Gene Therapy

Factor IX Deficiency

Since its inception, gene therapy has been characterized more typically by problems than by successes. The field has been limited by the characteristics of the gene delivery vehicles (vectors), and in some instances, the diseases targeted by gene therapists, particularly sickle cell disease and cystic fibrosis, have presented formidable challenges in terms of efficient entry of the vector into the appropriate target cells.

Gene therapy is still a young field, the first patients having been treated in 1990, and the early history of such innovative medical treatments as bone marrow transplantation was also marked by frequent setbacks and disappointments. Although there have been no clear-cut successes with gene therapy, neither have there been serious adverse events. Efforts to develop a gene therapy approach for hemophilia stand out against this discouraging landscape and have led to the recent initiation of clinical trials.

Hemophilia is characterized by frequent spontaneous bleeding episodes, which can be caused by a mutation in the gene for either factor VIII or factor IX. The major morbidity of the disease stems from repeated bleeding into joints and soft tissues, eventually leading to a crippling arthropathy. The major mortality results from bleeding into other critical closed spaces such as the intracranial space or the retroperitoneal space.

Clotting factor concentrates are the cornerstone of hemophilia therapy but have several disadvantages. First, the proteins have short half-lives, on the order of 12 to 18 hours. Second, concentrates prepared from pooled plasma can transmit bloodborne viral diseases. Introduction of more effective inactivation techniques and development of recombinant clotting factors has greatly reduced but not eliminated the risk of viral infection. Third, concentrates must be infused intravenously, and during the inevitable delay between the start of bleeding and the infusion, damage to tissues has begun. Finally, currently available clotting factor concentrates are expensive; an adult with severe hemophilia may spend $50,000 per year on concentrates alone.

Hemophilia has four characteristics that favor the success of a gene therapy approach. First, biologically active clotting factors can be synthesized in many tissues, including endothelial cells, fibroblasts, and muscle cells. Indeed, commercially available clotting factor concentrates are synthesized in hamster kidney and ovary cells. Thus, from a gene therapy standpoint, hemophilia affords wide latitude in choice of a target cell.

Second, in contrast to diseases that require precise regulation of the levels of gene expression, clotting factors in hemophilia have a remarkably wide therapeutic window, ranging from just above 1% of normal levels to about 150%. A generation of experience with clotting factor concentrates has established that maintenance of trough levels in the range of 1 to 4% prevents most spontaneous bleeds.

Third, the availability of animal models that closely resemble the human disease is a major asset for any attempt to develop new therapeutic strategies; in the case of hemophilia, both murine and canine models exist, and the murine and canine factor VIII and factor IX genes have been cloned.

Finally, therapeutic end-points are well defined and easy to measure. Circulating levels of clotting factor correlate well with disease phenotype and can be determined in any coagulation laboratory.

Early attempts at gene therapy for hemophilia were characterized by either short-term gene expression or long-term expression at levels too low to be therapeutic. The goal of long-term expression at levels high enough to result in shortening of clotting times has only recently been achieved in a large animal model. An advance that proved crucial was the observation that an adeno-associated viral vector could direct sustained expression of a transgene following introduction into skeletal muscle.

Adeno-associated viral vectors, which efficiently enter nondividing target cells, are engineered from a naturally occurring parvovirus with a small (4.7 kb) single-stranded genome. The wild-type virus is not pathogenic in humans and is replication-defective, requiring a helper virus such as adenovirus to generate infectious particles. Adeno-associated viral vectors do not contain any viral coding sequences, because these have been replaced with the therapeutic gene, reducing the likelihood of a major stimulus to the immune system. Their principal disadvantage is their inability to accommodate DNA inserts larger than about 4.7 kb.

The first demonstration of long-term expression of therapeutic levels of factor IX in an animal model was reported in May 1997 and involved an adeno-associated viral vector expressing human factor IX introduced into intramuscular sites in the hindlimb of a mouse. Similar findings were demonstrated when vector was introduced into the livers of mice via the portal vein.

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The stringent test of gene therapy strategies for hemophilia, however, is demonstration of success in a large animal. Such a model is much more realistic than an inbred strain of laboratory mouse because it requires solution of such problems as efficient scale-up of recombinant vector production, immune response to the transgene product, and efficient transit of the transgene product from the site of synthesis to the circulation. The scale-up factor from a mouse to a dog is about 400 to 800-fold, while the scale-up from a dog to a human is only 3 to 10-fold.

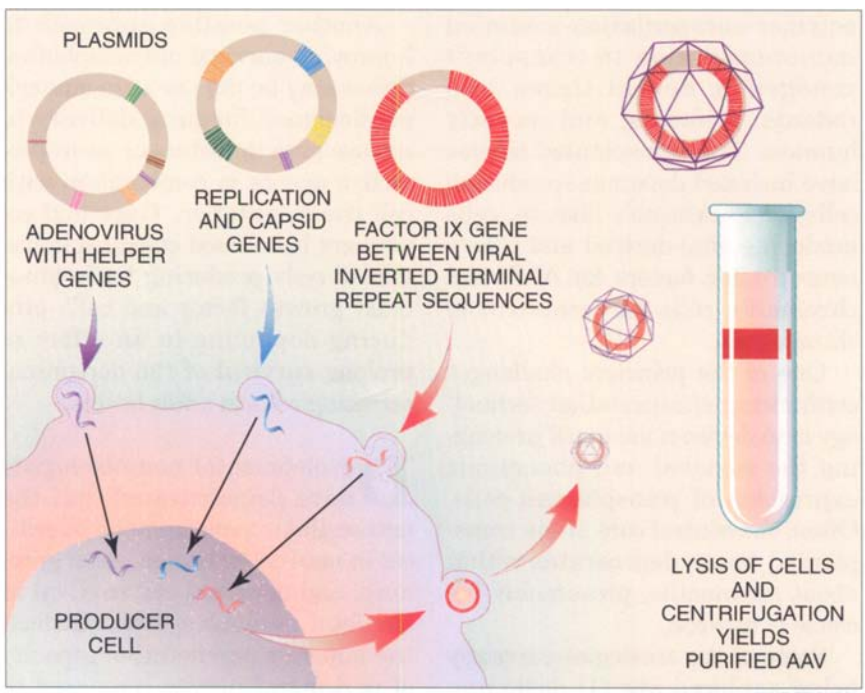
Two groups have reported sustained expression of canine factor IX at levels above 1% of normal human factor IX levels in hemophilia B dogs treated with adeno-associated viral vectors. In the first study, two dogs were infused intraportally, and in the second, five dogs were injected intramuscularly at multiple sites but at the same time. Neither study showed any local or systemic toxicity associated with administration of the vectors.

Depending on the dose injected via the intraportal route, one dog expressed 20 to 30 ng per ml of factor IX and the other 60 to 80 ng per ml, which represented approximately 0.5% and 1% of biological activity, respectively. Both animals displayed partial correction of the whole blood clotting time and activated partial thromboplastin time.

Via the intramuscular route, partial correction of the whole blood clotting time was achieved in all five animals, with canine factor IX levels ranging from 3 to 70 ng per ml. The levels of expression have been stable for at least 23 months, with no signs of a fall in factor IX levels. Biological activity of the circulating protein was shown by sustained partial correction of the clotting studies.

An important aspect of these studies in dogs addressed the possible development of an inhibitor to the factor IX transgene product. As has been well described for protein-based therapy, development of an antibody that prevents the biological activity of the protein (termed an inhibitor clinically) is a serious complication of treatment.

In the intramuscular study, only one of the five dogs developed an inhibitory antibody, which was present only transiently and which prolonged the clotting times during the first two months post administration. In another dog, a transient noninhibitory antibody was detected, which prolonged the clotting times for two weeks, presumably by enhancing the clearance of factor IX. Neither antibody response occurred later, even when the dogs were rechallenged with plasma containing canine factor IX.



ANNE ERICKSON

Both studies have shown that it is possible to achieve long-term gene expression in an immune-competent large animal model of hemophilia. The muscle approach is in some ways more attractive because of easy access to muscle mass and the relative safety and ease of the procedure. A high percentage of adults with hemophilia are infected with hepatitis B or C, or both, a fact that makes the liver approach more problematic. A clinical trial using an adeno-associated viral vector expressing human factor IX injected into skeletal muscle is currently underway.

Concerns for clinical trials are the possibility of inhibitor development and the possibility of integration of vector sequences into chromosomal DNA. Long-term experience with clinical trials will be needed to look for the development of oncogenic transformation induced by integration and to determine the risk of inhibitor formation. So far, no evidence of tumor formation has been observed in ongoing clinical trials with integrating vectors, such as retroviruses.

Another area of concern is the risk of inadvertent germline transmission of vector sequences, which may occur if vector disseminates from the site of injection to the gonadal tissues. It is unclear at present whether adeno-associated viral vectors can integrate into germline cells, but the issue needs to be addressed in the design of clinical trials, and more data will be needed from animal models.

PAUL A. FIELDS
KATHERINE A. HIGH
The Children's Hospital of Philadelphia