PART I

in a 3-part series on Gene Therapy

exploring gene therapy:

OVERVIEW AND INTRODUCTION

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In the last two years, three clinical trials of gene therapy in patients with hemophilia have begun. With this milestone, gene therapy has moved from the realm of science fiction to actual clinical reality. There are still some significant hurdles to overcome before gene therapy is a treatment option for most patients with hemophilia, but that day definitely appears to be drawing nearer. As gene therapy approaches become more common, it is essential for those who are considering this treatment, either for themselves or for family members, to have an understanding of the benefits and risks it might pose. This article will describe the goals, techniques, and possible drawbacks of gene therapy, with an emphasis on the progress that has been made in gene therapy for hemophilia.

Terminology

Like many other scientific fields, understanding the terminology is half the battle to grasping the concepts behind gene therapy. Throughout this article, italicized words can be found in the accompanying glossary.

Simply put, *gene therapy* is a means of treating human disease by transferring a specific *gene* into a patient's non-reproductive (*somatic*) cells. This is accomplished by means of a *vector*, a vehicle for transferring genes into a cell. As will be discussed later, most vectors are viruses or virus derivatives. Viral vectors enclose the genes and protect them until they are safely inside a cell. The therapeutic gene carried by the vector is referred to as the *transgene*. For hemophilia A, the relevant transgene is the factor VIII gene, while for hemophilia B, it is the factor IX gene. In most cases, the transgenes are modified somewhat from the gene form found in the body. The process of introducing the gene into the cells is called *transduction*.

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

Hemophilia and Gene Therapy

Why Hemophilia?

Hemophilia has long been considered one of the best candidates for gene therapy for a number of reasons. First, the genetic basis of the disease is well understood and, in the vast majority of cases, only one gene is involved. Hemophilia A is due to a *mutation* (genetic change) in the factor VIII gene, while hemophilia B is due to a mutation in the factor IX gene. Second, the exact levels of expression of factors VIII and IX are not tightly regulated: even a little bit can help promote clotting, and too much clotting factor may not be a problem. Third, a variety of organs can be used to secrete clotting factors into the circulation. Most clotting factors are normally produced by the liver, but other tissues, such as muscle or spleen, also work. Fourth, excellent animal models of hemophilia exist. These allow researchers an avenue in which to test possible gene therapy strategies. Finally, gene therapy could represent an advance over currently available therapies and potentially improve the quality of life of patients with hemophilia. The need for repeated intravenous (IV) injections and restrictions on the patient's lifestyle might be reduced or eliminated, and the cost could be less. In fact, gene therapy could potentially be a "cure" for hemophilia, in that one treatment with a gene therapy vector could possibly confer lifelong protection. However, a true cure would require replacement of the altered gene responsible for hemophilia with an intact gene. This is not yet feasible.

Hemophilia A vs. Hemophilia B

Although the clinical symptoms of hemophilia A and hemophilia B are similar, the genes that encode factor VIII and factor IX are not. The molecular differences between hemophilia A and B, particularly in the size of the gene involved, have important ▶

PICTUREQUEST

PHOTODISC

TABLE 1.
COMPONENTS OF AN IDEAL VECTOR SYSTEM

- Efficient and specific transduction when administered via a clinically acceptable route
- Sustained and controlled transgene expression
- Repeat administration
- No toxicity
- Ability to remove the vector in the event of an adverse reaction
- No risk to society or the environment
- Feasible manufacturing, storage, pricing, and treatment regimens

▶ consequences for gene therapy. Normally, factor VIII requires 7000 *basepairs* of coding information (the gene itself is much larger, but not all of that information is needed to make the protein). The protein made by this gene is not made as a finished product, but must undergo complex enzymatic processing inside the cell. Because of this requirement for accurate processing, it is possible that factor VIII will not be efficiently secreted by tissues such as muscle.

In addition, factor VIII requires von Willebrand factor for stability in the circulation. Normal levels of factor VIII are low (100 nanograms/milliliter of blood), but historically it has been hard to express factor VIII from cells. Factor IX requires only 1500 basepairs of coding information. It is readily secreted into the blood, and although normal levels of factor IX are about 50 times higher than factor VIII (5000 nanograms/milliliter of blood), it has been relatively easy to express factor IX in cells. Because of these features, much of the early gene therapy work was performed with factor IX. At this point in time, however, work on factor VIII has come close to "catching up" with the factor IX studies.

The Complexities of Gene Therapy

The concept behind gene therapy—overcoming a gene defect by introducing a healthy copy of the gene—is so simple that it can be easy to overlook the technical difficulties of such a strategy. An intact *genome* (the complete set of genes contained in each cell) is critical to our health, and so our bodies have developed numerous ways to protect it. For instance, following injection into the bloodstream or a tissue, the vector can be destroyed by the immune system. If it survives this assault, the vector must be able to reach and bind to the correct cells. It must then be internalized into the cell and get into the cell's nucleus, where the genome is found. Once in the nucleus, the vector must be able to express the transgene. This can be a key "shut down" point, as some cells are capable of "turning off" vectors, apparently by inactivating the genetic information that allows the vector to express genes. Finally, the cells expressing the transgene may be recognized as foreign and destroyed by the immune system.

There are two primary approaches by which gene therapy can be accomplished: ex vivo, which involves modifying cells outside of the body and then reinfusing the genetically modified cells, and in vivo, in which gene transfer occurs through systemic treatment or direct injection of a vector carrying the transgene (Figure 1). In the late 1980s, the ex vivo approach received the most attention. Bone marrow, fibroblasts, or muscle cells were removed from a patient's body and modified to express a clotting factor in a tissue culture dish. These cells were allowed to grow in culture, and then were reimplanted in the body. The in vivo approach, which is the one primarily being studied today, is somewhat easier because it allows the same vector preparation to be used for all patients requiring treatment. For in vivo gene therapy, the vector carrying the transgene is injected IV or into a specific tissue.

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Gene Therapy Systems

Table 1 lists a number of features of an ideal vector system. Many of these features exist in current vectors, but some challenges remain. At this point in time, efficient vector systems are available, but their specificity leaves something to be desired. Some current vectors may allow sustained transgene expression in humans, but controlled expression still presents a challenge. Safety, the most critical requirement, is being investigated. Although it is not known yet how to remove a vector in case of any problems, scientists are working on this.

Five types of systems to introduce *DNA* have been used for hemophilia gene therapy: retroviral vectors, adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, and naked DNA. The features of these systems are summarized in Table 2 and will be discussed in more detail in next issue's installment.

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FIGURE 1.
FEATURES OF GENE THERAPY SYSTEMS

VECTOR	ADVANTAGES	DISADVANTAGES	TARGET ORGANS*
Retrovirus	■ Integrate into host cell DNA ■ Potential for life-long expression ■ Potential for insertional mutagenesis	■ Can only transduce dividing cells ■ May be subject to promoter inactivation	■ Fibroblasts (ex vivo) ■ Muscle cells (ex vivo) ■ Bone marrow (ex vivo)
Adenovirus	■ High efficiency transduction in vivo ■ Can transduce nondividing cells ■ High level transgene expression	■ Short- to medium-term expression ■ Early generation vectors can cause liver damage ■ Antibodies can block transduction	■ Liver
Adeno-associated virus	■ Potential for long-term expression ■ Can transduce nondividing cells ■ No toxicity	■ Small packaging capacity ■ Difficult to manufacture	■ Muscle ■ Liver
Lentivirus	■ Integrate into host cell DNA ■ Potential for life-long expression ■ Can transduce non-dividing cells	■ Relatively new vector system ■ Difficult to manufacture ■ Potential for insertional mutagenesis	■ Muscle ■ Liver ■ Spleen ■ Bone marrow (ex vivo)
Naked DNA	■ No viral genes or proteins required ■ Can enter any cell	■ Poor in vivo transduction efficiency ■ Inefficient in vivo expression	■ Muscle ■ Liver ■ Blood vessels ■ Fibroblast (ex vivo)

* Following *in vivo* administration unless otherwise noted.