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The Eyes Have It

In this month's *Molecular Therapy*, Simonelli *et al.* describe the 18-month follow-up data for the first three patients with Leber's congenital blindness treated with adeno-associated viral (AAV) vector gene transfer of the *RPE65* gene.[1] Together with the authors' description of their complete study in the *Lancet*[2] and the initial report of the first three patients in the *New England Journal of Medicine*,[3] these data represent one of gene therapy's most impressive clinical accomplishments to date. Few who have seen the Web videos of a blind child being restored to sight can fail to be impressed by the power of gene therapy or moved by its benefits (http://www.nature.com/mt/journal/vaop/ncurrent/extref/mt2009277x3.avi). Although it may be a challenge to extend these accomplishments to other genetic causes of blindness, the fundamental properties of the eye in terms of its accessibility to local gene delivery and the extraordinary benefits to quality of life of even a limited restoration of sight will ensure that extensive efforts will be made to use gene therapy in a much broader range of retinal diseases.

Although the achievements of Simonelli *et al.* are impressive, I am an immunologist by training, and so for me one of the most curious features of this clinical trial is what did not happen. As with the dog that "did nothing in the night-time" (from Arthur Conan Doyle's story "Silver Blaze"), it is the apparent lack of a response from the immune system that is so striking. Since their inception, most gene transfer trials have made use of viral vectors, whose attraction is their relatively high efficiency of gene transfer, the wide range of cells that can be transduced, and the potential of several vectors—including AAV—to permanently integrate into host cell DNA. Unfortunately, these benefits are offset by the fact that vectors derived from viruses are subject to the full rigor of the immune system. Both innate and adaptive immunity are deployed to deny entry of viruses to target cells and to destroy infected cells before they can spread disease. Indeed, so aggressive can these antiviral vector responses be that they sometimes have fulminant and fatal consequences for the vector recipient.[4]

I think it fair to say that many in the gene therapy community were long in a state of semi-denial about the immunological consequences of injecting viral vectors into human subjects. One can readily find investigator rationalizations that explained how and why these responses would be unimportant for the specific application proposed, often based on animal studies in which the biology of the virus from which the vector was derived was quite different from that in humans. Undoubtedly, our willingness to pay limited attention to the immune response was facilitated by difficulties in measuring specific T-cell immunity to vectors, leading to a focus on assessments of neutralizing antibodies. Although increases in antibody titer may impede repeated vector administration, by limiting distribution and cell entry, it is cell-mediated immunity that is likely to be more relevant to gene therapy, because it is this that will recognize vector and transgene proteins expressed by the successfully transduced cells. As the ability to measure cellular immunity to vectors has become more widespread, it has become clear that such responses can lead to the speedy termination of gene expression. Certainly the presence of a potent cellular immune response contributed to the transient benefits observed in gene therapy studies for hemophilia using AAV, leading to a destructive inflammatory response in the transduced liver.[4] We now know that cellular immune responses to the hexon and penton proteins of adenoviral vectors can be even more aggressive[5] and indeed may be exploited to complement the therapeutic effects of these viral vectors when they are used to treat cancer.

Given the above background, it is notable that current reports of gene therapy for the eye[2,3] show limited local or systemic immune responses in the form of neutralizing antibody to the vector or T-cell responses to viral capsid or the therapeutic transgene. There are several possible explanations. The first is trivial and relates to the relatively small doses of vector administered, although this should have been ample to trigger at least a local response. More important, however, is the particular immune status of the eye. The anterior chamber has long been known as an immune-privileged site, but it is

apparent that the subretinal space too may lack an inflammatory immune response even after exposure to viral vectors. A limited immune response may not be considered so remarkable for AAV, because injection of this vector into certain other (extraocular) sites such as muscle may produce only modest immune stimulation, but the observation that adenovectors are similarly tolerated is much more striking. Thus, children with retinoblastoma who received repeated intraocular injections of an adenoviral vector containing the herpes simplex virus thymidine kinase had little evidence of significant local inflammation and no change in systemic cellular or humoral immunity to the adenoviral vector (C. Ildefonso, R. Hurwitz *et al*., personal communication, 2010), a sharp contrast to the effects of injecting the same vector into peripheral malignancies such as prostate cancer.[6] The mechanisms for this privilege have been unclear, and until recently the focus has been on physical barriers within the retina, such as Bruch's membrane. But it is now evident that the immune system can be actively downregulated, for example, by regulatory T cells (Tregs) and by cytokines, including interleukin-10 and transforming growth factor-β (TGF-β). Ocular administration of viral vectors may thus actively generate immunoregulatory responses, with increased Treg activity and associated cytokines, an effect that will not be detected by conventional interferon-γ ELIspot assays.[2,3] It is possible that infection by replication-competent viruses and associated inflammation is required to reverse such immune regulation.

What relevance does all this have for gene therapy outside the eye? Irrespective of the mechanism of ocular immune privilege, the ability to influence the Treg environment may be an important future component of successful viral vector–mediated gene transfer to other organ sites. At present, investigators are tackling the problem of unwanted immunogenicity by administering broad-spectrum immunosuppressive drugs with all their attendant adverse effects. A more sophisticated approach might include incorporation within a viral vector of immunoregulatory genes such as TGF-β that can induce a local regulatory environment and block an inflammatory immune response. Hence, the remarkable success of gene therapy for a rare disorder of the eye may turn out to have surprisingly broad implications for the field as a whole.

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