Gene Therapy (1998) 5, 725–727

http://www.stockton-press.co.uk/gt

Editorial

Adenoviral vectors: not to be sneezed at

Adenoviral vectors have been used in about 15% of gene therapy clinical trials, compared with over 60% using retroviruses. However, publications relating to adenovirus have overtaken those to retrovirus in the gene therapy field in the past 2 years (data from ISI literature search); the growing appreciation of the interplay of vector and host and consequent sophistication in the development of adenovirus vectors suggest this trend will continue into the clinical arena.

Adenoviruses offer the advantages of high titre, stability, the ability to achieve gene transfer and expression in a wide variety of cells independent of the cell replication status, and a capacity for foreign genes which is ample for many purposes. Although there are 51 serotypes of human adenovirus, the vast majority of studies have used vectors based on Ad5, one of the more commonly occurring serotypes, which mostly causes mild upper respiratory infections in infants. Adenovirus molecular biology is well documented; the genome is a linear double-stranded DNA of approximately 35 kb; that of Ad5 and several other serotypes of human and animal origin have been completely sequenced. When the linear viral genome is delivered to the nucleus, expression of the E1A gene is required for transactivation of other viral genes and consequent initiation of the virus lytic cycle. Deletion of the E1 region in adenoviral vectors renders them replication-defective, so they have to be propagated in cells which have been engineered to provide E1 functions *in trans*. The standard complementing cell line is the human embryonic kidney cell line 293, which carries a chromosomally integrated segment of Ad5 DNA from the left end of the genome, encompassing the E1 region. The icosahedral viral capsid can package viral genomes between 75 and 105% the length of wild-type viral DNA; in 'first generation' vectors with an E1 deletion and, usually, a deletion in the non-essential E3 region also, this corresponds to a payload capacity of approximately 8 kb of foreign DNA. Isolation, purification and concentration of adenoviral vectors is uncomplicated: the conventional method is to extract virions from infected cells by cycles of freezing and thawing, followed by purification and concentration of virions by equilibrium centrifugation. An alternative approach involving ion exchange chromatography may be more amenable to large-scale production for clinical trials and therapeutic use.[1]

Weighing against the positive features are concerns about potentially harmful inflammatory responses to high doses of adenoviral vectors; immune response to the input virion; decreased efficacy of gene delivery particularly with repeat doses (attributed to elevated titres of neutralising antibodies); and rapid elimination of infected cells by T cell responses against transgene-encoded foreign proteins or leaky expression of viral proteins. An article in *Gene Therapy*[2] attributes inflammation to virion-mediated activation of the transcription factor NFκB; this was seen at a high MOI (1000 p.f.u. per cell) in a vector lacking a transgene, even when the virus was inactivated by UV irradiation. This NFκB activation, and its downstream effect on a pro-inflammatory response (ICAM-I up-regulation), were only observed at a high MOI, and could be blocked by inhibition of IκB degradation. These observations are broadly consistent with an earlier report in which a relatively low input MOI stimulated the Raf/MAPK pathway and IL-8 secretion,[3] reinforcing the concept that the input virus particle is not merely an inert vehicle. If inflammation is proportional to MOI, it could be minimised if sufficient transgene expression can be obtained at a low MOI, or it might be susceptible to pharmacological control. Also, the requirement to avoid inflammation will depend upon the clinical application; what is clearly undesirable in the treatment of cystic fibrosis or other genetic diseases may arguably be advantageous in some approaches to cancer therapy – thus the incidence and consequence of inflammation will need to be evaluated for each clinical application.

Many experiments have shown a longer duration of transgene expression following gene transfer into immunodeficient mice compared with immune competent animals, apparently because of the elimination of adenovirus-infected cells in the latter by cytotoxic T lymphocytes (CTL). Some of these CTL responses are directed against the transgene, and so the extent of 'foreignness' may determine the extent of the problem. A careful analysis by Michou *et al*[4] used E1/E3-deleted adenoviruses expressing either *E. coli* β-galactosidase or human factor IX. In immunocompetent mice exposed to the Ad-βgal virus, there was a CTL response to the enzyme, and all β-gal-expressing cells were eliminated in 3 weeks. However, mice receiving the factor IX virus mounted a humoral response to the transgene product and, although this was accompanied by a CTL response to virus proteins, infected cells were still present beyond 7 weeks. These experiments suggest that the pre-existence of CTLs directed against adenoviral epitopes need not compromise the use of adenoviral vectors in patients.

Others have attributed greater importance to CTL responses against adenoviral genes, which may be expressed from the vector despite the absence of E1 functions. Vectors with additional viral genes removed have been made in order to address this problem; E2A-

deficient vectors which do not express the DNA binding protein (DBP) essential for DNA replication appear to be substantially less immunogenic and show improved transgene persistence and decreased inflammatory response in animal models. Vectors lacking E2B as well as E1 and E3, so that the DNA polymerase and terminal protein (both esssential for DNA replication) are not present, achieve a similar effect.[5] Such vectors have to be produced in cells which provide the missing E2 proteins *in trans*, and in some cases toxicity in the complementing cells has been problematic. The construction of a 293 cell derivative which expresses E4 as well as E1 has allowed removal of the entire E4 region from the vector, which effectively blocks DNA replication and late gene expression in target cells. An alternative approach which does not require a new complementing cell line, is to retain just the essential E4 ORF6 gene within the vector. However, there is no consensus as yet on the effect of E4 deletion on prolonging transgene expression (reviewed in Ref. 6). Second generation vectors which have prolonged transgene expression profiles in immune competent hosts represent an important advance for therapeutic applications where CTL elimination of the transduced cells is undesirable. However, for some applications, notably cancer, even a minimum approximately 10-day window of expression as provided by first generation vectors may be more than adequate, for example, for expression of a prodrug-activating (suicide) enzyme, p53 or cytokine to have its desired effects in tumour cells.

Taking gene removal to extremes, vectors have been produced with a 25 kb Cre–Lox-mediated deletion, or so-called gutless vectors retaining only terminal adenovirus sequences required for replication; these need a helper virus in order to generate virus particles in the permissive cell line. However, the biological properties and potential utility of vectors with such large deletions, in addition to the practicality of their large-scale production and separation from helper virus remain to be unequivocally demonstrated.

As an alternative to viruses which are intended to be completely replication defective, are recent developments in the use of conditionally replication-competent viruses; the paradigm is Ad5 dl1502 which lacks the E1B 55K gene (the 'Onyx-015 virus') and cannot complete a replication cycle in cells expressing wild-type p53, but is lytic if p53 is mutant or absent.[7] Another novel development is the creation of a hybrid adeno–retro virus, which may manage to incorporate all the useful features of both viruses without all their separate disadvantages.[8]

A general concern with propagating adenovirus vectors for gene therapy is the generation of replication-competent adenovirus (RCA) in the stocks, which comes about by recombination with the resident E1 sequences in 293 cells. A cell line [PER.C6] has been developed which encodes the minimum necessary E1 complementing sequences, for use in conjunction with a vector that does not share any overlapping sequence with the cell insert.[9] This useful development is expected to eliminate the generation of RCA by homologous recombination.

Adenovirus vectors are widely used because of their ability to express transgenes in a wide variety of cells, however, expression in cells other than the intended target may be problematic, and the subject of virus targeting is of much current interest. Adenoviruses use two cell surface interactions to attach to and enter cells; primary attachment is via the fibre component of the virion, and so far, two cell surface components have been shown to bind Ad5 fibre (but not Ad3); one is an Ig-like protein designated CAR which mediates binding of some adenoviruses and some coxsackie viruses and the other is the MHC class I α domain. For internalisation, the penton base of the virion interacts via an exposed RGD motif with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. The RGD motif of penton base has been mutated such that different integrins are recognised, thereby achieving specific targeting to lymphoid and haematopoietic cells. A bispecific antibody that recognises both the FLAG epitope and α_v integrin has been used to direct a virus with the RGD motif replaced by the FLAG epitope to endothelial and smooth muscle cells.

Genetic modifications of the fibre have used C terminal fusion of peptides, for example, gastrin releasing peptide, polylysine to target heparan-containing receptors, RGD motif (reviewed in Ref. 10). Another approach has been to block the fibre binding to its receptor using an anti-fibre antibody conjugated to a different targeting moiety; a folate conjugate redirects the virus to cells expressing folate receptors,[11] and an FGF2 conjugate targets cells expressing FGF receptor, including Kaposi's sarcoma,[12] ovarian tumour cells, and vascular endothelial and smooth muscle cells.[13] The tropism of different adenovirus serotypes can be used to advantage; Ad5 and Ad3 do not share a receptor, and substitution of the Ad3 knob domain sequence into the Ad5 fibre gene alters the tropism of the recombinant virus.[14] Two Ad11 genotypes with distinct tropism for the respiratory and renal tracts show differences in binding affinity and infectivity of various cell types in culture.[15] This serves as a reminder of the underexploited pool of human and animal adenoviruses which may harbour useful attributes for further vector development.

Although the initial promise of adenovirus vectors for gene therapy has not yet been fully realised, current efforts to understand the important details of their interaction with the host cells and animal models, and consequent improvements in vector, design are providing the solid framework that is needed to take adenovirus successfully into the clinic.

PF Searle and V Mautner
University of Birmingham
CRC Institute for Cancer Studies
Birmingham B15 2TA, UK

References

1 Shabram PW *et al*. Analytical anion-exchange HPLC of recombinant type-5 adenoviral particles. *Hum Gene Ther* 1997; **8**: 453–465.
2 Clesham GJ *et al*. High adenoviral loads stimulate NFκB-dependent gene expression in human vascular smooth muscle cells. *Gene Therapy* 1998; **5**: 174–180.
3 Bruder JT, Kovesdi I. Adenovirus infection stimulates the Raf/MAPK signaling pathway and induces interleukin-8 expression. *J Virol* 1997; **71**: 398–404.
4 Michou AI *et al*. Adenovirus-mediated gene transfer: influence of transgene, mouse strain and type of immune response on persistence of transgene expression. *Gene Therapy* 1997; **4**: 473–482.
5 Amalfitano A *et al*. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol* 1998; **72**: 926–933.

6 Leppard KN. E4 gene function in adenovirus, adenovirus vector and adeno-associated virus infections. *J Gen Virol* 1997; **78**: 2131–2138.
7 Bischoff JR *et al*. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996; **274**: 373–376.
8 Bilbao G *et al*. Adenoviral/retroviral vector chimeras: a novel strategy to achieve high-efficiency stable transduction *in vivo*. *FASEB J* 1997; **11**: 624–634.
9 Bout A *et al*. PER.C6: a novel packaging cell line for RCA-free production of E1-deleted recombinant adenoviral vectors. *Cancer Gene Ther* 1997; **4**: 324.
10 Kovesdi I, Brough DE, Bruder JT, Wickham TJ. Adenoviral vectors for gene transfer. *Curr Opin Biotechnol* 1997; **8**: 583–589.
11 Douglas JT *et al*. Targeted gene delivery by tropism-modified adenoviral vectors. *Nat Biotech* 1996; **14**: 1574–1578.
12 Goldman CK *et al*. Targeted gene delivery to Kaposi's sarcoma cells via the fibroblast growth factor receptor. *Cancer Res* 1997; **57**: 1447–1451.
13 Rancourt C *et al* and Reynolds P *et al*; B Sosnowkski personal communication.
14 Stevenson SC, Rollence M, Marshall-Neff J, McClelland A. Selective targeting of human cells by a chimeric adenovirus vector containing a modified fiber protein. *J Virol* 1997; **71**: 4782–4790.
15 Mei Y-F, Lindman K, Wadell G. Two closely related adenovirus genome types with kidney or respiratory tract tropism differ in their binding to epithelial cells of various origins. *Virology* 1998; **240**: 254–266.