PICTUREQUEST/PHOTOTAKE

Viral Vectors for Hemophilia Gene Therapy Part I: Potential Immune Consequences

Part I of this two-part gene therapy series will focus on the adaptive immune responses to adenoviral vectors.

While a gene therapy treatment of hemophilia remains an elusive goal, much progress has been over the last decade. Several gene delivery systems, called vectors, have been developed for both factor VIII (FVIII) and factor IX (FIX) gene delivery. Additionally, mouse models of hemophilia A and B, as well as naturally occurring canine hemophilia models, have significantly improved the preclinical evaluations of novel gene therapy strategies prior to moving into the clinic. Ideally, a gene transfer vector should be easily administered to a person with hemophilia, should deliver its gene to an appropriate target organ and should mediate a therapeutic level of clotting factor expression. The therapy should either mediate life-long expression of the clotting factor or be easily re-administered when warranted. Most importantly, the gene therapy should be safe; that is, toxicity associated with the vector or clotting factor expression should be minimal or absent. While no ideal vector system currently exists, the gene delivery systems in use in preclinical or clinical applications each have inherent advantages and disadvantages for hemophilia therapy. ▶

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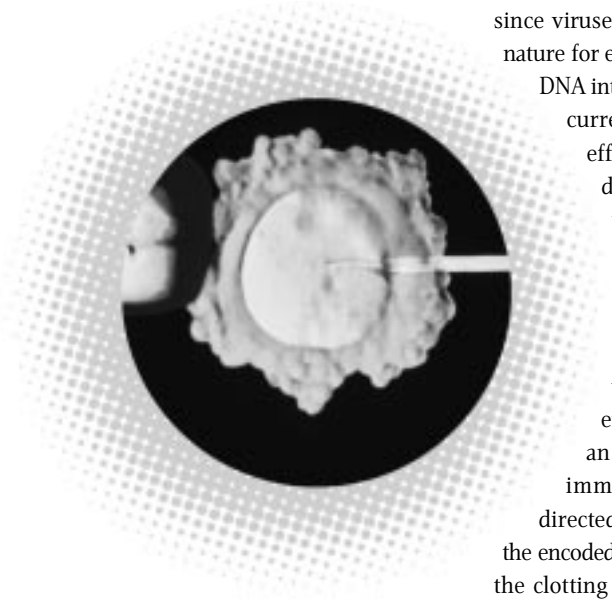
▶ Four gene therapy clinical trials for hemophilia treatment are in progress. Three utilize disabled viral vectors as vehicles for clotting factor gene delivery. The viral vectors are injected directly into the patient. The fourth trial employs the patient's own skin cells, which are modified outside the body to express the missing factor and then implanted back into the patient. These clinical trials are at an early stage, phase I, and are designed to assess safety issues, rather than the effectiveness of the therapy. This review will focus on the immune consequences of viral vector administration, which has a bearing on the safety of the therapy.



Two companies, Avigen and GeneStar Therapeutics Corporation, are conducting the clinical trials that utilize viral vectors. To date, Avigen has treated 10 patients, eight with intramuscular delivery and two with hepatic artery delivery. GenStar has treated one patient via intravenous delivery. Avigen's strategies, designed to treat hemophilia B, employ a gene transfer vector derived from adeno-associated virus (AAV). AAV is a virus that commonly infects humans and has no known pathological consequences. The native virus integrates into, and persists in, the DNA of the infected cells. GenStar's strategy, designed to treat hemophilia A, utilizes a vector derived from adenovirus. Adenoviruses are endemic in the human population and cause self-limited "common colds." Whereas the viral vectors in the current hemophilia trials have been derived from AAV and adenovirus, in both cases, all of the viral genes have been removed and replaced by the FIX and FVIII clotting factor genes, respectively. AAV vectors have the advantage of achieving sustained clotting factor expression, while adenoviral vectors generally achieve higher level expression.

Many gene therapy clinical trials, including those of Avigen and GenStar, use vectors derived from viruses since viruses have been designed by nature for efficient transfer of foreign DNA into cells. Thus, viral vectors currently provide the most effective means of introducing genes directly into patients. However, a disadvantage to the use of viral vectors stems from the fact that viruses are normally recognized by the immune system as foreign, and, as such, induce an immune response. This immune response can be directed at the viral particles or the encoded gene product, in this case, the clotting factor.

There are two general types of immune responses, adaptive and innate. The adaptive immune response consists of the development of immunity following an initial exposure to the foreign substance, in this case, the viral vector. In contrast, the innate immune response is an immediate reaction to the invading foreign agent, resulting in the release of specific biological messengers that induce inflammation and tissue injury. While the adaptive immune response has been well studied, especially with respect to adenoviral vectors, the innate immune response is not as well understood and is receiving increasing attention in gene therapy research. In general, the immune response is more pronounced for adenoviral vectors than for AAV vectors.

Adaptive Immunity

The adaptive immune system has two major weapon systems that are used to defend against viruses, termed humoral and cell-mediated immunity. The humoral immune system uses antibodies to directly attack the invading virus, while the cell-mediated response uses T-lymphocytes to recognize and kill cells that harbor an active virus. With the first exposure to a virus, the adaptive immune response is relatively slow and limited. However, the immune system is capable of remembering the virus and upon second exposure has a faster, more robust response termed anamnestic. There are several ways by which the adaptive immune system can interfere with adenoviral-vector mediated gene therapy.

Injection of an adenoviral vector into an animal or person results in the generation of circulating antibodies which peaks after two to three weeks. For the most part, these antibodies bind to the vector particle and neutralize it or facilitate its removal from the blood. Thus, antibodies induced by a first vector injection serve to block the effectiveness of a second treatment. Since adenoviral vectors do not mediate life-long clotting factor expression and multiple vector administrations may be required, the generation of anti-vector antibodies provides a significant hurdle for this vector system. Several strategies to enable repeat administration are currently being explored using animal models. Most investigators have used broad spectrum immunosuppression to block an antibody response, but the relevance to human therapy remains to be determined.[1,2] A second strategy involves coating the vector particle with a polymer such as polyethylene glycol[3] to shield it from antibodies. While most studies to date have been only partially effective, recent data are encouraging[4].

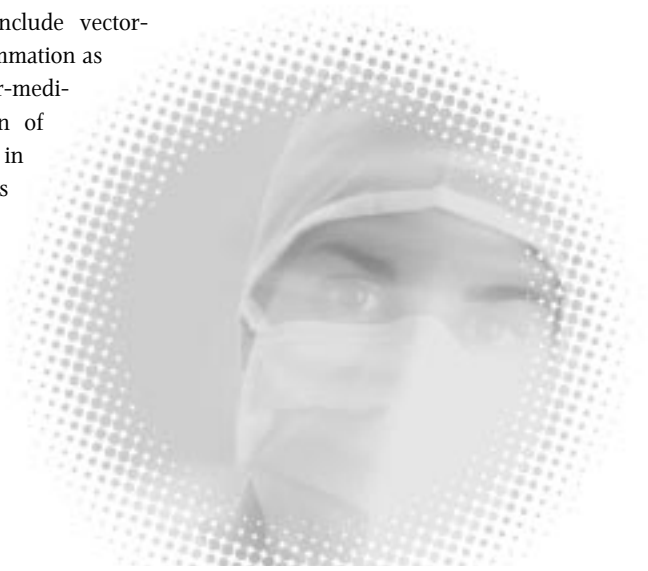
The issue of circulating anti- ▶

bodies against adenoviral vectors is further complicated by the fact that adenoviruses are endemic in the human population and most people have pre-existing antibodies, prior to even the first vector treatment. It is unlikely that immunosuppression will enable therapy in these patients. Strategies to address this issue include coating the vector and the use of vectors derived from uncommon adenovirus strains. An alternative strategy involves removing antibodies from the blood via an extracorporeal process called plasmapheresis.

The second way in which the adaptive immune system can interfere with therapy is through the cell-mediated response, which would eliminate the cells harboring the vector, resulting in loss of clotting factor expression. This response is most active against cells that express immunogenic viral genes and was problematic for early generation adenoviral vectors that retained viral genes.[5,6] Improved adenoviral vectors, deleted of all viral genes, such as the vector in the GenStar trial, are less likely to elicit a cell-mediated immune response.[7]

The third way in which the adaptive immune system can interfere with gene therapy for hemophilia is through the generation of an antibody, or inhibitor, to the expressed clotting factor. The generation of inhibitors has been variable in preclinical studies and, to date, the predisposing factors are poorly understood. For adenoviral vectors, two risk factors have been identified which include vector-mediated inflammation as well as vector-mediated expression of clotting factor in specialized cells of the immune system called antigen presenting cells. Encouragingly, recent studies in mice demonstrated that vector-mediated clotting factor expression may be less immunogenic than protein administration.[8,9,10] To date, no inhibitors have been observed in the ongoing hemophilia clinical trials. While these data are reassuring, clotting factor levels in these trials have been low. It will be important to carefully assess inhibitor development as clotting factor expression is improved.

In Part II of this series, the authors will discuss the innate immune response to adenoviral vectors, as well as other vector-mediated toxicities and the immune issues associated with AAV vectors.



REFERENCES

1. Smith TA, White BD, Gardner JM, Kaleko M, McClelland A. Transient immunosuppression permits successful repetitive intravenous administration of an adenovirus vector. *Gene Ther.* 1996;3:496-502.

2. Hariharan MJ, Rahman A, Podar E, Lillegren LL, Jaworski DO, Muralidhar G, Fang X, Sobol RE, Chiang YL. Transgene expression in the presence of anti-vector antibodies and re-administration of a helper dependent adenoviral vector with immune modulation. *Molecular Therapy* 5(5), 271-271. 6-1-2002. Abstract

3. O'Riordan CR, Lachapelle A, Delgado C, Parkes V, Wadsworth SC, Smith AE, Francis GE. PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody in vitro and in vivo. *Hum Gene Ther.* 1999;10:1349-1358.

4. Croyle MA, Miao C, Toietta G, Beaudet AL, Pastore L. PEGylated helper-dependent adenoviral vectors: highly efficient vectors with an enhanced safety profile. *Molecular Therapy* 5(5), 466-466. 6-1-2002. Abstract

5. Yang Y, Ertl HC, Wilson JM. MHC class I-restricted cytotoxic T lymphocytes to viral antigens destroy hepatocytes in mice infected with E1-deleted recombinant adenoviruses. *Immunity.* 1994;1:433-442.

6. Yang Y, Nunes FA, Berencsi K, Furth EE, Gonczol E, Wilson JM. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA* 1994;91:4407-4411.

7. Kochanek S, Schiedner G, Volpers C. High-capacity 'gutless' adenoviral vectors. *Curr Opin Mol Ther* 2001;3:454-463.

8. Bristol JA, Gallo-Penn A, Andrews J, Idamakanti N, Kaleko M, Connelly S. Adenovirus-mediated factor VIII gene expression results in attenuated anti-factor VIII-specific immunity in hemophilia A mice compared with factor VIII protein infusion. *Hum Gene Ther.* 2001;12:1651-1661.

9. Chao H, Walsh CE: Induction of tolerance to human factor VIII in mice. *Blood* 2001;97:3311-3312.

10. Fields PA, Armstrong E, Hagstrom JN, Arruda VR, Murphy ML, Farrell JP, High KA, Herzog RW. Intravenous administration of an E1/E3-deleted adenoviral vector induces tolerance to factor IX in C57BL/6 mice. *Gene Ther.* 2001;8:354-361.