Gene Therapy

Hemophilia

Several thousand inherited diseases have already been characterized and are potential targets for gene therapy. The coagulation disorders hemophilia A (factor VIII deficiency) and B (factor IX deficiency) are two among them. Because the level of coagulation factors in vivo is not tightly regulated, and because delivery of therapeutic agent is required only in the circulation, hemophilia is an attractive target for gene therapy. In addition, the high health care cost that hemophilia generates is an important consideration.

Hence, a large number of gene therapy research groups worldwide have directed their efforts to treating hemophilia. As a result, almost every gene therapy approach developed has been applied to hemophilia, with varying degress of success.

Current treatment for hemophilia relies on regular infusions of purified coagulation factors. During the past decade, the AIDS epidemic has hit the hemophilic population particularly hard. As a result of the use of plasma-derived factors, as many as 70% of all severe hemophiliacs in the U.S. became HIV-positive.

The advent of recombinant factor VIII, and the recent availability of recombinant factor IX, could basically eliminate the risk of infection by blood-borne pathogens. However, the high cost of the recombinant products may prevent their widespread use. Therefore, an alternative mode of treatment would be highly desirable.

The most critical element for a successful gene therapy protocol is the use of a vector able to achieve efficient delivery of the desired therapeutic protein in vivo. Gene therapy for hemophilia is no exception. Hence, particular attention has been given to the design of efficient vectors for the different gene therapy approaches.

The factor IX gene is not large, with a cDNA of about 1400 base pairs, so it is easily amenable to genetic modifications and cloning into virtually any expression vector. However, the high normal level of factor IX in humans, about 5 μg per ml, has represented a serious hurdle for the delivery of therapeutic amounts of the coagulation factor.

The first successful attempt to deliver factor IX to spontaneously hemophilic dogs achieved less than 0.1% of physiological levels. Nonetheless, it was encouraging to observe that even this low level was enough to affect the hematological parameters and reduce whole blood clotting time in the dogs. This was achieved with the administration of a retrovirus vector following partial hepatectomy to promote liver regeneration, as retrovirus infects only dividing cells.

Although the field has seen the widespread use of retrovirus vectors, experiments using adenovirus, a vector that can transduce nondividing cells, were the first to attain therapeutic levels of factor IX in hemophilic dogs and mice. Indeed, adenovirus vectors are able to deliver supraphysiologic levels of human factor IX in animals.

However, delivery of recombinant proteins with current adenovirus vectors is of a transient nature. Immune responses to both the vector and the transgene product cause the delivery to gradually decline. In addition, the immune response makes any subsequent readministration ineffective.

Although the coadministration of immunosuppressing drugs can somewhat prolong the delivery of factor IX, this currently represents a significant barrier to the treatment of life-long diseases such as hemophilia. There is a great research effort underway aimed at reducing the immune response to adenovirus vectors by eliminating viral genes from the vectors.

The most promising results to date come from the direct injection of adeno-associated virus (AAV). After intraportal injection, recombinant AAV-factor IX vector was targeted to the liver, and mice showed therapeutic levels of human factor IX (40% of physiological) in the circulation for at least a year. In spite of the use of immunocompetent mice, an immune response was not observed.

In a similar study that used the same mouse strain, intramuscular injections of AAV-factor IX vector also allowed for the delivery of therapeutic levels of human factor IX, albeit slightly lower levels. Interestingly, however, this mode of delivery did elicit antibodies to human factor IX in the animals. If AAV treatment in humans fails to elicit an immune response, this approach could be a promising avenue leading to the advent of human trials, as AAV is considered a benign virus.

Clearly, to date, the highest levels of factor IX have been achieved using viral vectors. Another in vivo gene therapy strategy that has been described for hemophilia is the intravenous injection of factor IX DNA enclosed in complexes of liposome formulations to extend the DNA half-life in vivo, or taking advantage of the presence of tissue-specific cellular receptors. Linking factor IX DNA to a receptor binding ligand allows targeting of DNA to the desired cells, such as

"Gene Therapy" is edited by Joanne T. Douglas and David T. Curiel of the Gene Therapy Program, University of Alabama at Birmingham.

hepatocytes. Direct injection of recombinant allogeneic cells, such as myoblasts, that secrete factor IX, and the implantation of biocompatible immunoisolation devices enclosing recombinant cells, have also been proposed.

Even though approximately 200 human gene therapy trials have already been approved or are under consideration for hemophilia, only one human factor IX gene therapy trial has been conducted to date. In that trial, which took place in China, fibroblasts were obtained from the patients and transduced in vitro with a retrovirus containing factor IX DNA. After cellular expansion in vitro, the factor IX-secreting fibroblasts were subsequently injected back into the patient. Four patients have been treated so far, with no side effects reported. Partial improvements in the hemostasis parameters of the patients were observed.

Although the level of factor VIII in humans is low, about 100 ng per ml, its expression has been more difficult than that of factor IX, primarily because of the presence of inhibitory sequences within the factor VIII gene that hinder its expression and the large size of the gene, which has a cDNA of 9000 base pairs. Gene therapy for hemophilia A has therefore lagged behind that for hemophilia B.

In order to facilitate the expression of factor VIII, the DNA coding for a large part of the factor called the B domain can be removed, reducing the size of the resulting protein by about 30% and making it more amenable to cloning manipulations. Its biological activity is not altered in any detectable way.

Recombinant B domain-deleted human factor VIII is currently in clinical trials for the treatment of hemophiliacs, and it is currently the factor VIII form used by most research groups engaged in hemophilia A gene therapy. Advances in vector development have achieved higher expression of recombinant factor VIII.

The approaches taken for the delivery of factor VIII have been largely similar to those for factor IX. Following on the success of adenoviral delivery of factor IX, in vivo adenovirus injections also resulted in impressive levels of factor VIII in the circulation. Indeed, there are reports of supraphysiological levels of factor VIII shortly after adenovirus treatment. These findings have been reproduced by different research groups. However, following the pattern observed with the delivery of factor IX, the delivery eventually declined. Once again, the transient nature of adenovirus was caused by an immune response.

Retroviruses are not antigenic and have also been used as vectors in factor VIII gene therapy experiments. Although the levels typically attained with the use of retroviral vectors have not been as high as those described using adenovirus, immune response to retrovirus is not a significant factor, so that the delivery achieved is not transient. Furthermore, repeated treatments may be possible.

Recently, Chiron, Inc. has reported the delivery of therapeutic and persistent levels of human factor VIII in rabbits and dogs following intravenous injection of retrovirus. The clinical findings also include a significant reduction of the blood clotting time that lasted for days in hemophilic dogs. Prolonged delivery was achieved in rabbits, but dogs developed antibodies to human factor VIII, and the delivery of factor VIII ceased.

The cDNA of factor VIII is too large to be cloned into adeno-associated virus vectors even with the B domain deleted.

The therapeutic levels of coagulation factors VIII and IX that can be achieved using viral vectors open the possibility of starting human trials. However, with the advent of recombinant coagulation factors, there is currently an effective, although suboptimal, treatment for these clotting factor deficiencies. Any gene therapy protocol must therefore meet stringent safety guidelines.

Another potential concern is that most vectors for in vivo gene therapy protocols target the liver. A large proportion of hemophiliacs, particularly those with severe cases, have liver damage because of viral infections (such as hepatitis) commonly suffered following the infusion of blood-derived coagulation factors. Indeed, more studies are necessary to determine what effect, if any, this pathology may have on the efficacy of the proposed gene therapy protocols.

Certainly, the long-term human response to viral vectors still remains to be established. Finally, it is relevant to remember that the hemophilic animals used to develop gene therapy protocols are not perfect models of human hemophilia. In fact, they often develop antibodies to human coagulation factors. As more data from other human gene therapy trials become available, safety concerns may be addressed appropriately, and hemophilia gene therapy trials may become a reality.

GONZALO HORTELANO

Department of Pathology
McMaster University

Sci & Med 5(4):4-5, 1998.