (17). Oral prednisone can improve weight gain, decrease hospital days and increase pulmonary function in some patients with CF, but the long term use of higher doses of prednisone can lead to side effects such as diabetes and poor growth (18). Thus, the use of systemic corticosteroids has largely been abandoned for routine treatment of CF. There have been several small studies of inhaled glucocorticosteroids in patients with CF, but these not have produced definitive evidence of effectiveness in reducing lung inflammation or improving pulmonary function (19).

Oral ibuprofen, at a dose achieving tissue concentrations of 60 to 100 μg/mL, increased weight gain and slowed decline in pulmonary function in young patients with mild CF lung disease (20). Ibuprofen metabolism is highly variable and serum concentrations need to be tightly maintained, because low serum concentrations have been associated with increased activation of airway inflammatory cells, while concentrations greater than 100 μg/mL have been associated with significant side effects. Although the cost of the medication is low, the inconvenience of monitoring has led to relatively few CF patients being chronically treated with ibuprofen.

One of the most important proinflammatory mediators released by activated neutrophils is human neutrophil elastase. This serine protease is highly destructive and induces mucus hypersecretion. Secretory leukocyte protease inhibitor has been evaluated in a pilot study, and over two weeks, it decreased human neutrophil elastase and interleukin-8 in bronchoalveolar lavage fluid (21).

An important concept in understanding the hyperinflammatory response is immunomodulation, which refers to reducing a hyperimmune state that causes tissue destruction without suppressing the baseline level of active host immunity (immunosuppression). The best studied of the immunomodulatory agents are the macrolide antibiotics (22). Studies in Japan dating back more than 20 years have shown that the 14-member macrolides all have significant immunomodulatory activity and are effective for treating diffuse panbronchiolitis, a chronic inflammatory airway disease seen most commonly in Japan and Korea (23). Patients with diffuse panbronchiolitis have intense airway inflammation, sinobronchitis, hypersecretion of mucus and progressive destructive lung disease often associated with chronic *Pseudomonas* tracheobronchitis. Clinically, this disease is similar to CF, except that onset tends to be later in life and it has not been associated with CFTR gene defects. Recent studies using the macrolide clarithromycin and the 15-member azalide azithromycin have shown similar effects in patients with CF (24). Azithromycin has been shown to be highly effective, particularly in patients homozygous for the ΔF508 gene defect (the most common CF gene defect) and those who are not taking dornase alfa (Pulmozyme, Genentech, USA). Most of these patients can expect a 10% or more improvement in pulmonary function after six months, while patients taking placebo would experience declining lung function over the same period of time (25).

ALTERED ION TRANSPORT

The CFTR protein localizes to the apical epithelium of cells, and regulates salt and water transport (26). The P2Y2 agonists typified by uridine triphosphate can activate the (non-CFTR) calcium-dependent chloride channel. Other agents such as duramycin may induce chloride and water transport through the airway epithelium by forming new channels in the airway epithelium. It is hoped that by stimulating chloride and water transport, the CFTR ion transport defect can be bypassed (26).

Mucolytic agents have been studied that reduce the viscoelasticity of airway secretions by depolymerizing DNA (dornase alfa), F-actin (thymosin beta$_4$) or mucin (Nacystelyn). In general, agents that have an effect on the DNA or actin may be more effective in the treatment of CF in which there appears to be no increased mucin in the airway secretions (27).

Airway secretions in CF are adhesive, probably due to surfactant dysfunction (28). In a randomized, placebo controlled trial, it has been shown that the aerosol of surfactant can improve pulmonary function by increasing the clearability of secretions (29).

CF GENE REPLACEMENT THERAPY

The challenges of gene replacement are to package CFTR complementary DNA (cDNA) into a vector that targets abnormal cells and then effectively produces normal CFTR protein. CFTR protein should then be transported to the surface of the cell and function as a chloride channel (30). The earliest efforts at gene transfer using an adenovirus were effective in vitro, but binding of the viral capsule to the airway epithelium induced an intense inflammatory response, making this both ineffective and dangerous. The adeno-associated virus (AAV) is a small, nonpathogenic, single-stranded parvovirus that is replication defective. This virus needs coinfection with a helper virus to replicate. The *rep* gene product allows wild-type AAV to integrate into a specific safe area on chromosome 19. However, the packaging capacity of the AAV is small, making it difficult to insert the cDNA for CFTR along with the regulatory elements needed for safe gene transfer (31).

Vectors introducing cDNA into airway stem cells should achieve persistence of gene transfer without the need for repeat administration. Lentiviruses are retroviruses (like the human immunodeficiency virus) that can transfect even undifferentiated basal cells. If it were possible to target these cells in vivo,

all cells that differentiate from these cells would also carry the CFTR gene. However, lentiviruses insert at random into the genome with the potential for insertional mutagenesis (32).

The most commonly studied nonviral vectors have been liposomes. Cationic liposomes are lipid capsules that form complexes with the lipid bilayer of cells. The more efficiently liposomes fuse with the cell membrane, the more dangerous they are to cell viability, making gene transfer efficiency poor with earlier generation liposomal gene transfer (33).

PROTEIN MODIFICATION

It may not be necessary to affect gene transfer of normal CFTR if the abnormal CFTR protein can be 'taught' to efficiently transport salt and water. The ΔF508 mutation is responsible for 70% of abnormal CF genes. Thus, 50% of all patients with CF are homozygous for ΔF508. CFTR ΔF508 protein is recognized by the endoplasmic reticulum as being abnormal and is degraded there rather than being glycosylated and transported to the epithelial surface. If CFTR ΔF508 protein reaches the cell surface without degradation, it exhibits partial function as a cAMP-activated chloride channel. This has led to the development of strategies to partially restore ΔF508 CFTR function. Class I CFTR mutations (null mutations) do not produce CFTR protein because of a premature stop signal in the CFTR DNA. This can be corrected by aminoglycosides such as gentamicin that cause the aberrant stop signal to be skipped (34). Mutations leading to a CFTR protein that attains an unstable structure shortly after translation in the endoplasmic reticulum form class II. Class II mutations can be restored to the protein trafficking pathway by manipulation of chaperone protein/CFTR interactions with chemical chaperones (35) or drugs that affect gene regulation such as the butyrates (36). Production of a CFTR with reduced chloride transport on the basis of abnormal regulation of the chloride channel is the basis of class III. Genistein can overcome this block in regulation (37). Mutations that partially reduce chloride conductance through CFTRs (class IV) can be stimulated with milrinone, which is a phosphodiesterase inhibitor. Finally, mutations that lead to a severe reduction in normal CFTR protein form class V. Increased levels of CFTR could be generated with the butyrates or supplemented with gene therapy. Such gene activation or gene modification strategies may be among the most important novel treatments for CF lung disease.

LUNG TRANSPLANTATION

With end-stage CF lung disease, lung transplantation is an effective therapeutic alternative. CF is the most common reason for double lung transplantation in North America (38). Long term survival of CF patients after lung transplantation is similar to survival in patients transplanted for other diseases, with a five-year survival rate of over 50% (39). Early morbidity is due to lung transplant rejection, with early and late morbidity attributable to infection. It is important to remember that immunosuppression is required to retain the transplanted organ, but CF patients all have chronic sinus infections that can be a significant source of pathogens in the immunocompromised host. Late rejection, infection and transplant-related cancers such as post-transplant lymphoproliferative disease are problems following CF lung transplantation, just as they are in other patients.

It has been an exciting 17 years since the CF gene defect was first described, and the tremendous amount of information gained by our understanding of CFTR function makes the promise of a cure or significant control for this disease highly likely.

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