Gene Therapy: If At First You Don't Succeed . . .

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"Study Details Success of First Gene Therapy"
Headline, *Los Angeles Times*, October 20, 1995

"Scientists Report the First Success of Gene Therapy"
Headline, *New York Times*, April 28, 2000

"Teen Dies Undergoing Gene Therapy"
Headline, *Washington Post*, September 29, 1999

Gene therapy represents the culmination of medical research and its application to human health. Less than 60 years ago, Linus Pauling and others[1] described at the molecular and genetic level what was to be the first of many molecular diseases. In the most simple form, a mutation of just one base pair in a gene can alter the production or function of a protein that is vital to health and life. A "rough draft" of the human genome sequence has been completed and, with this, the sequence of every human gene is now theoretically available to scientists, physicians and the general public. Subsequently, the mapping of diseases to individual genes will be only a matter of time and effort. Over the past 50 years, investigators have teased out the mechanisms of many diseases at the physiologic and genetic levels and have made great progress in developing pharmacologic drugs to alleviate these maladies. The next step, already in progress, is to use genes themselves as the drugs—replacing or altering the expression of defective or misregulated genes—to treat patients at the molecular level.

Unfortunately, while gene therapy may be to the 21st century what antibiotics were to the last, we have a long way to go before success is at hand. As can be seen in the headlines above, gene therapy has had multiple "first" successes followed by the realization that much of the enthusiasm for each success has been perhaps premature or overstated. Further, much of the early excitement about this approach has been dampened following the recent death of a young male involved in one clinical trial, as noted in the third headline above. However, as with all discoveries and new fields, problems do exist, and they need to be identified, studied and overcome. Indeed, these are exciting times to practice medicine, but much of the initial unbridled enthusiasm for gene therapy has worn off, and now the real work has begun.

Gene therapy began in the mid-1970s when it was discovered that DNA could be manipulated in the test tube. Forward-thinking investigators quickly realized that the possibility of expressing normal or foreign genes in human cells (and ultimately in humans themselves) could usher in a new era of medicine. Theoretically, mutant genes, in patients with such diseases as sickle cell disease and cystic fibrosis, could be replaced and the diseases corrected by transferring the wild type, or normal, genes into these patients.[2] Other diseases (e.g., cancer) might be treated with genetic therapy by adding genes to increase antitumor immune responses or by inhibiting angiogenesis and cutting off the tumor's blood supply.[3] Furthermore, infectious diseases might be combated by altering the body's immune response.[4]

As more and more of the human genome was discovered, new associations between genes and diseases were identified, and the possibilities for genetic medicines became apparent. All of these treatments require moving desired genes into the appropriate cells in both animals and people—and this is where problems have been encountered over the past 10 years.[5,6]

Two major approaches have been taken to ▸

developed, and as soon as a second dose of the viral vector is given, the virus and the cells it infects will be targeted for destruction when a second dose of the viral vector is administered, thereby greatly limiting the expression of the transferred therapeutic gene. Although these drawbacks may seem daunting, investigators are actively engaged in moderating these responses and finding ways to circumvent the problems. To this end, novel and modified viruses are being tested and show great promise in terms of significantly reduced inflammatory responses and the ability to administer repeated doses for multiple gene therapies.

The second approach for gene delivery and therapy is to use nonviral systems. The latter include plasmid DNA that is either delivered directly (termed "naked DNA") or complexed with carriers such as liposomes that encase the DNA, add stability and increase cellular delivery and entry.[9] Based on design, plasmids have the ability to express in dividing and nondividing cells, thus making them appropriate for administration to all cells in the body. Further, the lack of viral proteins and genes that the body sees as foreign and worthy of reaction means that little inflammation is induced; thus, no immune response is mounted against the DNA, which, in turn, makes multiple effective treatments possible. Unfortunately, the level of expression obtained with nonviral methods is usually much lower than that occurring with viral methods.

Other potential delivery vehicles that produce increased gene expression are in development. They include delivery to the skin by Star-Trek–like pneumatic guns and the use of electric fields and sound waves to drive DNA into cells.

Although the levels of nonviral DNA delivery and gene expression are much lower than those obtained using viruses, the lack of an immune response may lead the field to favor these systems over the potential hazards associated with viral methods. As time goes on, it will most likely be found that each system has ▶

deliver genes to cells: viral and nonviral. In the case of viral delivery, the vectors (delivery agents) are modified viruses that have had their genomes largely replaced with therapeutic genes.[5] This allows the desired genes to be packaged into virus particles that are effective at entering cells within the body. By removing most of the viral genes, the chance for viral replication and aberrant infection in the host is, hopefully, removed. Thus far, adenoviruses and retroviruses are the two families of viruses that have been used in the majority of clinical trials.

Adenoviruses are good at infecting the nondividing or very slowly dividing cells that make up most of our tissues. Conversely, the application of retroviruses is limited because they can infect only dividing cells; however, this application results in life-long expression of the carried gene because it actually incorporates itself into the host's chromosome.[7] Unfortunately, such effective gene delivery comes at a price: viral vectors, especially adenovirus, can induce significant localized and systemic inflammation, and a sustained immune response. While each person will vary in inflammatory response to viral administration, the response can range from unnoticeable to severe.

Indeed, in the recent case at the University of Pennsylvania, administration of recombinant adenovirus carrying a gene to alleviate ornithine transcarbamylase (OTC) deficiency in a 17-year-old male resulted in activation of his innate immune system and fever, and ultimately led to his death.[8,9] Although the preceding 17 patients in the same study had received the same viral vector, they did not exhibit anywhere near these levels of inflammation or response, thus revealing no indication predictive of the patient's death. This case only exemplifies the variations that are possible.[9]

Another result of an immune response to viral vectors is that the response limits the number of times the virus can be administered. Once antibody and T-cell responses are ▸

its unique applications and both may ultimately find their way to the physician's office.

So, has gene therapy succeeded? In the realm of theory and laboratory experimentation, the answer is a resounding yes. Results from experiments have demonstrated that genes can be transferred to animals and humans and can exert therapeutic effects. In animal models, diseases can be corrected transiently and, in some cases, even on a long-term basis. In the earliest "first success" of gene therapy, Blaese and colleagues[11] transferred the gene for adenosine deaminase (ADA) using a retrovirus to T cells isolated from two young females with severe combined immunodeficiency as a result of ADA deficiency. When it was transfused back into the patients, small increases in their ADA levels were detected, as were minor increases in T cells and immune responses. However, the benefit was incomplete and did not fully "cure" the patients.[11]

In a more recent "first success," researchers in France took a similar approach to this earlier work to treat a different form of severe combined immunodeficiency but, rather than target T cells, they targeted stem cells isolated from the bone marrow of three different infants, also using retrovirus.[12] When transfused back into the infants, the successfully transduced stem cells propagated and expanded, outgrowing and replacing the endogenous defective cells. Indeed, success may be at hand, but only time will tell. Thus far, more than 390 other clinical trials involving gene therapy have been conducted over the past 10 years.

In conclusion, how close are we to using gene therapy in the family practice setting? When will an injection of recombinant virus or a plasmid infusion be used to control hypertension, sickle cell disease or cancer instead of using the traditional approaches? When will the first success of clinical importance occur? Hopefully soon, but to be realistic, definitely not before you have to take the Boards again.

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