Gene Therapy—So Near and Yet So Far Away

Genes transferred into cultured cells often work very well but getting good expression of foreign genes in live animals is another matter

THE development some 2 to 3 years ago of efficient viral vectors for introducing new genes into cells raised hopes that gene therapy for human hereditary diseases might soon be feasible. Since then, researchers have shown that genes often work well when they are transferred into cells growing in laboratory dishes. In the latest such development, transferred genes have for the first time directed the synthesis of active proteins in human bone marrow cells, the cell type that will be the target for the gene therapy trials when they begin.

The initial trials are likely to be directed at alleviating a severe hereditary immune deficiency caused by the lack of an enzyme called adenosine deaminase (ADA). Transfer of a good copy of the ADA gene has now been shown to "cure" the enzyme defect in cells taken from patients and grown in culture.

Nevertheless, one big problem must be solved before gene therapy can be tried in the patients themselves. More often than not, investigators have found that foreign genes are inactive when they are transferred into live animals by methods similar to those envisioned for eventual human use. Without the possibility of good expression, clinical experiments on humans would be futile, not to say unethical, and such experiments are still on hold.

Some results have been encouraging, however. Within the past several months, a few groups have obtained expression of foreign genes in animals. The successes include the expression of the ADA gene in monkeys, even though the amount of ADA made would probably not be sufficient to cure the deficiency in human patients. Although firm estimates of when clinical trials of gene therapy might begin are hard to come by, the more optimistic researchers suggest perhaps in a year or two. For perspective, this estimate is similar to the one put forth in early 1984.

Gene therapy will be attempted first for defects that primarily affect cells originating in the bone marrow, simply because bone marrow can be readily removed from the patient with a needle and syringe, maintained in culture where the healthy gene can be introduced into the cells, and then reinjected into the patient. Investigators have had trouble demonstrating that foreign genes function after transfer into cells from human bone marrow, but that situation now appears to be changing.

Randy Hock and A. Dusty Miller of the Fred Hutchinson Cancer Research Center in Seattle recently found that the products of two drug resistance genes were made after the genes were introduced into human bone marrow cells with a viral vector that was developed by Eli Gilboa of Princeton University. "We get about 5 to 20% of the cells infected with the virus and they become drug-resistant," Miller says.

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The Seattle workers encountered a problem while growing the vector containing one of the drug resistance genes, however. Viral vectors for gene therapy are designed so that they cannot be transmitted further once they enter a target cell. But the cell line in which the one vector was growing began making an active "helper" virus that could be transmitted to a recipient animal with the vector, thus allowing further reproduction and transmission of both viruses.

For gene therapy to be effective, a transferred gene must be functional in the bone marrow stem cells that give rise to the red blood cells and to all of the various types of white blood cells. The methods of the Seattle workers did not allow them to determine whether the genes they transferred entered the stem cells, but the genes were expressed in the bone marrow progenitor cells that are more advanced in development than true stem cells but are still immature.

Alan Bernstein of the Mount Sinai Hospital Research Institute in Toronto, Robert Phillips of Toronto's Hospital for Sick Children, and their colleagues have also transferred the *neo* gene, which codes for resistance to the antibiotic neomycin and was one of those used by Hock and Miller, into progenitor cells from human bone marrow. Although the Gilboa vector works both in mouse and in human cells, the Toronto group encountered a different situation with their own vectors. They tried without success for 2 years to put a gene into human bone marrow cells with one of the same vectors that had worked well for transfers into mouse cells. They eventually had to develop a new vector for the human cells. "The lesson is that we can't assume that what works in the stem cells of mice, or of any species, will necessarily work in humans," Bernstein notes.

Transferred genes have been found to work in the cells primarily affected by ADA deficiency. These are the T and B lymphocytes, which originate in the bone marrow and are needed for fighting off infections. Without a functional ADA enzyme the cells die, thereby causing a severe immune deficiency that usually kills the patients in the first few years of life.

David Williams and Stuart Orkin of Harvard Medical School and Richard Mulligan of the Massachusetts Institute of Technology and, independently, W. French Anderson and his colleagues at the National Heart, Lung, and Blood Institute (NHLBI) have now shown that they can "cure" lymphocytes obtained from patients with the ADA deficiency by introducing a new ADA gene into the cells. The enzyme is produced by the transferred gene and protects the cells against a chemical that would otherwise kill ADA-deficient cells.

Even though genes transferred into cultured cells frequently work very well, investigators who have attempted to use viral vectors to introduce new genes into the bone marrow of living animals have often found that the genes are not active. Howev-

er, late last year Bernstein, Phillips, and their colleagues, a second group including Gordon Keller of the Basel (Switzerland) Institute for Immunology and Erwin Wagner of the European Molecular Biology Laboratory in Heidelberg, and a third group consisting of Anderson and his colleagues, all reported success at introducing a functional *neo* gene into the bone marrow of mice. Not only was the gene product made in relatively high concentrations, but the results established that the transferred gene entered the bone marrow stem cells.

Why these three groups have obtained better expression of transferred genes in living animals than other investigators is currently not clear, although the design of the viral vectors may be important. Both the Anderson and the Keller-Wagner groups used the Gilboa vector. The Toronto workers used one that they had made but its design is similar to that of Gilboa's vector.

More recently, Anderson, with Arthur Nienhuis of NHLBI and Richard O'Reilly of Memorial Sloan-Kettering Cancer Institute, has gone on to use the Gilboa vector to introduce the the human ADA gene into the bone marrow of living monkeys. The gene product is made in bone marrow–derived cells of five of the seven monkeys treated, although the levels of expression are no more than 10% of those thought to be needed for successful gene therapy of the ADA deficiency. "We are basically making the observation that we have a long way to go [to human trials]," Anderson says.

Williams, Orkin, and Mulligan attempted a similar transfer of the human ADA gene into mice with their vector but were unable to detect any expression of the gene even though the animals' cells carry the new DNA in intact and unrearranged form. "The gene works magnificently in cultured cells," Orkin says, "but when you infect the animal, it [the gene] is nearly dead."

Persistent expression of a transferred gene in the living animal requires that the gene be functional in the bone marrow stem cells, which can then continually replenish the body's red and white cell populations. Differences in the properties of the stem cells and of ordinary cells grown in culture may account for the trouble that investigators have encountered in obtaining good expression of transferred genes in living animals. "The genes are not rearranged. It seems to be a biological problem," Orkin notes.

Work by the Bernstein-Phillips group indicates that the transferred genes are expressed shortly after their entry into stem cells but are later inactivated. The reason why the transferred genes are turned off in stem cells is currently unclear, but there are indications that the cells can specifically inactivate genes regulated by the viral control sequences, as has been the case with the first-generation vectors.

The hereditary anemias, which include sickle cell disease and the thalassemias, are caused by defective genes coding for the hemoglobin proteins. The anemias would be good candidates for gene therapy, except that transferred globin genes have so far not worked particularly well even in cultured cells. Their expression is often low and not subject to the normal regulatory influences in the recipient cells.

According to Nienhuis and Stephan Karlsson of NHLBI, that situation may also be changing. When they introduced a hybrid globin gene with its own regulatory sequences into mouse erythroleukemia cells, the amount of messenger RNA (mRNA) made by the transferred gene was about 10 to 40% of the amount of mRNA made by the natural globin genes. In addition, control of the transferred gene paralleled that of the endogenous genes. "We are encouraged by these results," Nienhuis says. "Previously investigators hadn't seen much mRNA made." Nevertheless, and for reasons that are still poorly understood, the efficient production of mRNA by the transferred genes translates into very little protein synthesis.

Investigators clearly have to do more tinkering with their vectors to improve the expression of transferred genes. At the same time, they have also begun addressing a potential safety problem. In animals at least, insertion of the viral regulatory sequences in the genome may cause a cell to become cancerous if they go in near an oncogene and activate it.

No one knows whether this will also happen in bone marrow cells that have been infected with a viral vector, but Gilboa, Bernstein, and Mulligan, among others, are designing their newer vectors to minimize the possibility. The idea is to modify the vector in such a way that the gene-activating sequences will be lost during integration. Genes carried by such vectors would then have to be controlled by regulatory sequences other than those of the viruses, but this may well be a good idea if some of the expression problems are caused by inactivation of genes under viral control. ■

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ADDITIONAL READING

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D. A. Williams, S. H. Orkin, R. C. Mulligan, "Retrovirus-mediated transfer of human adenosine deaminase sequences into cells in culture and into murine hematopoietic cells *in vivo*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2566 (1986).

Briefing:

Unexpected Size Pattern in Bacterial Proteins

While he was examining data on *Escherichia coli* proteins, Michael Savageau, a biologist at the University of Michigan, came across an unexpected pattern. Rather than being spread over some kind of continuous size distribution, the proteins instead were bunched into groups, which were multiples of a molecular mass of 14,000 daltons (14 kD).

Perhaps this 14-kD unit represents some kind of previously unrecognized fundamental structure, imposed by some aspect of gene architecture or protein chemistry? In which case, suggests Savageau, it might have functional and evolutionary implications.

The observation undoubtedly falls into the "If true, then interesting" category. But how important it might be must then depend on how extensive the pattern is and what exactly underlies it, according to Gregory Petsko of the Massachusetts Institute of Technology.

The idea that proteins might be assembled from basic structural units with a molecular mass of about 18,000 goes back to the 1930's, when Svedberg and others proposed the existence of an underlying unit architecture. Known as the cylcol hypothesis, the suggestion was overwhelmed by strong criticism and eventually was abandonned. If Savageau is correct in his current observations, it may be that he is seeing more clearly what others had perceived earlier but had been unable to substantiate.

Savageau's data come from the extensive catalog of protein information that now exists for *E. coli*. Of the 3000 or so proteins that this bacterium probably manufactures, details of about 1000 are now in the catalog and therefore offer a reasonable sample for statistical inference. A frequency distribution of these proteins shows the expected skew to smaller sizes. But it also reveals an unexpected "fine structure," which analysis shows to be a 14-kD periodicity. "This period of 14 kD is evident for four cycles and then disappears as the data at higher molecular masses become sparse," notes Savageau.

In order to check whether the periodicity might be an artifact of the gel methods used to measure the protein size, Savageau looked at data for *E. coli* genes. GenBank contains data on 146 *E. coli* genes suitable for analysis, and, according to Savageau, these cluster at "lengths of 400, 800, 1200, and 1600 base pairs, corresponding approximately to proteins of 14.6, 29.3, 44.0, and