

Can viruses be modified to achieve sustained gene transfer

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Gene therapy, which aims to temporarily or permanently replace a faulty or missing protein at therapeutic levels, requires efficient transfer of genes into a host cell. The gene transfer vehicle has to withstand enzymes in blood, find the right cells, enter them and then their nucleus. Viruses meet these criteria to the dot; their capsids protect the genome, ensure that they enter cells and then facilitate transfer of the gene into the nucleus.

Gene therapists have been attempting to take advantage of viruses' talents for gene transfer focusing on viruses that commonly infect humans. During the milleniums, well before primates climbed down from trees evolution has selected for cellular organisms that can fight off viral infection first through primitive innate immune responses and then about 410 million years ago through an adaptive immune system and its high specificity and ability to remember. Living beings have thus evolved for billions of years to combat viruses. The evolution of gene therapy pales in comparison; the first humans were treated a mere 21 years ago.

Gene therapy can roughly be divided into two fields, transient and permanent gene therapy. The latter can be divided into *ex* and *in vivo* therapy. Transient or *ex vivo* gene transfers have their own sets of problems, but the virtually insurmountable challenges caused by anti-viral immune responses are mainly faced upon direct injection of a viral vector for *in vivo* correction of a gene.

The two viral vectors most commonly used are based on adeno- (Ad) and adeno-associated viruses (AAV).

Humans encounter different serotypes of Ad viruses early in life and develop neutralizing antibodies. Prevalence rates of such antibodies vary depending on the Ad serotype and the geographic region ([Chen et al., 2010](#)).

Neutralizing antibodies prevent the virus from entering its target and thus reduce transduction rates. This can be overcome, e. g., by using alternative Ad serotypes. CD8⁺ T cells pose the bigger problem. Humans have robust numbers of Ad virus-specific CD8⁺ T cells ([Chen et al., 2010](#)), which are activated and are chock-full of granules containing lytic enzymes ([Hutnick et al., 2010](#)). Even immunosuppression, short of complete T cell ablation, would have no effect on the ~1 billion Ad virus-specific CD8⁺ T cells of the average adult, which can commence lysis immediately.

In AAVs vectored for use in gene therapy, the ORFs are removed and replaced with the gene of interest flanked by the ITRs. Conversion of the ssDNA genome of AAV vectors into double-stranded (ds)DNA is rate limiting in transduction and delays onset of gene expression ([Ferrari et al., 1996](#)). DsAAV vectors have been developed and shown to achieve per vector genome copy higher levels of transgene product with accelerated onset of expression ([McCarty et al., 2001](#) ; [Nathwani et al., 2006](#)).

Prevalence rates of neutralizing antibodies to the different AAV serotypes vary ([Calcedo et al., 2009](#)). Neutralizing antibodies, even if present at only low titers, readily blocks AAV-mediated gene transfer. Humans have circulating CD4⁺ and CD8⁺ T cells to AAV capsid at low frequencies of approximately 0. 1% of a given subset. They less activated than those to Ad

viruses and belong predominantly to the effector memory or central memory subset ([Li et al., 2011](#)).

Adeno-associated viruses-mediated gene transfer readily results in sustained transgene expression in experimental animals ([Song et al., 1998](#) ; [Mount et al., 2002](#) ; [Gao et al., 2006](#)) presumably reflecting that AAV vectors are not particularly immunogenic. Based on successes in animals, AAV vectors were tested in clinical gene transfer trials. If given at moderate doses to an immunoprivileged site, they resulted in some correction of disease ([Maguire et al., 2009](#)). But if given systemically at the high doses needed to encode beneficial levels of a transgene product, such as factor (F) IX in hemophilia B, ssAAV vectors induced a capsid-specific CD8⁺ T cell response accompanied by loss of the therapeutic protein ([Manno et al., 2006](#) ; [Mingozi et al., 2007](#)). The potential success of AAV-mediated gene transfer in humans may turn out to be a numbers game. Transgene product expression by AAV vectors with a dsDNA genome is more efficient and in a recent trial a dsAAV8 vector achieved near therapeutic levels of factor FIX in hemophilia patients without evidence of T cell activation if given at a dose of 2×10^{10} vg/kg ([Nathwani, 2010](#)). In a preceding trial with ssAAV2 vectors for FIX, doses of 8×10^{10} vg/kg failed to elicit a T cell response but they also did not achieve detectable levels of FIX. At the next tested dose of 4×10^{11} vg/kg, T cell expansion was observed although levels of FIX still remained below detection. FIX levels together with increases in circulating AAV capsid-specific T cells were not observed till the final dose of 2×10^{12} vg/kg ([Manno et al., 2006](#) ; [Mingozi et al., 2007](#)). The T cell response to the capsid

proteins of AAV vectors is dictated by the input dose as without *de novo* synthesis of capsid proteins, T cells rely on presentation of degrading capsids, which are limiting both in terms of quantity and duration. Assuming that AAV vector doses below $\sim 10^{11}$ vg/kg can dodge recognition by T cells, gene therapists should concentrate on the use of maximally efficient AAV vectors. Nevertheless, this assumption, which is based on low numbers of AAV vector treated patients, has to be viewed with caution. Different serotypes of AAV vectors or even ss versus dsAAV vectors may differ in the degradation rate of their capsids. Processing of capsid for presentation of peptides with MHC class I molecules is mediated by proteases, which in turn are upregulated by proinflammatory cytokines. Capsids that are processed more rapidly may result in a bolus of epitopes, which may favor T cell recognition unlike capsids that are degraded slowly. Frequencies of circulating AAV capsid-specific CD8⁺ T cells in humans vary and humans with higher frequencies might be more prone to mount effective recall responses to AAV gene transfer than humans with low frequencies. Recommending firm doses for AAV-mediated gene transfer will thus not be feasible till larger cohorts have been tested and one can at this stage only advise a very conservative dose escalation.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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