Gene Therapy (2004) 11, 229–230

www.nature.com/gt

Adenoviral vectors

Adenoviral vectors, breaking a barrier to gene therapy?

G Eric Blair
School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

Gene Therapy (2004) **11,** 229–230. doi:10.1038/sj.gt.3302168

One of the most significant barriers to successful adenoviral gene therapy is the inability to perform repeated administrations due to the development of an immune response against the virus particle. This leads to production of neutralizing antibodies to the virus and the rapid clearance of transduced cells by the cellular immune system, resulting in cessation of transgene expression. Irrespective of developments in the design of adenovirus vectors, efficient gene delivery by the virus depends on intact virus particles; therefore, strategies to overcome the immune response against the virus are urgently needed. In this issue, Haegel-Kronenberger *et al*[1] report a targeted approach to transient suppression of the immune system in a primate model of adenovirus gene therapy, using chimeric human monoclonal antibodies against two key molecules involved in the stimulation of the immune response, namely CD40 and CD80. Rhesus monkeys were treated with antibodies against both molecules at the time of systemic administration of the first recombinant adenovirus. This led to a decrease in neutralizing antibody production and the successful expression of a transgene from a second recombinant adenovirus. This research expands possible approaches towards the goal of repeated adenovirus gene therapy and has important potential applications in the gene therapy of chronic diseases.

The immune response against adenovirus particles is mediated by interaction between virally transduced cells and T lymphocytes. In addition to presentation of viral antigens by the major histocompatibility class I and class II pathways, T-lymphocyte activation depends on a further interaction between costimulatory molecules present on the antigen-presenting cell (APC) and the corresponding receptors on T lymphocytes. In the absence of this interaction, T lymphocytes are not activated and do not develop into cytotoxic T lymphocytes that will kill APCs. The activation of T lymphocytes is also required to generate immunological memory in the form of memory T cells and to cooperate with B lymphocytes in the production of antibodies against the viral antigens. Therefore, targeted disruption of costimulatory interactions can have profound consequences in both suppressing production of neutralizing antibodies and preventing a cellular immune response to the viral vector. This would then permit readministration of the viral vector and expression of the transgene.

The major costimulatory pathways are the CD40–CD40L and CD28–CD80/86 pathways.[2,3] CD40 receptor molecules are present on the surface of B lymphocytes and so-called professional APCs such as dendritic cells and macrophages. CD40L (also termed CD154) is induced on activated T lymphocytes following antigen recognition and the CD40–CD40L interaction is essential for B-lymphocyte development and antibody production. CD28 (and its related receptor CTLA4) is present on T and B lymphocytes and interacts with CD80 and CD86, leading to lymphocyte activation. Previous attempts to suppress the immune system during adenovirus-mediated gene transfer have used either immunosuppressive drugs,[4] blocking of immune cell function with a nondepleting antibody against surface CD4 molecules of T lymphocytes,[5] or subversion of the CD40–CD40L interaction with either anti-CD40 antibodies or soluble forms of CTLA4.[6,7] These studies, performed in mice, showed prolonged transgene expression[4] and diminished neutralizing antibody production, permitting readministration of an adenovirus vector.[5,6] There is a clear need for these immunological approaches to be translated into a more appropriate model system prior to use in humans. A previous study blocked the CD40–CD40L interaction in rhesus monkeys using an anti-CD40L antibody and reported success in readministration of an adenovirus vector,[8] but no systematic blockade of both the CD40–CD40L and CD28–CD80/86 pathways has been reported in non-human primates.

Haegel-Kronenberger *et al* adopted an approach in which monkeys were treated either with human anti-CD40 or a mixture of anti-CD40 and anti-CD86 therapeutic antibodies. These were administered prior to, at the time of, or 3 days following inoculation with recombinant adenovirus (encoding human soluble CD4). After 32 days, the control and antibody-treated animals were given a second recombinant adenovirus expressing a different transgene (mouse interferon-gamma, mγIFN) and the expression of the transgene product assayed in serum samples. As expected, control animals that received no antibody treatment developed neutralizing antibodies against the adenovirus vector and failed to express the transgene product from the second recombinant adenovirus. In contrast, both groups of antibody-treated animals showed greatly reduced levels of neutralizing antibodies, and expression of the second transgene product (mγIFN) was detected in serum samples. However, much higher levels of mγIFN were detected in animals treated with both anti-CD40 and anti-CD86 than in the anti-CD40-treated animals. Treatment with either set of therapeutic antibodies also prolonged transgene expression driven by the first recombinant adenovirus. Reduced infiltration of CD8+ T lymphocytes in liver biopsies of antibody-treated animals was also noted compared to untreated controls, again with fewer CD8+ cells detected in animals treated with both anti-CD40 and anti-CD86. These results suggest that blockade of both CD40 and CD86 prevents both humoral and cellular immune responses against adenovirus vectors, as well as improving transgene expression from both first and

second administrations of recombinant adenovirus.

This work gives us hope that repeated administration of recombinant adenoviruses, coupled with transient blocking of costimulatory pathways, can result in prolonged expression of transgenes. However, this is very much a first step towards that goal. While expression of the second transgene could be detected in antibody-treated animals, this was at best around two orders of magnitude lower than that obtained in naïve animals. Clearly, more optimization is needed to boost expression of the repeat vector-derived transgene. The authors made an interesting observation in one monkey that received one-third less adenovirus in the initial administration (along with anti-CD40 and anti-CD86). This animal developed the lowest level of neutralizing antibodies and the highest level of mγIFN following second administration of recombinant adenovirus. The relationship between the dose of adenovirus and blocking monoclonal antibodies needs further study and could prove to be an important parameter for therapy. The stability of the therapeutic antibodies also deserves optimization, since prolonged expression of the transgenes appeared to correlate with levels of the antibodies in serum, indicating that prolonging the block to costimulation improved the transgene persistence. Finally, perhaps the key question is how many rounds of adenovirus administration along with blocking antibodies can be performed, and what levels of transgene expression can be achieved? ■

Correspondence: Dr G Eric Blair, School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.
e-mail: G.E.Blair@leeds.ac.uk

1 Haegel-Kronenberger H *et al*. *Gene Therapy* 2003; **11**: 241–252.
2 Grewal IS, Flavell RA. *Ann Rev Immunol* 1998; **16**: 111–135.
3 Sansom DM, Manzotti CN, Zheng Y. *Trends Immunol* 2003; **24**: 313–318.
4 Jooss K, Yang Y, Wilson JM. *Hum Gene Ther* 1996; **7**: 1555–1566.
5 Chirmule N *et al*. *J Immunol* 1999; **163**: 443–455.
6 Yang Y *et al*. *J Virol* 1996; **70**: 6370–6377.
7 Kay MA *et al*. *Proc Natl Acad Sci USA* 1997; **94**: 4686–4691.
8 Chirmule N *et al*. *J Virol* 2000; **74**: 3345–3352.