Gene Therapy

Gene therapy for cystic fibrosis is conceptually an attractive alternative for this disabling illness, which is the most common fatal inherited disease in Caucasians. Cystic fibrosis affects 1 in 3000 newborns in the United States, where 30,000 people suffer from the disorder. The major life-threatening complication of cystic fibrosis is severe airway disease, although significant pathology also occurs in the sinuses, pancreas, intestines, liver, vas deferens, and sweat ducts.

Current treatments have been successful in extending the survival of cystic fibrosis patients, many of whom now live into their late 20's, but have not effected a cure. The cause of cystic fibrosis is mutation of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which was discovered and characterized in 1989. Over 700 mutations in the gene are known, and consequent abnormalities of CFTR function give rise to the disease. By virtue of their accessibility, the airways are amenable to in vivo gene therapy strategies.

Early descriptions of the potential for gene therapy coincided with discovery of the CFTR gene. The anticipation and indeed expectation from cystic fibrosis patients and their families was that a cure for CF would be offered in the not too distant future. CF is an autosomal recessive disorder, so the goal would be simply to deliver the normal CFTR gene to the airway epithelium, where its expression would overcome the physiological deficit that causes the disease.

Pursuit of this goal, although not yet affording a cure, has taught many important lessons. The pathophysiology of cystic fibrosis is much more complicated than was expected, and understanding of the biology of the airways has advanced. In addition, impediments to successful gene therapy for CF and other diseases of the lung as well as of other organs have been identified, allowing strategies to be devised to overcome them.

Prior to discovery of the CFTR, physiologic studies had shown that the epithelial defect in CF was of a chloride channel or of the regulation of chloride channels. Characterization of the CFTR confirmed that it is primarily a cAMP-dependent chloride channel, but it also regulates other cationic and anionic channels. In cystic fibrosis, besides poorly functioning chloride channels, there is overactivity of sodium channels at the apical surface of the airway epithelial cell.

How defects in salt channels cause the pathological manifestations of cystic fibrosis, which in the lung are principally airway inflammation, infection, obstruction, and ultimately destruction, remains unknown. The simplest though not necessarily the most likely explanation is that lack of chloride secretion onto the luminal surface of the airway together with excessive sodium and water reabsorption leads to a dehydrated, viscous mucus layer that obstructs airways and forms a nidus for infection. These concepts form the basis of clinical management of CF, which is focused on clearance of airway secretions and treatment of bacterial infection.

The hypothesis does not explain the propensity of CF patients to infection with *Staphylococcus aureus*, *Haemophilus influenzae*, and particularly *Pseudomonas aeruginosa*, the most important pathogen in established CF airway disease. Recently, abnormal ionic conditions in the airway surface liquid have been shown to inhibit the function of natural peptide antibiotics called defensins, which usually inhibit *Pseudomonas aeruginosa* growth on epithelial surfaces. In vitro reconstitution of CFTR function by gene transfer has restored defensin function in CF airway cells.

Abnormal ionic conditions may also affect neutrophil function, inhibiting phagocytosis and bacterial killing. Moreover, Gerald Pier and colleagues in Boston have shown that the CFTR acts as a *Pseudomonas aeruginosa* receptor and aids internalization and clearance of this organism. Low or absent CFTR diminishes this function.

Abnormal attachment of *Pseudomonas aeruginosa* to CF airway epithelial cells, possibly because of undersialylation of cell surface receptors, has also been observed and can be corrected by transfer of the normal CFTR to these cells. Abnormal sialylation and sulfation of macromolecules may be related to as yet poorly defined intracellular functions of the CFTR.

Which of these abnormalities is most important in cystic fibrosis remains uncertain. To date, gene therapy has concentrated on reversal of the primary defect in chloride conductance. At least some of the secondary phenomena are also reversed by this approach.

Expression of CFTR in airways is maximal in submucosal glands of the proximal airway, less but significant in subpopulations of distal airway epithelial cells, and low or absent in proximal epithelial cells. Driven at least in part by practicality, the principal target for in vivo CF gene therapy has been the ciliated columnar epithelial cell. Direct airway instillation or aerosolization can achieve delivery to this target relatively easily.

In vitro studies of CF epithelial monolayers have shown that only 6

to 10% of these cells need to have normal *CFTR* expression to correct the chloride conductance defect, although the abnormalities of sodium transport require much higher *CFTR* expression. The degree of gene transfer and expression required to correct the other abnormalities has not been determined, nor is it known whether all of the secondary pathophysiological abnormalities need to be reversed if there is to be clinical benefit.

Likewise, the potential therapeutic implications of not correcting the CFTR defect in the mucosal glands is unknown. Targeting the basal cells or mucosal gland cells is more difficult, requiring an intravenous or in utero approach. The potential for in utero therapy, as discussed in an earlier article by Janet Larson and colleagues (see the January/February 1998 issue), highlights another uncertain aspect of gene therapy for CF: When should the gene be delivered?

In cystic fibrosis, the airways are relatively normal at birth, but inflammation and infection soon ensue. Although most protocols to deliver the normal CFTR gene to CF airways have so far concentrated on patients with established disease, it may well be that earlier intervention, before there is significant irreversible airway damage, is more rational.

Consistent with clinical observations is the hypothesis that there are two major phases of lung disease in CF. The first, classified as "CF disease," is driven by CFTR dysfunction and consequent abnormalities of airway fluid electrolytes and mucus. Subsequent infection and inflammation lead to "lung disease," which progresses independent of the abnormal CFTR. A vicious cycle of infection and airway destruction is the usual cause of death, which supports earlier use of gene therapy.

It is possible, however, that gene therapy will retard progression of the "lung disease" phase. In addition, gene therapy for cystic fibrosis need not be restricted to the CFTR. Delivery of genes for antiproteases, antioxidants, or cytokines may help to reduce lung destruction and improve airway defenses.

Despite many uncertainties, a number of clinical studies are in process. In these trials, the normal CFTR gene is delivered either to the nose, as a model of the airways, or directly to the lower airways by adenovirus, adeno-associated virus, or cationic liposomes. Each of these vectors has benefits and limitations.

Adenoviral vectors are tropic to airways, have the ability to transduce nondividing cells, and are efficient in vitro. In vivo, however, their use has been hampered by airway inflammation, transient gene expression, and low efficiency. Removing residual viral genes combats the inflammation, which is in part due to an MHC class I response to expressed viral proteins. Reducing the immunogenicity of the adenovirus may also allow repeated treatments, which will likely be necessary given airway cell turnover and transient expression of the delivered genes.

The reduced in vivo efficiency of adenoviral vectors is coming to be understood. A number of groups have shown that well differentiated airway epithelium is less efficiently transduced than poorly differentiated cell cultures. Reasons for this include low expression of both the adenoviral fiber receptor and the integrins that mediate adenoviral binding and entry to the cell. Nonspecific transport of molecules across the apical surfaces of airway epithelial cells is also low, further limiting vector uptake. Investigators are modifying the adenovirus to redirect it to other cellular receptors.

Adeno-associated virus vectors induce long-term expression of delivered genes that integrate in vitro into the host cell chromosome. However, they are difficult to produce, and in vivo they do not appear to integrate. How these problems might be overcome is uncertain.

Gene delivery by cationic liposomes has been shown to successfully correct the chloride channel defect in CF cells in vitro as well as in vivo in some studies in the nose. By virtue of their charge, liposomes envelop the gene to be delivered and bind to cell surfaces, which should enhance uptake of DNA into the cell. However, liposomes are taken up poorly by well differentiated airway epithelium, and in high doses, liposomes are directly cytotoxic. Modifications of liposomes are being developed to try to overcome these limitations.

Combinations of nonviral and adenoviral vectors, capitalizing on the complementary advantages of each, are promising as gene delivery vehicles. Another prospect is to combine the advantages of different viruses in a single viral vector. For example, the ability of a retrovirus to integrate genetic material into host cell DNA might be added to an adenovirus.

That gene therapy has not yet delivered a cure for cystic fibrosis has been a disappointment to patients and those who care for them. Progress in the field has in fact been extraordinary compared to development of new drugs for this common and severe illness. The difficulties encountered are being addressed, and enthusiasm for gene therapy in CF remains high.

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