CYSTIC FIBROSIS

# Vehicles for gene therapy

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FOUR years ago the gene responsible for cystic fibrosis (CF) was identified, since when there has been intense research into ways of treating the disease through gene therapy. The pace of development has been unprecedented in medicine, and there are now four clinical trials underway in two continents. They involve, as delivery vehicles, either modified viruses or liposomes. The latest news comes from Zabner *et al.* in *Cell*[1], where the authors report on a trial in humans that employed recombinant adenovirus. Their results are very encouraging. But whereas both the viral and liposome approaches are promising, both have their potential limitations.

The gene defective in CF encodes a chloride channel, called the cystic fibrosis transmembrane conductance regulator (CFTR), that is expressed in epithelia of several organs. The lung has been the initial target for CF gene therapy because the pulmonary manifestations of the disease — characterized as airway obstruction, recurrent infections, and ultimately respiratory failure — are the most serious and life-limiting. Ultimately one can envisage ways of reconstituting gene expression by inhalation of the recombinant gene construct, but complexities in the biology of the diseased lung pose unique challenges. The CF gene is regulated in a highly cell-specific manner in the adult human lung, its product — the CFTR — being expressed in subpopulations of cells throughout the superficial epithelia of both conducting and respiratory airways[2], as well as in cells of the submucosal glands[3]. It will be difficult to reconstitute precisely the normal expression of the CF gene using available gene-transfer techniques, although this may not be necessary to achieve clinical efficiency. Another consideration is the stability of recombinant gene expression in the recipient — until the pulmonary progenitor cells responsible for repopulating lung epithelia are identified and made accessible for gene transfer, patients will probably require repeated treatment.

The primary goal of CF gene-therapy research in both the commercial and academic sectors is the development of a vehicle for delivering the normal CFTR gene directly into the lung. The demands on that vehicle are high. It must efficiently transfer the gene into nondividing airway epithelial cells in a manner that is safe and clinically practical. Furthermore, recombinant gene expression must be prolonged, and the vehicle must not elicit an immune response that is either destructive or precludes repeated administration.

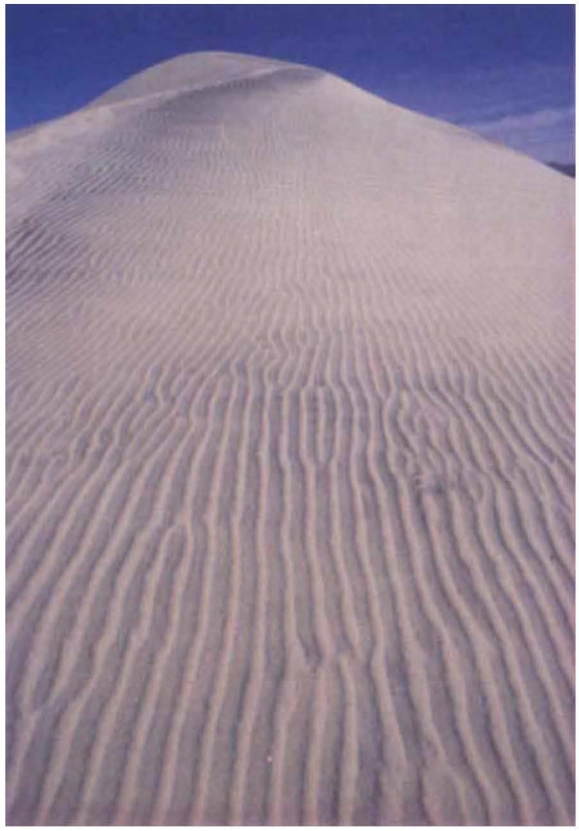
In principle, recombinant adenoviruses have the appropriate biological properties for such vehicles. This family of viruses, which normally causes infections of the upper respiratory tract, can be modified for gene therapy by replacing a gene that controls viral replication (*E1a* and *E1b*) with a normal CFTR minigene. Experiments in the cotton rat, an animal that resembles humans with respect to the biology of human adenoviruses, have shown that recombinant adenoviruses can indeed transfer recombinant CFTR genes into cells of the airway[4]. Human CF respiratory epithelia reconstituted *in vitro*[5] have helped in demonstrating efficient adenovirus-mediated gene transfer and correction of the bioelectric properties of the epithelia.

Zabner *et al.*[1] describe results of a clinical trial in which recombinant adenoviruses, containing the normal CFTR gene, were introduced into nasal epithelia of three CF patients. The rationale for evaluating the consequences of gene transfer in the nose is that nasal epithelium is an extension of the epithelium of the lung and is accessible for harvesting cells and performing direct measurements of function. The defect in chloride transport, as measured by transepithelial voltages, was corrected in all three patients. No toxicity has been observed in another clinical trial, underway here at the University of Pennsylvania, when similar doses of adenovirus were instilled into the airways of two CF patients (J.M.W. and J. Engelhardt, unpublished data).

The observation of biological efficacy in nasal epithelia with doses of virus that do not cause toxicity in lung is heartening in view of previous studies in a variety of animal models, including non-human primates, in which there was substantial inflammation in lung with higher doses of virus[6]. But the biological effect of gene therapy in the patients of Zabner *et al.* was transient, lasting less than three weeks, which is consistent with studies in animal models. These preclinical and clinical studies suggest that therapeutic applications of the available adenoviral technology will require repeated administrations, which may lead to confounding immunological responses. An alternative strategy is to modify the recombinant virus to facilitate more stable expression of the CFTR transgene or to make the virus less immunogenic.

Liposome-mediated gene transfer has emerged as another promising way of treating CF lung disease. Several groups have used mouse models of CF generated by site-directed mutation of the CFTR gene in embryonic stem cells, to evaluate the biological efficacy of liposome–DNA complexes. Hyde *et al.*[7] have analysed

## The case of the coarsened crests



**In a pattern within a pattern, the sand grains in these ripples are sorted so that the coarsest are at the crests and the finer grains in the troughs. The effect resembles the sorted stone stripes found on some hillsides (*Nature* 361, 117; 1993). In this case, though, the sand grains hop into position under the influence of the wind blowing across the ripples, whereas 'sorted' rocks are levered from their beds by the periodic growth of ice crystals. Cellular-automaton techniques applied to the stone stripes again prove successful here: grains represented by computer pixels are individually shifted according to a simple set of physical rules. Given two distinct grain sizes and a reasonable picture of how the grains leap and scatter one another on impact, R. S. Anderson and K. L. Bunas find they can pin down the cause of the sorting. And the answer? See page 740 of this issue.** LM