PART II

in a 3-part series on Gene Therapy

exploring gene therapy: { VECTORS }

In the last issue of *HĒMAware*, the authors discussed the basics of gene therapy. The following installment describes and explains the five types of systems used to introduce DNA that have been used for hemophilia gene therapy.

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RETROVIRAL VECTORS

Retroviral vectors have a significant advantage over many other vectors: they integrate into (insert themselves into and become one with) the host cell DNA. Once integrated, these vectors are part of the host cell DNA and will continue to exist and, hopefully, express the transgene throughout the life of the cell. If the cell later divides, a copy of the vector will be found in both of the resulting cells. However, the drawback of retroviral vectors is that they can only transduce dividing cells, and most of the cells in an adult body are not dividing. This problem has been circumvented by using ex vivo techniques and stimulating the cells to divide in tissue culture before adding the vector. Once these cells are put back in the body, however, the retroviral vectors are sometimes turned off for reasons that are not yet clear. Finally, a theoretical concern is that the retrovirus might insert into a critical part of the genome and cause mutations with deleterious consequences. Although this is a potential issue with any vector that inserts into the genome, it has not turned out to be a significant problem.

Retroviral vectors have been used to transduce the factor IX gene into mouse fibroblasts.[1] When these cells were injected into mice, good factor IX expression was seen for the first few days, but by seven days factor IX was no longer expressed due to vector inactivation. Longer-term expression was achieved with mouse myoblasts (muscle cells),[2] but the levels of factor IX were 10- to 25-fold lower than needed to achieve clotting. Even less encouraging results were observed in larger animals and in humans.

Scientists have recently begun working on ways to use retroviral vectors for in vivo transduction. Although these vectors do not transduce nondividing cells, administration of the vector with a protein that stimulates the growth of certain types of cells resulted in efficient in vivo gene transfer to liver cells.[3] Retroviral vectors have also been shown to successfully transduce cells in vivo when they are injected into newborn mice, as young animals have a significant number of dividing liver cells.[4] Finally, injection of very high levels of retroviral vector into adolescent or adult animals has been shown to result in in vivo transduction.[5]

ADENOVIRAL VECTORS

Adenoviral vectors were introduced to the field of gene therapy in the early 1990s. These vectors are extremely efficient at transducing cells, even nondividing cells, and they achieve high-level transgene expression. Unfortunately, adenoviral vectors are associated with some disadvantages as well. Adenoviral vectors do not integrate into the genome, and thus they eventually disappear from cells. In addition, many people have been exposed to adenovirus, a common cold virus, and thus have antibodies to it in their blood, which can attack and neutralize the vector before it reaches the target cells.

Liver cells are the target for adenoviral vectors: almost 100% of liver cells are transduced following injection of the vector into a ▶

GLOSSARY

- **Basepair:** the smallest unit of information in a double-stranded DNA molecule
- **DNA:** deoxyribonucleic acid, the chemical substance that makes up genes
- **Ex vivo:** outside of the body
- **Gene therapy:** a means of treating human disease by transferring genes into a patient's somatic cells
- **Gene:** a segment of DNA that contains all of the genetic information required for a particular product, such as a clotting factor
- **Genome:** the complete set of genes contained in each cell
- **In vivo:** inside the body
- **Mutation:** a change in the information contained in a gene. It can involve one basepair or many basepairs
- **Transduction:** the process of transferring a gene into a cell
- **Transgene:** the therapeutic gene carried by the vector
- **Vector:** the vehicle for transferring genes into a cell

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mouse's tail. Early experiments in mice demonstrated that very high levels of factor VIII could be achieved in a mouse model of hemophilia.[6] Although a drop-off in expression occurred over time, factor VIII levels remained above the human therapeutic level for a year. However, when similar vectors were used in hemophilia A dogs, factor VIII expression was only detected for about 10 days, and liver toxicity was a significant problem.[7,8] This "shut down" was believed to be due, at least in part, to an immune response against the liver cells expressing the vector.

To solve this problem and reduce liver toxicity, "gutless" adenoviral vectors were made. The key feature of these vectors is that all of the adenoviral genes have been removed. In mice and baboons, gutless adenoviral vectors result in higher levels of transgene expression, longer-term expression, and less toxicity than adenoviral vectors with their genes intact.[9,10] Gutless adenoviral vectors may thus represent a major advance in gene therapy.

ADENO-ASSOCIATED VIRAL VECTORS (AAV)

Although it is not yet known whether AAV vectors integrate, it is clear that they result in long-term expression. AAV vectors can transduce nondividing cells, although not quite as well as adenoviral vectors, and they do not appear to be associated with any toxicity. AAV vectors target muscle and liver. The drawback to this vector is its size: it cannot hold large quantities of genetic information, and thus it has been difficult to get factor VIII into AAV, although this has recently been achieved.[11]

In studies with a factor IX transgene, an AAV vector resulted in expression of therapeutic levels of factor IX in mouse liver for over a year.[12] Slightly lower levels were observed in the muscle of dogs with hemophilia, but these amounts were still sufficient to mediate improvements in clotting time.[13] Factor VIII AAV constructs have recently become available and result in high-level expression in mice.[11]

LENTIVIRAL VECTORS

Lentiviral vectors are derived from the human immunodeficiency virus (HIV). However, virtually all of the backbone viral genes have been removed: vector preparations do not contain any live HIV virus. Like retroviruses, to which lentiviruses are closely related, lentiviral vectors integrate into the host DNA and thus have the potential for lifelong expression. Their advantage over retroviral vectors is that they transduce both dividing and nondividing cells, so that in vivo therapy is much easier to achieve. The target cells for lentiviral vectors are muscle, liver, spleen, and, for ex vivo therapy, bone marrow. In the liver, not only hepatocytes appear to be transduced, but also other cells such as capillary endothelial cells,[14] which are useful targets for expressing clotting factors into the circulation. Also like retroviral vectors, lentiviral vectors have the potential to cause mutations when they insert into the genome. Other drawbacks to the lentiviral system include difficulties in manufacturing these vectors and a general lack of knowledge concerning their effects, as they are the newest gene therapy approach to be studied.

Early studies with lentiviral vectors demonstrated that these vectors allow the sustained expression of genes when delivered directly into liver or muscle.[14] Lentiviral vectors containing human factor VIII are currently being developed.

NAKED DNA

Unlike viral vectors, naked DNA gene therapy systems do not require a vehicle in which to enclose the gene. Although some scientists are using naked DNA to mediate in vivo gene therapy, the best-studied approach to date involves ex vivo transfer of naked DNA. For instance, one of the companies conducting clinical trials in patients with hemophilia (see following section), Transkaryotic Therapies, is introducing naked DNA into fibroblasts from a patient's body by an electrical technique that creates

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▶ temporary "holes" in the cell membrane referred to as electroporation. They then select cells that are expressing particularly high levels of factor VIII, grow these in tissue culture, and then return them to the patient. The use of naked DNA avoids some of the problems associated with viruses, but creates some of its own. For instance, vector-related toxicity is not a problem with this approach, but naked DNA may be broken down before reaching cells and inefficiently taken up into and expressed by cells during in vivo therapy, while ex vivo techniques are labor intensive.

Next issue: Clinical trials in gene therapy for hemophilia

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