Gene therapy made difficult

One of the driving forces behind the characterisation of single gene diseases such as cystic fibrosis and complex disorders such as restenosis after angioplasty is the hope that novel therapies may emerge from a precise knowledge of the molecular pathophysiology. The development of a number of vector systems designed to deliver foreign genes to cells set the stage for the emergence of experimental gene transfer to investigate the function of individual genes, soon to be followed by gene therapy. In the early 1990s a number of review articles on the “gene therapy made easy” theme described the basic mechanisms by which studies with replication deficient adenoviruses and synthetic vectors could open up new possibilities for the treatment of vascular diseases. However, despite enormous enthusiasm, the application of gene transfer techniques in animal models has been problematic, and to date meaningful clinical benefit has not been realised in human studies. Moreover, fundamental difficulties in the basic biology of gene transfer techniques need to be addressed before real progress can be made in the application of this fashionable concept to the clinical arena.

Gene delivery

Genes are large polar molecules that are not readily taken up by cells, and considerable efforts have been made to develop suitable vectors to deliver DNA to the nuclei of target cells. These include a number of synthetic compounds such as cationic liposomes and cationic polymers, which complex with DNA and fuse with cell membranes to gain entry. Replication deficient adenoviruses can be engineered to carry genes of interest, but these vectors have to be individually constructed and, because of the ability of these genetically engineered infectious agents to form aerosols, cumbersome and expensive safety procedures must be employed. The fact that many investigators have resorted to these viruses attests to the ineffectiveness of synthetic vectors, even under optimised conditions. Effective gene transfer can be accomplished in cultured cells with adenoviral vectors, but very high titres are often required to achieve modest levels of gene expression in vivo. In particular, the medial smooth muscle cell of an intact artery appears to be a relatively resistant target for gene transfer.

Inflammation after vector delivery

A local inflammatory response at the site of vector delivery is a common finding with adenovirus mediated gene transfer both in animal models and in clinical studies. This inflammatory response appears to be dose dependent and is generally observed using the sort of adenoviral loads needed to achieve expression of the transgene. The ability of defective and inactivated adenoviral particles to induce inflammation in murine lungs demonstrates that this process is independent of viral protein or transgene expression. In keeping with these findings, we have been able to demonstrate that high loads of a replication deficient adenoviral vector expressing no transgene were able to activate the transcription factor nuclear factor κB (NFκB), an important mediator of inflammation. Activation of NFκB by this mechanism was able to induce expression of the pro-inflammatory adhesion molecule intercellular adhesion molecule-1 (ICAM-1). Thus, inflammation following adenoviral vector infection appears to be an inevitable host response to high viral loads.

Regulation of gene expression

Successful gene transfer relies on adequate expression of the foreign gene following delivery to the target cell nucleus. Promoters such as the human cytomegalovirus immediate early promoter (CMV-IEP) and the Rous sarcoma virus long terminal repeat (RSV-LTR) have been widely used in gene transfer vectors on the assumption that these viral promoters are sufficiently active in a wide variety of target cells to drive detectable transgene expression. High adenoviral loads are generally required to produce detectable recombinant gene expression after infection of human vascular smooth muscle cells, and because of the perceived effectiveness of these “constitutively active” viral promoters, failure of transduction using lower adenoviral titres has generally been interpreted as a failure of viral delivery to the nucleus. We have been able to exploit inducible elements within the CMV-IEP to augment promoter performance after adenoviral gene transfer into human vascular smooth muscle cells. This strategy resulted in the demonstration of transgene expression after infection with much lower titres than those normally required to achieve transduction. The identification of promoter performance as a critical limiting factor after adenovirus mediated gene delivery raises the prospect that the identification of more suitable promoters may allow effective gene transfer using much lower viral titres, which in themselves may provoke much less of an inflammatory response.

Immune responses to gene transfer

The ability to deliver DNA to the nucleus of infected cells is exploited when viruses are used for gene transfer. However, the immune responses that accompany natural viral infections have emerged as obstacles to this process. Reports of improved efficacy in immunosuppressed animals focused attention on the role of the immune response in gene transfer. The so called first generation adenoviral vectors were rendered replication deficient by the deletion of adenoviral genes (E1 region) usually required to initiate the regulated cascade leading to viral replication. Although these vectors were significantly disabled, continuing low level expression of native adenoviral genes was shown to induce a cytotoxic T cell response resulting in the elimination of virally infected cells. Although initially promising, further deletions have not revolutionised the efficacy of these vectors despite the fact that adenoviral vectors containing virtually no viral
genes can now be constructed.\textsuperscript{12} In addition, immune responses to the transgene have been described, which may play a role in limiting the duration of expression of non-autologous proteins.\textsuperscript{13}

**Unanswered questions**

A number of critical issues remain unresolved in the field of experimental gene transfer. The ability of adenoviral and other gene transfer vectors to deliver genes to cell nuclei and the identification of viral DNA in vivo have not been carefully studied. For example, it is not known whether the resistance of the arterial media to transduction is because of a failure of gene transfer vectors to penetrate the arterial wall and deliver DNA to nuclei, or a failure of gene expression in these vascular smooth muscle cells after successful gene delivery. In addition, it is not clear what happens to adenoviral DNA after entry into cells and why gene expression with adenoviral and plasmid vectors is transient. The traditional explanation that episomal (non-chromosomal) DNA does not direct long term gene expression does not appear to be supported by recent observations with adeno-associated virus where prolonged expression was observed without integration into the host genome.\textsuperscript{14}

On a broader level it is unclear what gene therapy may ever offer in single gene disorders caused by an abnormal protein product that interferes with normal protein function. These dominant negative mutations have been described in hypertrophic cardiomyopathy and Marfan’s syndrome and would not be amenable to treatment by overexpression of the normal gene. There is at present no prospect of being able to downregulate and replace these disease genes in patients.

**Conclusions**

It is 25 years since Friedmann and Roblin\textsuperscript{15} speculated that gene therapy could be accomplished by the use of synthetic and viral vectors to replace defective genes with “good” DNA. Despite an explosion in the understanding of the basic biological processes underlying many human diseases, the prospects for the widespread use of direct gene transfer for human gene therapy are not good. If one considers that the uptake and regulated expression of foreign genes is a highly abnormal process for host cells, then a more fundamental understanding of these basic mechanisms is required if meaningful progress is to be made. The ability to introduce new genes into human cells may one day be routinely used to treat patients. Whether this will happen soon or in 25 years is likely to depend on major advances rather than minor refinements in the technology of gene transfer.

G J CLESHAM

Cardiac Unit, Papworth Hospital, Cambridge CB3 8RE, UK


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G J CLESHAM

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