HUMAN GENE THERAPY 25:178–179 (March 2014)

DOI: 10.1089/hum.2014.2506

What Is Suppression of Anti–Adeno-Associated Virus Capsid T-Cells Achieving?

Terence R. Flotte and Christian Mueller

In general, recombinant adeno-associated virus (rAAV) vectors so far have been found to be safe and persistent. However, anticapsid immune responses have been observed in various clinical trials. Specifically, these are the development of neutralizing antibody responses, which may interfere with readministration and the development of anti-AAV capsid responses. Based on data arising from a number of rAAV factor IX clinical trials (Manno *et al*., 2006; Nathwani *et al*., 2011), a correlation has been made between effector T-cell responses to AAV capsid epitopes and the loss of cells that have been transduced *in vivo*. Particular emphasis has been placed on the presence of AAV capsid epitope-specific IFN-gamma ELISPOT results in peripheral blood. On the basis of this correlation, a number of investigators have included either prospective immune suppressive drug regimens or therapy with corticosteroids in the posttransduction period into their clinical trials of rAAV vectors. It has been suggested that the effectiveness of such facilitating therapies would be deemed successful initially by abrogation of anticapsid ELISPOT results. The results presented in an article in this issue of *Human Gene Therapy* by Ferreira *et al.* (2014) call this reliance on IFN-gamma ELISPOTs into question and raise the broader issue of whether pharmacologic suppression of effector T-cell responses is always necessary. In fact, the report published here was the basis for the licensure of intramuscular (IM) rAAV1-Lipoprotein Lipase (Glybera), the first human gene therapy licensed in the Western world, an agent whose European Medicines Agency (EMA) label indicates a need for immune suppression. In this report, despite the use of immune suppression, 4/5 subjects had positive IFN-gamma ELISPOT to AAV1 capsid epitopes and more significantly had CD8+ cellular infiltrates at the site of injection. However, the systemic and local immune responses did not seem to affect transgene expression or pose a safety risk.

It is vital in considering this report to keep in mind that the correlation of anticapsid T-cell responses with transduced cell loss has only been observed clinically when the liver is targeted, as in the two hemophilia programs mentioned above. In fact, the only other IM rAAV1 product for which there is significant immune response data, the rAAV1-alpha-1 antitrypsin (AAT) vector product, has included a series of trials performed without immune suppression. Two successive studies have demonstrated that vector expression persists for at least a year despite effector T-cell responses (Brantly *et al*., 2009; Flotte *et al*., 2011). Perhaps more informative is the recent finding that rAAV1-AAT elicits an AAV capsid-specific regulatory T-cell (T_{reg}) response (Mueller *et al*., 2013), which was documented both *in situ* and in the peripheral blood. In that study there was also evidence of PD-1 T-cells at the injection sites, indicating that very long-term capsid epitope persistence may have led to effector T-cell exhaustion, which contributed to the development of the robust T_{reg} response. In the current AAV1-lipoprotein lipase study by Ferreira *et al*., the muscle biopsies also stained positive for FOXP3+ CD4 T-cells. Thus, we believe that positive anticapsid ELISPOTs may be better interpreted in the context of this T_{reg} response. The abundance of T_{regs} in the rAAV1-AAT study (10% of all infiltrating T-cells in the injected muscle) would suggest that the T_{regs} are present in sufficient numbers to locally suppress any effector response that might have eliminated transduced myofibers.

Taken together, the findings in the study published here, along with the other IM rAAV1 clinical immunology data, suggest that IFN-gamma ELISPOTs do not predict loss of vector expression and do not serve as a reliable indicator of the need for immune suppression. Further studies are clearly needed to determine whether IFN-gamma ELISPOTs are predictive of undesirable elimination of vector-mediated expression with other rAAV serotypes and other routes of delivery. Until then, given the obvious risks associated with immune suppression, it seems prudent not to rely on ELISPOTs alone as the indication for immune suppression, but rather to seek first to correlate such laboratory findings with actual limitations on vector performance.

References

Brantly, M.L., Chulay, J.D., Wang, L., *et al*. (2009). Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. Proc. Natl. Acad. Sci. USA 106, 16363–16368.

Gene Therapy Center and Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA 01655.

Ferreira, V., Twisk, J., Kwikkers, K., *et al.* (2014). Immune responses to intramuscular administration of alipogene tiparvovec (AAV1-LPLS447X) in a phase II clinical trial of lipoprotein lipase deficiency gene therapy. Hum. Gene Ther. 25, 180–188.

Flotte, T.R., Trapnell, B.C., Humphries, M., *et al*. (2011). Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing alpha1-antitrypsin: interim results. Hum. Gene Ther. 22, 1239–1247.

Manno, C.S., Pierce, G.F., Arruda, V.R., *et al*. (2006). Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. Nat. Med. 12, 342–347.

Mueller, C., Chulay, J.D., Trapnell, B.C., *et al*. (2013). Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. J. Clin. Invest. 123, 5310–5318.

Nathwani, A.C., Tuddenham, E.G., Rangarajan, S., *et al*. (2011). Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N. Engl. J. Med. 365, 2357–2365.

Address correspondence to:
Dr. Terence R. Flotte
Office of the Dean
University of Massachusetts Medical School S1-340
55 Lake Avenue North
Worcester, MA 01655

E-mail: terry.flotte@umassmed.edu