Gene Therapy: Into the Home Stretch

After a tortuous review process, proposals to use genes to treat cancer and immune deficiency have been approved; the first tests should begin soon

TUMORS CANNOT LIVE WITHOUT BLOOD. Shut off the blood vessels that feed a tumor and the tumor will turn black and shrivel away. That simple idea lies behind the first attempt to cure a disease by gene therapy, expected to take place at the National Cancer Institute in the next few weeks.

When it does, it will test a technique that worked like a charm in mice. According to NCI surgeon Steven A. Rosenberg, when a potent natural killer called tumor necrosis factor, or TNF, is injected into the bloodstream of mice, it begins to shrink tumors "within hours, sometimes even minutes." Not known for downplaying his theories, he declared last month at a momentous meeting of the National Institutes of Health's human gene therapy subcommittee: "It is almost miraculous."

But so far, attempts to recreate that miracle in people with cancer have not fared as well. Rosenberg and his colleagues have given TNF intravenously to more than 35 patients in experiments that he frankly says were "an abysmal failure." Undaunted, Rosenberg is about to try another route—gene therapy. He hopes to deliver TNF in much larger doses directly to a tumor by packaging the gene for TNF inside special lymphocytes that have a natural affinity for cancer.

On 30 July, the National Institutes of Health's human gene therapy subcommittee approved Rosenberg's protocol. The next day, the higher ranking Recombinant DNA Advisory Committee (RAC) also told Rosenberg he can go ahead. Thus, one of the first attempts to use human genes as medicine will be put to the test in cancer.

At the same meetings, the two committees—each comprised of scientists, ethicists, lawyers, and lay people—voted "Yes" to a proposal from R. Michael Blaese, also of the cancer institute, and W. French Anderson of the National Heart, Lung, and Blood Institute, to try gene therapy in children with a rare, inherited, and often lethal immune system disorder known as ADA (adenosine deaminase) deficiency (see story, p. 975). The votes at the back-to-back meetings of the country's two most important gene review committees means that human gene therapy—long promised—has finally arrived.

Observers called it a historic moment in medicine. Rosenberg demurred. "It will be a historic moment only if the experiments work," he told *Science*.

A debate that began nearly a decade ago has reached at least tentative resolution, but it did not come easily. During the past few months, as the two gene protocols were reviewed and then reviewed again and again, committee members grappled with questions of ethics, safety, politics, money, and even professional competition as they inched toward approval. Throughout, the debate over the protocols has been dominated by a clash between two cultures—physicians anxious to treat dying patients and laboratory scientists insisting on having every scientific "i" dotted and "t" crossed before they signed off on the experiments.

The debate carries special significance because physicians with gene therapy protocols for a variety of genetic diseases are beginning to line up for permission to begin experiments. One already in the review process is a proposal to use marker genes to study the course of bone marrow transplantation in patients with cancer. This proposal, from Malcolm K. Brenner and his colleagues at St. Jude Children's Research Hospital in Memphis, and NIH's Anderson as a collaborator, will get a second review when the gene subcommittee meets in November.

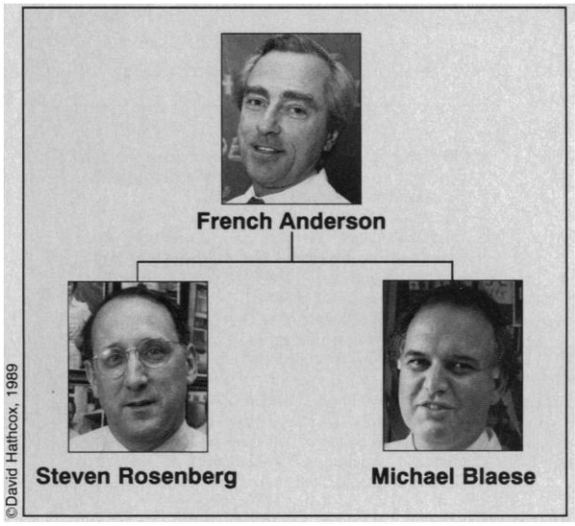
Another bone marrow–related study from University of Wisconsin researchers is expected to be in for review by October, as is an experiment from researchers at the University of Pittsburgh that involves inserting a marker gene into lymphocytes. It is possible there will be even more.

Rosenberg's TNF gene experiment and the Blaese-Anderson ADA protocol are the direct intellectual descendants of studies the NIH gene triumvirate has been carrying on since May 1989 when Rosenberg first infused a patient dying of malignant melanoma with tumor-infiltrating lymphocytes (TIL) bearing a marker gene (in this case, for neomycin resistance) that would reveal where the TIL went inside the patient's body.

Lymphocytes that have infiltrated a tumor are taken from surgically removed pieces of the tumor and then grown in large numbers in the presence of interleukin-2 (IL-2), a potent growth stimulant that is infused along with the laboratory-grown lymphocytes to keep them replicating in vivo.

The cloned gene for TNF is attached to a retrovirus that no longer carries the genetic machinery it needs to replicate itself. The engineered virus then infects TIL in culture, depositing its genes into the TIL's own DNA. Thus, the debilitated retrovirus is nothing more than a vector or vehicle for getting the gene into the TIL.

In this week's issue of *The New England Journal of Medicine*, the Rosenberg team reports on the first five patients. (An additional three have been treated to date.) Those patients have laid the groundwork for the TNF trials. "We have been able to demonstrate that, in those patients who respond to TIL, the TIL home to the tumor. . . . Also, the TIL that don't go to the tumor are quickly cleared; they don't accumulate in



Gene triumvirate. *Three NIH researchers and their colleagues from separate labs have joined forces to make human gene therapy a reality.*

ADA Gene Therapy Enters the Competition

Around the world, some 70 children are members of a select and deadly club. Born with an immune deficiency so severe that they will die of infection unless their immune systems can be repaired, they have captured the attention of would-be gene therapists who believe that a handful of these kids—the 15 or 20 who lack functioning levels of the enzyme adenosine deaminase (ADA)—could be saved by a healthy ADA gene.

A team of gene therapists is ready to put the theory to the test. In April 1987, a team of NIH researchers headed by R. Michael Blaese and W. French Anderson came up with the first formal protocol to introduce a healthy ADA gene into an unhealthy human. After 3 years of line-by-line scrutiny by five review committees, they have permission to go ahead. Blaese says that two or three children will be treated in the next year.

Blaese and Anderson, in collaboration with NIH colleagues Kenneth Culver and Steven Rosenberg, will infuse patients with T lymphocytes carrying the gene for ADA. If the experiment works, the ADA gene will begin producing normal amounts of ADA.

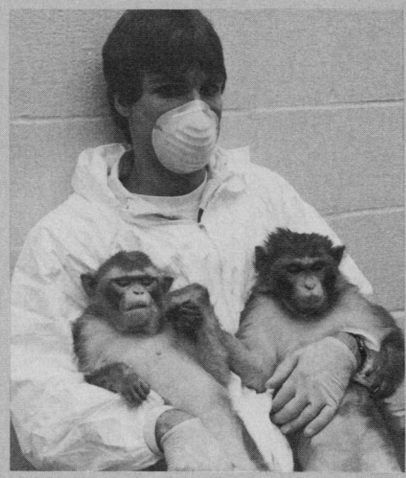
An interesting feature of ADA deficiency, that makes it ideal for initial gene studies, is that the amount of ADA one needs for a healthy immune system is quite variable. Hence, once inside a patient's T cells, the new ADA gene needs only to express the enzyme in moderate amounts. No precise gene regulation is necessary.

Because it is an experiment in gene therapy, the ADA protocol faced special scrutiny under rules that require the researchers to prove that (i) alternative therapy does not exist and (ii) that they have data showing how the therapy is expected to work. Until last spring, when the Food and Drug Administration approved a a new drug called PEG-ADA, there was no alternative therapy—the natural enzyme cannot be used for treatment because it has a half-life of minutes. But if you coat the enzyme with polyethylene glycol, or PEG, it survives in the blood for days, enhancing the children's ability to fight infection as long as they get weekly injections. But PEG-ADA is not a cure and, at $60,000 a year, it is expensive therapy.

Still, the arrival of PEG-ADA on the scene almost derailed the ADA gene therapy trial as reviewers wrestled with conflicting opinion about just how good the drug is and whether it qualified as an alternative therapy. Michael Herschfield, a Duke University researcher who has been coordinating clinical trials of PEG-ADA for Enzon, the New Jersey–based biotechnology company that makes this particular PEG drug, attended the NIH review meetings as an observer but, when the debate became heated, he became a vocal, if hardly disinterested, participant. Although clinical data on the 14 patients who are taking PEG-ADA have not been published, Herschfield suggested that PEG-ADA is effective enough to make gene therapy unnecessary. Certainly, he said, it would be dangerous to take PEG-ADA away from children who showed some improvement on it.

The reviewers settled on a compromise that Blaese accepted—the children he treats with the ADA gene will receive PEG-ADA at the same time. Because PEG-ADA does improve children's immune function enough to allow them to lead reasonably normal lives, withdrawing it raised ethical questions in many reviewers' minds.

Once that issue was resolved, the committee turned its attention to safety concerns. Several members worried that the recombinant DNA retrovirus that will be used to carry the ADA gene into the patient's cells may pose a special risk to children who will receive repeated injections of gene-bearing T cells. (The only way to effect a permanent cure is to insert the ADA gene in self-perpetuating bone marrow stem cells—a feat that is gene therapists' ultimate goal. Shorter lived T cells are an interim step.)



Monkeys for safety. *Animals "infected" with the disabled retrovirus used to carry genes into human T lymphocytes are healthy months after safety studies began.*

Would repeated injections expose children to an undue risk of getting cancer? Suppose the viral vector, which integrates the cells' DNA at random, landed next to an oncogene, unleashing its potential to produce tumors? Heads nodded in shared worry, even though all available data indicate that retroviruses, specially disabled to prevent replication, are safe.

But then Brigid Leventhal, a pediatric oncologist at Johns Hopkins, offered a counter-argument: "Listen," she said, "no matter what we do, these children are likely to get lymphoma. Anyone with an immune deficiency is at risk for lymphoma."

As the review process headed toward its conclusion after 3 years, one scientific issue still needed resolution: What evidence is there that ADA, produced intracellularly by a foreign gene, will be better for the patient than extracellular ADA administered as the drug PEG-ADA?

The answer came from Italy.

French Anderson's network of collaborators extends far and wide. It includes A. Dusty Miller in Seattle who designed the retrovirus that will be used in the ADA experiment. It includes Eli Gilboa of Memorial Sloan-Kettering Cancer Center in New York, another expert at constructing retrovirus vectors, and Claudio Bordignon of Milan who has been using one of Gilboa's vectors—quite similar to the one that will be used by Blaese—to get a human ADA gene into immune-deficient mice.

Bordignon, who flew to Washington to present his data to the gene subcommittee, reported that mice whose cells had been transfected with the human ADA gene showed normal immune function for up to 3 months. The gene therapy had, in effect, given them an immune system. By contrast, Bordignon told the committee, administering ADA alone did not lead to long-term immune reconstitution in the animals.

The Bordignon-Gilboa study was not designed as part of the Blaese-Anderson work. Nevertheless, it produced data about the mechanism by which ADA gene therapy might work that gave the review committee the scientific basis they needed for approving a human test of the ADA gene.

D-day for the experiments has not been set. Blaese estimates October. ■ **B.J.C.**

normal tissue," states Rosenberg's paper. Furthermore, he says, "We've shown that lymphocytes can be used as cells for carrying genes," a notion that has been crucial to current gene therapy research (*Science*, 10 November 1989, p. 746).

Using PCR (polymerase chain reaction) analysis to detect the DNA of the neomycin marker gene, the researchers can show that TIL can survive at tumor sites for at least several months and that the TIL express the gene they carry. Equally important, the studies demonstrate that the insertion of a gene by a recombinant viral vector is safe.

Armed with data about the ability of TIL to deliver a gene directly to tumor, Rosenberg followed the lead to its logical extension. Because TIL have a clear antitumor effect in some patients, Rosenberg reasons that TIL bearing the potent TNF gene might be all the more powerful.

The TNF/TIL experiment is a third-generation study in a series of tests of what Rosenberg calls "adoptive immunotherapy" that have been going on at the cancer institute and elsewhere for several years.

The TNF proposal began its journey through the review process on 23 April when it went to the NIH's Institutional Biosafety Committee (IBC), which has first crack at all experiments based on recombinant DNA technology. On 2 May the IBC unanimously rejected Rosenberg's protocol, saying—among other things—that it lacked information on the safety of gene-bearing TIL, did not say much about the toxicity of TNF, lacked safety data from animals, and failed to say whether there are data to show that TNF, delivered locally, reduces tumors in people.

On 24 May, Rosenberg responded to the IBC.

The safety of TIL carrying foreign genes is evident from the TIL/neomycin experiments, he said, supplying the committee with the data that have just been published in the *New England Journal*. Two patients infused with the engineered TIL have died of their cancer, two have shown significant remission, and a fifth—a young woman of 26—appears to be virtually free of disease a year after treatment. There is no evidence that any of the patients had any reaction to the retroviral vector.

As to the toxicity of TNF—it is terrible. Rosenberg told *Science* that it is so toxic that "by injection, we just can't give people enough TNF to be effective." (Mice can tolerate up to 40 times the dose of TNF that is possible in man, however.) But three studies in patients in whom TNF has been injected directly into solid tumors have shown that TNF does cause regression. The limitation there, however, is that one can only treat tumors close to the surface. Presumably, TIL bearing the TNF gene will home to deep-seated tumors as well.

And as for animal studies, neither monkeys nor mice that have received the neomycin resistance gene have shown ill effects from the retrovirus that carried the gene into their cells.

On 6 June, the IBC gave Rosenberg's protocol provisional approval, as did the institutional review boards of the National Cancer Institute and the National Heart, Lung, and Blood Institute which also have jurisdiction. It then went on to the NIH gene therapy subcommittee where many of the IBC's questions were raised again.

Committee members focused on the retrovirus Rosenberg will use to shuttle TNF genes into TIL. Researchers have been working with debilitated retroviruses for more than a dozen years now, and there is no evidence that any of them will suddenly become activated. Nevertheless, there is what is called a "finite but not zero" risk that a retrovirus, when it integrates itself into the DNA of a host cell, could sit down next to a native oncogene and turn it on.

The special fear is that a retrovirus, out of control, might induce cancer. In many ways, this ingrained fear of a retrovirus gone wild (data notwithstanding) divides the "go for it" physicians like Rosenberg, Anderson, and Blaese from scientists whose research bent is to seek detailed answers and greater guarantees. It is well documented but often forgotten that standard radiation therapy and chemotherapy are likely to induce secondary tumors in patients years after initial treatment.

"If this study was for AIDS, people would be beating down the door telling us to approve it."

—Abbey S. Meyers

R. Scott McIvor of the University of Minnesota, a member of the gene subcommittee and the RAC, was among those whose attention to detail guaranteed that the proposal did not get just rubber-stamp approval. McIvor said he could imagine "a variety of scenarios" in which the viral vector might locate itself in the host cell in an "unanticipated site." That, he suggested, could be "aberrant to the health of the individual, even though it is an unlikely event that has been looked at over years." Rosenberg says simply that widespread melanoma is pretty aberrant to health.

McIvor wondered about the toxicity of TNF and said that even though the calculations regarding dosage in the protocol were accurate (McIvor had checked the math), "you can't predict" exactly what will happen. McIvor suggested it would be nice to have *more* data from mice. However, the mouse cells resist retroviral infection with foreign genes.

Rosenberg reviewed the data he has accumulated over the years from patients who have been treated experimentally with a variety of specific types of lymphocytes, including TIL plus IL-2—more than 900 all told. The toxicity data have been published in the medical literature. Someone suggested it would be nice to have toxicity data from IL-2 in monkeys. There was a feeling in the air that the TNF protocol might be sent back to the drawing boards. Rosenberg said it would be a "nightmare" to give monkeys continuous infusions of IL-2. You'd have to strap them down for days to keep them from pulling out the IV lines, he noted.

Then subcommittee member Brigid Leventhal, a pediatric oncologist at Johns Hopkins, said out loud what Rosenberg's team had been thinking. "We have zillions of data about toxicity and safety in people," she stated. "Why do we want to fool around with monkeys?"

Abbey S. Meyers, a lay member of the subcommittee, added her own perspective. "There is another meeting going on down the hall," she said. "An AIDS meeting. If this study was for AIDS, people would be beating down the door telling us to approve it." In the end, both the gene subcommittee and the RAC voted unanimously in favor of the TNF/TIL experiment.

Rosenberg, who is already growing cells, is ready to begin as soon as he gets final approval from the acting director of NIH and from the Food and Drug Administration. He could treat his first patient sometime in October.

He will start with malignant melanoma because, in its advanced stages, it is impossible to cure. Regrettably, he says, he will have no trouble finding patients who are suitable for the experiment. "A decade ago, there were 8,000 new cases of melanoma a year," he told *Science*. "Last year, there were 25,000." No one knows why.

What are the chances that the TNF and ADA experiments will calm concerns about the use of retroviruses and genes in medicine? Rosenberg offers this assessment. "So far, gene therapy has been an abstract idea, and it is easy to think about the risks when there are no evident benefits. The climate will change if the experiments work—if we make sick people better," he says, harking back to his own view of what defines a historic moment in medicine. "It is historic when it works." ■ **BARBARA J. CULLITON**