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THE LATEST FROM THE LAB

5 Five different approaches to gene therapy have been sufficiently refined to enter the clinic. However, several new tactics are still brewing in the laboratory—methods that may one day provide reliable, safe treatment for patients. With each success, new ideas grow. If it works in the muscle, perhaps a similar concept in the liver would enhance expression. If it works with hemophilia B, maybe the same can be tried with hemophilia A. These are the thought processes that drive the experiments.

The adeno-associated virus (AAV) transporting the factor IX (FIX) gene, is the vector used in two ongoing clinical trials in patients with hemophilia B. In the lab, researchers are investigating the same vector to ferry the factor VIII (FVIII) gene. AAV is appealing for gene therapy as it is small and simply constructed, making it easy to manipulate. However, the gene coding for FVIII is too bulky to squeeze into the AAV package. In the past, scientists have skirted this obstacle by deleting the "B domain," a section of the DNA that can be sacrificed without losing function. Space constraints, however, still prevent researchers from including the desired promoters—those part of genes that encourage the cell to pump out as much protein as possible. ▶▶ TO | 65

BY JUDY BERLFEIN

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Divide and Conquer

Several labs are trying to circumvent the space issue by dividing the job. Scientists at Avigen in California split the FVIII gene in two, placing sequence for one portion of the protein in one vector and the other section in the second vector. Barry Byrne, of the University of Florida, and Cathryn Mah, an NHF Judith Graham Pool fellow, modified this approach. "Once you've committed to two vectors," Byrne states, "the question is how to best utilize the space it provides you." They are using one vector to carry the entire gene for FVIII, rather than splitting it up. The other vector carries all of the regulatory machinery, which includes a promoter and a transactivator. The promoter is designed to turn on particular genes in designated tissues, avoiding the potential problems that could occur if the wrong gene is activated in the wrong place. The transactivator functions as a switch to regulate factor synthesis. Rather than calling for a steady flow of FVIII, one can invoke production at will. The two vectors are injected simultaneously. When both successfully penetrate a cell in the appropriate tissue, the promoter turns on the FVIII gene and protein is synthesized.

Repairing, not Replacing

Chris Walsh from the University of North Carolina has also analyzed the dual vector-concept. While Walsh sees the merits of the dual-vector concept, he's putting his money on another horse. Rather than ferrying in a complete replacement gene, Walsh plans to simply repair the mistake. He is focusing on one of the more common mutations found in individuals with hemophilia A. In individuals with hemophilia A, the gene for FVIII is present; however, in one small section, the DNA sequence is reversed, preventing the protein from being made accurately. The error is smaller than the entire gene; therefore, it only requires a snippet of genetic material to fix it. Hence, AAV provides a suitable candidate for transport of these repair instructions. Walsh has tested this mechanism in mice and achieved high levels of functional activity. If the results can be replicated in higher animals, he sees great potential. By fixing the endogenous gene, one allows the body's machinery to complete the task of synthesizing factor, a job it's already wired and programmed to do. Also, it avoids having to prod an outside system to accomplish this complex job.

Addressing Inhibitors

To ensure the greatest level of safety, gene therapy trials have always excluded individuals with a history of inhibitor formation. Genzyme Corporation of Massachusetts hopes to serve individuals with a history of inhibitor formation who also encounter obstacles with conventional treatment. Genzyme Corporation is focusing on FVIIa, a protein that can induce clotting through an alternative pathway, thus avoiding the need for traditional factor replacement. People with hemophilia normally carry the gene for FVII–the precursor to the activated form, FVIIa. Their systems have seen the protein before; therefore they are not prone to developing inhibitors. Additionally, a large dose of FVIIa can induce clotting. Using cells cultured in the laboratory, Genzyme has been able to express high levels of FVIIa and hopes to reproduce this expression in animals. Because the project is still in preliminary stages, Samuel Wadsworth of Genzyme came to the workshop asking for feedback. He is hoping to determine the positives and negatives of such an approach early in the process. He posed several questions to those who are intimately familiar with the field, such as: "What are the obstacles?" "What are the risks of thrombosis?" "Would such a therapy, which could circumvent the risk of inhibitor formation, fulfill an unmet need in the community?"

Another group is focusing on gene transfer to treat those with inhibitors. Because of potential immunologic reactions, subjects in these early gene therapy trials are only permitted a single injection of treatment. Jude Samulski and his colleagues at the University of North Carolina have investigated creative ways of overcoming the single-injection hurdle. The AAV vector comes in several different flavors (serotypes) that function in a similar manner–they can all transport genetic material. However, the shell enveloping the genes varies sufficiently, such that exposure to one serotype will not elicit an immunologic reaction to another. Samulski theorized that patients could receive repeat treatment if they simply switched the package with each new injection, in essence, provide the same genetic information, but change the wrapping slightly. Not only did the theory prove accurate, but in the process, Samulski's

laboratory stumbled across exciting new findings. Because of a certain comfort level with serotype 2, scientists consistently relied on that particular serotype, as it was well characterized and understood. However, while studying the variations, Samulski's laboratory discovered that alternative serotypes significantly enhance factor expression–a bonus that no one had anticipated.

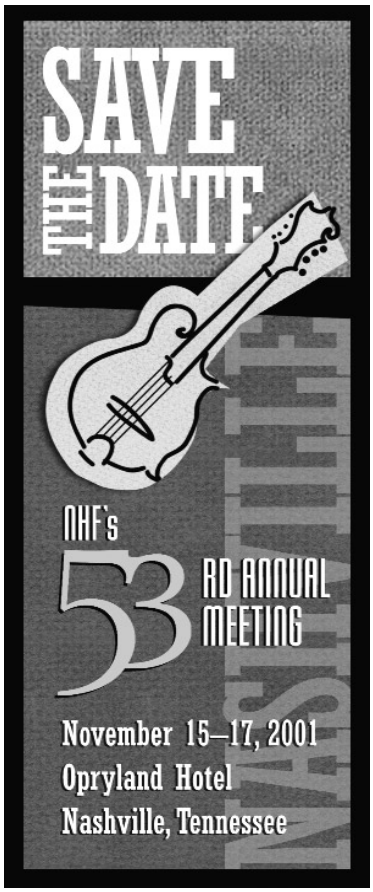
The Non-Viral Approach

Although a great deal of gene therapy research has focused on using viruses to ferry genes into cells, there are still several groups of researchers who are investigating non-viral approaches. Valentis, Incorporated is developing a non-viral approach that uses a plasmid containing FIX genes. The genetic material is injected directly into the muscle. A brief electrical pulse administered with the DNA creates pores in the cell membranes, allowing the plasmid to enter the cells. The company has progressed with its treatment from experiments with mice to experiments with dogs and scientists have observed successful factor production in both. However, its studies continue to be hampered by inhibitor formation in some dogs.

Still Another Technique

An alternative technique, still in its early phases, was presented by Robert Hebbel, of the University of Minnesota. Hebbel and his colleagues are investigating blood outgrowth endothelial cells (BOEC) as a vehicle for gene therapy. The team has inserted the genes for human FVIII into cells and injected them into immunodeficient mice. Following the injection, the scientists were able to detect therapeutic levels of FVIII in the circulation of the mice. Similar to the technique used in the clinical trial by Transkaryotic Therapies, Hebbel's method would allow individuals to be infused with material originating from their own cells, minimizing exposure to foreign substances.

Many pathways are being investigated by scientists. Only time and additional experiments will separate the wheat from the chaff—determining which therapies will proceed into the clinic and which will be relegated to a stack of old lab books. In the meantime, researchers continue to build upon their basic knowledge and hone the many strategies they are working with, thus increasing the odds of eventually developing a cure for hemophilia.



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