Gene Therapy Method Shows Promise

Researchers have developed highly efficient gene transfer methods that may enable them to correct certain inherited diseases

Molecular biologists at the Massachusetts Institute of Technology and elsewhere report that they are having unprecedented success in using viruses to transfer genes into cells of research animals. Scientists now predict that it will be only a few years before it will be technically feasible to treat patients with certain rare inherited diseases with this sort of technique. "We now have a system that is ideal for beginning to assess the feasibility of certain forms of gene therapy," says Richard Mulligan of MIT.

The idea of using viruses for gene therapy is not new but, until recently, investigators could see no way to overcome what looked like insurmountable problems with it. The viruses they wanted to use are retroviruses—RNA tumor viruses that are highly unusual because they do not kill cells like most other viruses do. Instead, they enter cells during periods of division and insert their viral genes into the cells' chromosomes. The goal, now close to attainment, is to use these viruses as a vehicle for carrying certain therapeutically valuable genes into a patient's defective cells.

The challenge has been to find a way to make a noninfectious virus that carries particular genes to be transferred but that can essentially do nothing but enter a cell and insert the genes. Researchers knew how to engineer such a virus and realized that they would have to produce it in a cell along with a "helper virus" that would supply it with a coat which it would need to enter cells. But they were baffled by the problem of separating the infectious helper from the uninfectious virus to be used for gene transfer. If both the helper and the uninfectious virus were released from the same cell and if they both were wrapped up in coats supplied by the helper, the two viruses should look identical.

About 1 year ago, Mulligan, Richard Mann, and David Baltimore of MIT found a way to avoid this helper virus problem. The key was their discovery that a particular segment of the viral RNA is essential for packaging an infectious virus in a protein coat. If that segment is missing, the virus can never leave the cell. The MIT group engineered a helper virus that was missing that piece of RNA. In this way, the helper could supply a coat for the recombinant virus but would not itself be wrapped in a coat and so would not be able to leave the cell or enter others. Although the recombinant virus can get into another cell and insert its genes into that cell's chromosomes, it cannot be transmitted any farther because it lacks the necessary viral genes.

The recombinant retroviruses theoretically should be capable of inserting their genes into a wide variety of dividing cells that are exposed to them. Mulligan and his associates David Williams and Ihor Lemischka of MIT and David Nathan of Harvard Medical School decided to test this theory with a recombinant retrovirus that carries, as a marker, a bacterial gene for resistance to neomycin-like antibiotics. They used this virus to infect mouse bone marrow cells. Cells of the bone marrow are easy to obtain and are ideal for gene transfer experiments because they contain stem cells (precursor marrow cells) that are immortal—they are not yet committed to a path of differentiation and they essentially divide indefinitely. If the stem cells take up and integrate the bacterial gene, the mice will continue to have the bacterial gene in every type of blood cell that is derived from the "immortal" stem cells that carry the gene. But if only the more differentiated cells take up the neomycin resistance gene the transferred DNA sequences will gradually decrease as those cells age and die.

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In human terms, transfer into stem cells eventually may be important in the treatment of certain hemolytic diseases, such as sickle cell anemia and thalassemia, in which there is a fundamental disorder in blood cell production. However, technical problems with controlling the regulation of transferred genes so far preclude attempting such therapy. Other diseases, such as certain immune deficiency diseases, may, however, be treated by gene transfer into bone marrow stem cells. These diseases are not diseases of blood cells but they may be cured by genes that are expressed in bone marrow stem cells, even though the genes are normally expressed elsewhere.

The stem cells constitute only 1 in 10,000 of the bone marrow cells and there is no way to separate them from the rest. They look like the other bone marrow cells and behave like them. So any method of gene therapy that can get genes into the stem cells must be highly efficient. The method that currently appears most promising for this sort of experiment is the retrovirus system.

Mulligan has supreme confidence in the retrovirus system. However, he and his associates decided to double-check their results to allay any doubts that other investigators might have.

First, they removed bone marrow cells from experimental mice. Then they infected these bone marrow cells with the genetically engineered retroviruses and returned the marrow cells to mice whose marrow cells had been killed by irradiation. To determine whether the stem cells among the extracted marrow took up the bacterial gene that was being transferred, they looked at the spleens of the mice. Every blood cell in the spleen is derived from stem cells, which form colonies. Mulligan, Williams, and Lemischka picked out the colonies and divided each in half. They examined one-half of each colony to be sure the bacterial gene had been inserted into the chromosomes and took the other half of the cells and injected them into another mouse, whose marrow population had been wiped out by irradiation. The second mouse then should have the neomycin resistance gene in all of its spleen colonies—and it did. Finally, the MIT investigators examined the spleen colonies from the second mouse and showed that the gene was in the same position in the second mouse's chromosomes as it was in the chromosomes of the first mouse.

Now, Mulligan and his associates are putting human globin genes and mouse immunoglobulin genes into retroviruses. They are using retroviruses containing these genes to infect the bone marrow cells of a number of strains of mice, one of which has thalassemia genes. These thalassemic mice, developed by French Anderson of the National Heart, Lung, and Blood Institute and his colleagues, are missing their gene for β globin. Mulligan is also working with Stuart Orkin of Harvard Medical School to put the human gene for adenosine deaminase into retroviruses and infect mouse bone marrow with it. People born without this gene have no immune system. The results of these experiments are not yet in.

Another way to get genes into animals is to put them in embryos. Mulligan and Rudolph Jaenisch of the University of Hamburg took mouse preimplantation embryos, infected them with a retrovirus carrying a bacterial gene for the enzyme guanine-xanthine-phosphoribosyl transferase, and replaced the embryos in other female mice. About 20 percent of the embryos took up the gene and integrated it into their germ lines. But they did not express the gene. The difficulty seems to be that the embryos need a control region along with the gene in order to express it.

Now Mulligan and his associates believe they may have found a way out of this dilemma. They have recently reconstructed retroviruses to include various "transcriptional enhancer sequences" shown by others to turn on genes in cultured teratocarcinoma cells, which are embryo tumor cells. When they include this "enhancer sequence" with the antibiotic resistance gene in their retrovirus, the antibiotic resistance gene is expressed by teratocarcinoma cells. Without the enhancer sequence, these cells take up but do not express the gene. Jaenisch and Mulligan are infecting preimplantation mouse embryos with the enhancer-containing retrovirus and expect that the embryos that take up the gene may express it as well. They hope to learn whether the gene will be expressed throughout the animals' lives or only when they are embryos.

Mulligan, however, is not the only researcher to vigorously pursue the retrovirus approach to gene transfer. For example, Anderson and his colleagues, working with Eli Gilboa at Yale, also are attempting to transfer β globin genes to the thalassemic mouse. In addition, C. Thomas Caskey and his associates at Baylor College of Medicine and, independently, Theodore Friedmann and Douglas Jolly of the University of California in San Diego, working with Inder Verma and A. Dusty Miller at the Salk Institute, are putting the human gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT) into retroviruses and are transferring it into cells of mouse bone marrow. People born without the HGPRT gene have Lesch-Nyhan syndrome, a serious and untreatable disease characterized by mental retardation, cerebral palsy, self-mutilation, and an accumulation of uric acid in the body.

These investigators are cautious, but optimistic, about eventually using retroviruses to cure genetic diseases in humans. "We wouldn't be spending our time on it if there weren't some hope for the technology being useful," Caskey remarks. The diseases they are hoping to treat are serious, debilitating, and terminal. Says Friedmann, "Traditional kinds of therapy are probably not going to be very much more effective than they already are." The only cure for adenosine deaminase deficiency is a bone marrow transplant, which requires a well-matched donor. There is no treatment for the mental retardation, cerebral palsy, and self-mutilation that characterizes Lesch-Nyhan syndrome.

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Despite the promise that the retrovirus method offers, most genetic diseases are not likely to be amenable to treatment by it. The vast majority of genetic diseases, notes Joseph Schulman of George Washington University Medical School, are caused by too much genetic material rather than the lack of a gene. For example, Down's syndrome is caused by an extra chromosome 21. No one has any idea how to selectively remove genes and chromosomes. And even those diseases that are caused by missing or nonfunctional genes may not yet be suitable for retrovirus treatment. The problem is that, so far, molecular biologists have no way of controlling where a transferred gene inserts itself or—in most cases—in which cells it is expressed.

A clear-cut exception is immunoglobulin genes. About 6 months ago, three groups of investigators found DNA sequences that specifically turn these genes on when they are in antibody-producing cells (*Science*, 19 August 1983, p. 735). Mulligan and others are confident that such tissue-specific enhancers are a common phenomenon and a search is now on to find others and exploit them to get gene expression in specific cell types.

But molecular biologists' current inability to precisely control the fate of genes that they transfer into cells means that diseases such as sickle cell anemia and thalassemia which, Anderson says, were initially thought to be most amenable to gene therapy, will probably not be among the first to be corrected. These inherited anemias are caused by defective globin genes. Although the human globin genes have been cloned, the problem is that the globin gene products are precisely regulated. It is not just β globin that is needed in sickle cell anemia. It is the correct proportion of β globin, produced in concert with α globin and produced in red blood cells. The same need for proportion is true for thalassemia. Because the retrovirus transfer method may not solve the regulation problem, it cannot as yet be used to treat these diseases.

It is only the genetic diseases that are caused by a lack of what molecular biologists call "housekeeping genes"—genes that are expressed in many types of cells and whose control does not seem to be tightly regulated—that may be treated soon. For these diseases, the expression of a therapeutically transferred gene in any cells might correct the disorder. In addition to adenosine deaminase deficiency and, possibly, Lesch-Nyhan syndrome, Friedmann points out several other human diseases that also should be suitable for the sort of gene therapy now being contemplated. These include purine nucleotide phosphorylase deficiency, an immune system disease similar to adenosine deaminase deficiency, and citrullinemia, a disease in which ammonia is not converted to urea. The disease leads to mental and physical retardation, coma, and death. The genes to correct these disorders have been cloned.

Lesch-Nyhan syndrome and adenosine deaminase deficiency might be ideal test cases for gene therapy for two other reasons as well. In both diseases, for reasons that are not clear, cells that carry the missing gene have a competitive advantage over cells that do not. And, in both diseases, there is reason to believe that even a little bit of the missing gene product can alleviate the disease.

Adenosine deaminase deficiency can sometimes be treated with blood transfusions, which supply the cells of the immune system with a small amount of

adenosine deaminase. Blood transfusions are not an ideal therapy, however, because eventually iron from the transfused blood accumulates in the heart, causing heart failure. But the transfusions show that even a small amount of adenosine deaminase can keep some immune system cells alive.

The reason for believing that even a little bit of HGPRT might be therapeutically useful is that persons who inherit a defective HGPRT gene and make only 1 percent of the normal amount of this enzyme have none of the neurological symptoms of Lesch-Nyhan syndrome. Their only clinical complaint is gout caused by high uric acid levels and that can be treated.

But there is no guarantee that the neurological symptoms of Lesch-Nyhan syndrome will be alleviated by gene therapy with the HGPRT gene. The symptoms seem to be caused by a lack of HGPRT in the brain, yet the cells that would get the HGPRT gene with this therapy are in the bone marrow. Whether much or any HGPRT would get from these blood cells to the brain is open to question. Finally, it may simply be too late for treatment once a child is diagnosed as having Lesch-Nyhan syndrome. Irreversible brain damage may already have occurred.

For these reasons, Mulligan feels very strongly that to emphasize gene therapy as a possible treatment for Lesch-Nyhan syndrome is to offer false hope. Others disagree. As Schulman points out, there are diseases such as Wilson's disease in which serious neurological symptoms, including intellectual impairments, disappear once a metabolic defect is corrected. The same may be true for Lesch-Nyhan syndrome. Therefore, Schulman says, "The only way to find the effect of gene therapy in Lesch-Nyhan syndrome is to try it. I favor trying because the risks are fairly minimal and the prognosis for the disease is so bad."

What happens, then, when investigators find that they are able to insert adenosine deaminase and HGPRT genes in mice and get them to function? At what point do they try treating human patients? "We will have to sit down and think pretty seriously," Caskey says. "We have to be certain that we will do no harm." But Caskey and the others doing this gene transfer work are confident that the day is not far off when patients will be treated. "We're obviously excited about the work, and the diseases are certainly worth the effort. Let's just say that I'm optimistic but I'm not unrealistic," he remarks.

—GINA KOLATA

National Networks for Molecular Biologists

After years of highly productive but somewhat dispersed programming efforts in DNA sequence analysis, a national facility is to be established

The National Institutes of Health has awarded $5.6 million over 5 years to a small Palo Alto company, IntelliGenetics, to establish a national computer resource for molecular biology. In addition to giving researchers ready access to national databases on DNA and protein sequences, the resource, named BIONET, will provide a library of sophisticated software for sequence searching, matching, and manipulation. An equally important aspect of BIONET, however, will be the development of further software, both by IntelliGenetics personnel and in collaborative ventures with outside researchers.

Peter Friedland, a Stanford computer scientist and a cofounder of IntelliGenetics, likes to emphasize a further stated BIONET goal: to establish a community of molecular biologists who can communicate rapidly, effectively, and frequently with each other over a computer network. "In my area, artificial intelligence, I can plug into 60 or 70 electronic bulletin boards, through which 400 or so people in the community can pose questions about problems they are stuck with, and get instant suggestions for answers," he says. "We hope molecular biologists will be able to do the same."

Richard Roberts, a molecular biologist at Cold Spring Harbor and a member of the site-visit team that reported to NIH on the IntelliGenetics proposal, endorses the community idea. "Molecular biologists need something like this. An effective communications network would be extremely valuable." And, as Allan Maxam of Harvard Medical School points out, a lot of people have been tackling similar problems in isolation, thus leading to a great duplication of effort. "There has been a great deal of reinventing the wheel," he comments.

With DNA sequencing proceeding apace in laboratories throughout the world, the need for effective data handling is inescapable, and has been for some time. The number of bases in sequences known so far is fast running up to 3 million, with the prospect of its doubling before very long. Efforts to have NIH underwrite a national DNA database were under way by the beginning of 1979, but the instruments of bureaucracy and a certain political uncertainty mired progress. It was not until August 1982 that a contract—$3 million over 5 years—was awarded to a Cambridge-based company, Bolt, Berenek and Newman, to set up the national database, now known as GenBank.

The NIH initiative to establish GenBank was supposed to be part of a coordinated effort with scientists at the European Molecular Biology Laboratory (EMBL), Heidelberg. But, frustrated by American tardiness, the Europeans finally went ahead alone: EMBL announced the availability of its Nucleotide Sequence Data Library in April 1982, 5 months before NIH agreed on funding for the U.S. version. The original notion of having a coordinated approach is, however, now almost achieved, with the two databases more or less harmonized and only some formatting disparities to be resolved. Future data collection will be shared between the two centers.

IntelliGenetics had been an unsuccessful contender for the GenBank contract. Unbowed, the company's scientists turned their attention to what had become known as project 2, which was to be the provision of a national facility for computer analysis of DNA sequences. For reasons of financial stringency, however, NIH was forced to abandon the idea, or so it seemed. Nonetheless, through the persistence of IntelliGenetics representatives and creative financing arranged by NIH personnel in the division of research resources, the BIONET proposal was approved and a $5.6 million, 5 year "cooperative agreement" awarded this month, just a little more than a year since the proposal was formally submitted. IntelliGenetics faced