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Moving straight to the target

Retroviral vectors for gene transfer experiments have their limitations — hence the attraction of using other viral vehicles to administer genes directly to affected organs.

THERE is growing evidence that the theoretical promise of human gene therapy for the treatment of cancer and a variety of hereditary disorders is finally being matched by positive results in both research and clinical settings[1–3].

To date, the dozen or more clinical trials employing viral delivery systems that have been approved in the United States have used retroviruses[2,3]; such vectors are highly efficient at delivering (and integrating) genes into dividing cells and, provided the vectors are adequately compromised, they seem to pose no serious safety hazard[3]. But the inability of retroviruses to infect non-replicating cells, coupled with the potential advantages of other delivery systems, has led several groups to look at alternative strategies of gene therapy. Two papers in the August issue of *Nature Genetics* illustrate progress in the application of different viral vectors for *in vivo* gene transfer, namely adenoviruses[4] and herpesviruses[5]. The levels of expression in both cases are still low, but these hurdles should be surmountable.

Recent work, notably from Ron Crystal's laboratory at the National Institutes of Health, has demonstrated the promise of adenoviruses in delivering genes to cells *in vivo*, for example the cystic fibrosis gene to airway epithelial cells in cotton rats[6]. Preliminary experiments transferring adenovirus, containing either the *lacZ* gene or a human α1-antitrypsin (α1-AT) complementary DNA, into cultured human endothelial cells produce significant levels of expression, which also bodes well for *in vivo* gene transfer to the endothelium[7]. Clearly, one of the most important tissues to be targeted is the liver, given its central role in metabolism, but most approaches are necessarily of the *ex vivo* variety (whereby hepatocytes are genetically modified in culture before replacement). Adenoviruses are not thought to be particularly tropic for hepatocytes; however, given that hepatocytes share an embryological origin with airway epithelial cells, and genes such as ornithine transcarbamylase (OTC) can be targeted successfully to rapidly growing, neonatal rat liver[8], an *in vivo* gene transfer strategy may be feasible.

Crystal and colleagues now report the successful transfer of *lacZ* and the human α1-AT gene to normal adult rat liver *in vivo*[4], by injecting the adenovirus constructs into the intraportal vein. Three days after exposure to the *lacZ* gene, some one per cent of hepatocytes stained positive for β-galactosidase (the *lacZ* gene product). In rats injected with the adenovirus α1-AT construct, serum levels of human α1-AT averaging about 300 ng ml^{-1} were detected for at least 28 days afterwards.

It should be stressed that the level of human α1-AT produced in these adult rats is some 3,000 times less than the endogenous α1-AT concentration (and that necessary for correction of the phenotype produced by α1-AT deficiency). In contrast, administration of OTC cDNA by means of adenoviruses to newborn mice suffering OTC deficiency did result in correction of the mutant phenotype in some cases[8]. Expression of OTC was not liver-specific, however, and OTC is synthesized at far lower levels than α1-AT in the normal liver. The results suggest that, with improved efficiencies, the *in vivo* approach may offer the possibility of treating a variety of disorders, including haemophilia and other errors of metabolism.

Retroviral gene transfer to post-mitotic cells in the central nervous system suffers from many of the limitations described above, including the requirement for actively dividing cells. One possible alternative would be to employ herpesviruses to deliver genes: after an acute infection stage lasting less than 10 days, herpesviruses become latent and transcriptionally silent except for production of the latency associated transcript (LAT). This transcript, which does not seem to be translated into protein, accumulates in the nucleus of latently infected neurons. So foreign genes downstream of the LAT promoter may be suitably expressed in the central nervous system. The mouse model chosen by Wolfe *et al.*[5] to test this strategy was for mucopolysaccharidosis VII, a lysosomal storage defect that is caused by an abnormality in the gene for β-glucuronidase (*GUSB*) and which causes a form of mental retardation known as Sly disease in humans.

Following corneal infection with the herpesvirus–*GUSB* constructs, GUSB-positive cells were evident in the trigeminal ganglia and brainstem of 11 out of 13 infected mice. As in the adenovirus hepatocyte transfer study, staining was generally confined to individual cells, although occasionally clusters of positive cells were seen. Positive cells were detected in 7 out of 8 latently infected mice, including one more than four months after inoculation. Although expression was low (too low to correct the defective phenotype) it does seem to be long-lasting. Improvements in the vector system coupled with targeting to other organs (at least in the case of mucopolysaccharidosis VII) does offer some hope for the future. Herpesviruses may also prove valuable for gene transfer to peripheral neurons. For example, given the successful phenotypic correction of 'retinal degeneration slow' (*rds*) mice — a model for retinitis pigmentosa — by creation of mice transgenic for the normal *rds* gene[9], modified herpesviruses may prove effective in transferring *rds* to the retinas of affected mice, and perhaps even humans.

In general, it is too soon to reach any firm conclusions about the clinical efficacy of retroviral gene therapy. So it is understandable that direct *in vivo* methods, exploring the use of other viral systems, should be so appealing. As W. French Anderson has put it: "Gene therapy will have a major impact . . . only when vectors are developed that can safely and efficiently be injected directly into patients as drugs [such as] insulin are now"[2].

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Also in this month's *Nature Genetics*: the mislocalization of the cystic fibrosis (CF) transmembrane conductance regulator in CF patients' sweat glands; mutations in the human *PAX6* gene in patients with aniridia; fragile X syndrome associated with a deletion of the *FMR1* gene; automated sequencing and analysis of a segment of the short arm of chromosome 4; the autosomal localization in mice of a gene which is pseudoautosomal in humans.

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