At Age 2, Gene Therapy Enters a Growth Phase

Last month, a research team at the National Institutes of Health (NIH) held a party to celebrate the second anniversary of the world's first attempt to cure an illness with gene therapy. The guests of honor were two girls, aged 6 and 11, who had been injected with white blood cells carrying a transplanted gene that makes adenosine deaminase (ADA), an enzyme that plays a crucial role in the immune system. Both girls were born with a defective ADA gene and consequently had virtually no natural immune defenses. Today, according to immunologist Michael Blaese of the National Cancer Institute (NCI), a member of the team that carried out the gene therapy, the two children have functioning immune systems. Although their disease might have confined them to a life in isolation, both girls are in public school.

The sense of achievement at last month's celebration wasn't just because this one treatment had worked, however. It reflected gene therapy's coming of age. Over the past 2 years, the field has exploded. Thirty-seven gene transfer experiments have been approved worldwide, 18 of which are designed to deliver an immediate therapeutic benefit, according to a count by gene therapy pioneer W. French Anderson, who recently left NIH for the University of Southern California. Researchers have flooded into the field, gene therapy centers have been established at the Universities of Michigan and Pittsburgh, and some agencies have even started asking grant applicants what relevance their studies might have for gene therapy. "Just a year or two ago, I don't think any of us in the field thought it would move so fast," said Albert Deisseroth, a physician at the University of Texas M.D. Anderson Cancer Center in Houston, who is developing a gene therapy treatment for different forms of leukemia.

Just how fast the field has moved was evident at a meeting held at Cold Spring Harbor Laboratory a week after NIH's party. More than 300 scientists showed up to discuss the latest developments in gene therapy, ranging from the creation of new vectors that carry genes into target cells to proposals to treat a variety of diseases—even arthritis—by gene transfer. But in spite of the progress, the meeting indicated that researchers still have numerous technical problems to solve before gene therapy becomes standard treatment.

Another sign that gene therapy is maturing is the fact that the often bitter clashes that preceded the first human gene transfer tests have ceased. Before those tests were approved, critics such as Richard Mulligan, an outspoken scientist from the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, who developed many of the gene transfer techniques, argued that the experiments were premature and that more basic research was needed to ensure that the transfer would work and that patients would not be harmed. Physicians such as Anderson countered that although the technology wasn't perfect, it was state-of-the-art, safe, and desperately needed to treat patients who had few other choices.

The two sides are no longer fighting—at least not in public. Indeed, Anderson and Mulligan, along with Theodore Friedmann, a long-time gene therapy visionary from the University of California, San Diego, co-organized the Cold Spring Harbor meeting. "The idea of doing human gene therapy is now more acceptable," says biochemist Inder Verma of the Salk Institute in La Jolla, California, adding, however: "I don't know why. It is not the success of experiments."

Basic problems remain. Verma was referring to the fact that the field remains plagued by many of the same problems that have stymied progress since the beginning. Gene therapy researchers still have difficulty making large quantities of the defective mouse retrovirus vectors that they often use to transfer genes into target cells. Moreover, the gene transfer efficiency of most vectors is low, with only about 10% of the treated cells actually acquiring the new gene; it remains a tricky problem to target the genes to the correct cells; and even when the gene transfer works, many transplanted genes have a tendency to turn themselves off after a few weeks.

Now that researchers are flooding into the field, however, novel solutions seem to be following. Several researchers at the Cold Spring Harbor meeting described new defective-virus vectors designed to improve cell targeting and gene transfer efficiency, including those made from the herpes virus, adenovirus, and even the AIDS virus (see p. 745). Researchers are also finding new ways to keep genes switched on for as long as the target cell survives. Verma's lab, for example, has constructed a two-pronged regulatory sequence that can produce high levels of the clotting protein Factor IX in cultured muscle cells for more than 8 months. To do this, the group first hooked a small bit of enhancer DNA from the muscle creatine kinase gene

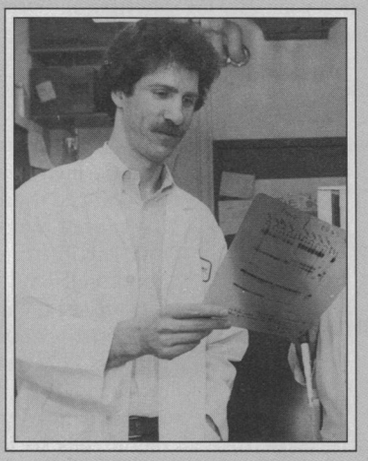
GENE THERAPY TRIALS

Disease	Gene Inserted	Principal Investigator
ADA deficiency	Adenosine deaminase	Michael Blaese, NCI
Advanced cancers	Tumor necrosis factor	Steven Rosenberg, NCI
Advanced cancers	Tumor necrosis factor	Steven Rosenberg, NCI
Advanced cancers	Interleukin-2	Steven Rosenberg, NCI
Liver disease	HDL receptor	James Wilson, U. Michigan
Ovarian cancer	Thymidine kinase	Scott Freeman, U. Rochester
Malignant melanoma	HLA-B7	Gary Nabel, U. Michigan
AIDS	Thymidine kinase	Phil Greenberg, U. Washington
Neuroblastoma	Interleukin-2	Malcolm Brenner, St. Jude's, Memphis
Brain tumor	Thymidine kinase	Kenneth Culver, NCI
Malignant melanoma	Interleukin-2	Eli Gilboa, Sloan-Kettering
Kidney cancer	Interleukin-2	Eli Gilboa, Sloan-Kettering
AIDS	HIV *env*	Douglas Jolly, Viagene
Cancer	Interleukin-4	Michael Lotze, U. Pittsburg
Lung cancer	antisense *ras/p53*	Jack Roth, M.D. Anderson
Hemophilia B	Factor VIII	Jerry Hsueh, Fundan Univ., Shanghai
ADA deficiency	Adenosine deaminase	Claudio Bordignon, Lab. of Hematology, Milan
ADA deficiency	Adenosine deaminase	Dinko Valerio, Univ. Hospital, Leiden

SOURCE: W. FRENCH ANDERSON

Researchers Test Gene Therapy Against AIDS

When Joseph Sodroski stepped up to the microphone at the Cold Spring Harbor Laboratory's meeting on gene therapy, few people in the audience had any idea what he was about to say. His talk, titled "Newer Strategies for Gene Therapy for AIDS," had no abstract, and even the session's chairman said he was as much in the dark as anybody about the presentation. It didn't take long for Sodroski, a virologist at the Dana-Farber Cancer Institute, to break the suspense, however: "We are considering HIV as a vector for [putting anti-AIDS genes into the cells of] people who are already infected with the HIV virus," Sodroski began. As a stunned murmur ran through the auditorium, Sodroski quickly noted that an HIV vector, gutted to make room for other genes, would be a toothless tiger unable to cause or worsen the disease.



Fighting AIDS with HIV. Would-be gene therapist Joseph Sodroski.

Fighting AIDS with the AIDS virus is just the newest twist on the idea of attacking the fearsome disease with gene therapy. Several groups have sought to transplant a variety of genes into the cells of AIDS patients to produce proteins that block HIV. Progress, however, has been slow. Part of the problem is getting the genes into the cells HIV attacks. In addition, the virus mutates so rapidly that it seems able to slip past the various blockers that researchers are trying to throw in its path. Sodroski's proposal is a dramatic effort to solve the first problem, while a string of speakers at the meeting described efforts to solve the second.

Strange as it may sound, Sodroski's plan to use a modified form of HIV to transfer anti-HIV genes into patients has a compelling logic. The vector would, for example, infect the same cells as the natural AIDS virus, targeting only those that need treatment or are likely to become infected. Moreover, HIV's efficient gene transfer mechanisms would integrate the antiviral genes directly into the DNA of the target cell. The viruses now being used for other types of gene therapy—such as the mouse leukemia virus—do not integrate into the chromosomes unless the cell is dividing.

Sodroski's group has several technical problems to work out before it can begin clinical trials, however. These include creating a cell line that produces high concentrations of HIV vector and maintaining long-term expression of the transplanted gene. Eventually, Sodroski envisions taking uninfected white blood cells out of an AIDS patient, inserting an antiviral gene, and then returning the now-protected cells to the body. And by the time Sadroski has worked the bugs out of his HIV vectors, other groups should have completed preliminary tests of the most promising antiviral genes to pack into them.

Several groups are experimenting with genetic decoys that would soak up key proteins produced by HIV. One target has been tat, a protein that stimulates HIV reproduction by binding to a specific region of the virus's genome. The decoy gene produces RNA segments that "act like sponges to remove all of the tat [protein]," said Clay Smith from the Memorial Sloan-Kettering Cancer Center in New York City. "With decoys, HIV replication is dramatically reduced in protected cells verses control cells" grown in the laboratory. But there's a catch; the effect is short-lived: "We do not see, in the end, a population [of cells] that is absolutely protected against HIV," because the virus eventually evades the blockade, Smith said. Scientists at Sandoz Forschungsinsitut in Vienna, Austria, the University of Michigan, and the National Cancer Institute (NCI) had similar difficulties with their anti-HIV decoy systems.

If decoy effectiveness can be boosted, that would be a promising way to interfere with HIV replication in infected people. But it may not be desirable to have the blocking genes constantly turned on, producing decoy RNA indiscriminantly. NCI's Julianna Lisziewicz has, however, devised a novel way to turn on a blocking gene only when the AIDS virus enters the cell and makes Rev, a DNA-binding protein that activates HIV genes and is associated with HIV replication. She accomplished this by hooking the HIV *rev* promoter, a stretch of DNA to which the Rev protein binds, to a *tat* decoy gene. The result: Whenever HIV makes Rev, the Rev protein binds to the decoy gene's promoter and stimulates production of the *tat* decoy, inhibiting HIV replication.

A team at the National Heart, Lung, and Blood Institute is following a similar approach, splicing the promoter for another HIV gene to a variety of genes that the group has transferred into target cells. These include a vector with "diphtheria toxin that only becomes active after HIV infection," group leader Richard Morgan told the meeting. Turning on the diphtheria gene kills the infected cell and the virus along with it.

These approaches are novel and may offer pinpoint precision, but they are not the most aggressive strategy for AIDS gene therapy now being tried. That honor goes to ribozymes, bits of RNA that have enzymatic properties, which could be used to slow down HIV. Flossie Wong-Staal from the University of California, San Diego, described how her group has built gene therapy vectors to deliver a ribozyme that inhibits 95% of the AIDS virus's activity in cells, when measured by HIV protein production.

Although most of the anti-HIV gene therapy systems have encountered problems, researchers are eager to begin clinical trials to determine whether the current approaches can block HIV in the body, since the virus seems to act differently in the laboratory, says Sloan-Kettering's Smith. Smith's first clinical protocol, however, was shot down earlier this year by NIH's Recombinant DNA Advisory Committee (RAC) because he had not demonstrated long-term protection in cell culture or in animals.

One biotech company, Viagene of San Diego, California, has already received approval from the Food and Drug Administration, however, and is planning to carry out the first attempt to treat AIDS patients with gene therapy by year's end. In the treatment, planned to be tested on 12 to 15 HIV-infected patients, fibroblasts will be removed from the patients' arms and genetically altered to manufacture gp160, a protein that is part of the virus's protective shell. These engineered fibroblasts will be irradiated to make them stop dividing (though they survive and continue producing gp160 for a few weeks), and then will be put back into the patient. Viagene's research director, Doug Jolly, said laboratory tests suggest that the cells should produce enough gp160 to activate the immune system's cytotoxic T cell response throughout the body, killing any cell showing the HIV protein on its surface.

Other teams are edging toward clinical trials, including groups at NIH and Columbia University. By the time results come in from these tests, Sodroski hopes to have his HIV vectors ready for clinical testing.

–L.T.

to a cytomegalovirus promoter. (The promoter is the gene's "on switch" and the enhancer further boosts the gene's activity.) The researchers then spliced that combination to the factor IX gene from a dog and placed this construct into a retroviral vector for transfer into immature muscle cells.

Even if the genetic regulation can be worked out, however, cell biology remains a problem. So far, gene therapy provides only a short-term cure that must be repeated every few months because the cells carrying the transplanted genes die off and must be replenished. Researchers have yet to find a way to put genes into long-lived cells, especially those immortal, primitive cells that differentiate into more mature cell types.

That's one reason why researchers at the Cold Spring Harbor meeting were excited by a report by Malcolm Brenner from St. Jude Children's Research Hospital in Memphis, Tennessee, of a gene marking experiment he conducted with bone marrow cells from children with acute myeloid leukemia or neuroblastoma. His primary goal was to use the marked cells to determine why radiation treatment generally fails to cure these cancers. But the experiment also provided the first hint that foreign genes can be transplanted into bone marrow stem cells in people. Gene transfer into stem cells has been the Holy Grail for researchers because these immortal cells produce all other blood cells.

"There is no definitive evidence that we are marking stem cells, but it is suggestive."

–Malcolm Brenner

Brenner's experiment was simple in concept. Patients with acute myeloid leukemia or neuroblastoma are often treated with massive doses of chemotherapy or radiation to kill the cancer, and then rescued from the deathly effects of treatment by transplanting their own bone marrow—collected before treatment—into their bodies. Usually, however, the cancer comes back. Brenner genetically marked the transplanted bone marrow cells to learn whether the recurrence is caused by cancer cells hiding in the retransplanted bone marrow or cancer cells surviving the toxic treatment. Of 10 patients treated with gene-marked marrow since September 1991, two have relapsed, and in both cases, the cancer cells carried the marker gene, showing conclusively that transplanted bone marrow caused the relapse.

The gene jockeys got excited because the marker genes were expressed for more than 12 months in 5% to 10% percent of the children's normal blood cells. "There is no definitive evidence that we are marking stem cells, but it is suggestive," Brenner said. A possible explanation is that chemotherapy used to purge cancer cells from the bone before it was removed may have caused the stem cells to divide. Current vectors require the target cell to divide in order to integrate their genetic cargo into its nucleus.

Treating cancer with genes. Brenner is among the vanguard of cancer physicians who have turned to gene therapy as a weapon against the disease. Indeed, the speed with which cancer researchers have begun testing gene therapy has surprised most genetic pundits, who predicted that gene therapy would be used primarily for inherited illnesses like hemophilia or muscular dystrophy. Several cancer trials are now under way, including those conducted by Steven A. Rosenberg at the National Cancer Institute (NCI). Rosenberg is trying to boost the immune attack on melanoma, a skin cancer, by inserting genes such as interleukin-2 into the tumor cells and then implanting the modified cells back into the patients. The tumor-produced IL-2 helps activate nearby immune cells to recognize the cancer cells and attack them. Those immune cells then go on to attack any other melanoma cells anywhere in the body—at least, that is the theory. "It's too early to draw conclusions," Rosenberg said.

A former associate of Rosenberg's, Kenneth Culver, who recently left NCI for Gene Therapy Inc., of Gaithersburg, is about to launch a novel attack on brain tumors. In animals, Culver can cure 80% of the tumors by injecting into the brain mouse cells a vector carrying the herpes thymidine kinase (TK) gene. Culver then treats the animal with the drug gancyclovir, which the TK enzyme turns into a poison that kills the cells. The unengineered tumor cells die of a poorly understood "bystander effect," in which products from the dying cells kill nearby cancer cells as well (*Science*, 12 June, p. 1550). Human trials should start before year's end.

Shortly before the Cold Spring Harbor meeting, Jack A. Roth from the M.D. Anderson Cancer Center won approval from NIH's Recombinant DNA Advisory Committee (RAC) to launch a dramatic assault on lung cancer, the most common cause of cancer death in the United States. Roth will try to stop the tumors in 14 patients by injecting two different genes. One, an antisense gene, is intended to block the effects of a mutated *K-ras* gene that causes the cell to grow out of control. The other, a *p53* gene lost in lung cancer, suppresses cell growth.

The next targets. Even as work progresses on cancer, several other diseases are now in the gene therapists' crosshairs. Cystic fibrosis (CF) is perhaps the most important inherited illness now being targeted. At least two groups, one led by Ronald Crystal at the National Heart, Lung, and Blood Institute, and one led by James Wilson at the University of Michigan at Ann Arbor, are racing to see who will be first to win permission to use gene therapy against that deadly disease. Both made the filing deadline for the next meeting of the RAC that will be held on 3 and 4 December.

Crystal has pioneered the use of adenovirus, a virus that commonly infects the upper respiratory system, the main target tissue for CF therapy, as a vector. Both Crystal and Wilson have successfully tested the adenovirus vector, which has been modified so that it can still get into cells but doesn't cause disease. Wilson made a CF animal model by layering human lung cells onto the inner surfaces of rat bronchial tubes transplanted into a nude mouse. This study showed that the vector infected up to 30% of the epithelium cells that line the surface of the lungs, but not the progenitor basal cells that produce the epithelium.

Animal trials, however, can only answer so much. "Until humans are done," Crystal said, "we are not going to know if all the cell types [in the lung] are infected." Both teams envision spraying the genetically engineered virus vector into the CF patient's lungs every 2 to 3 months to infect new epithelium as the old cells die off.

Probably the most unexpected use for gene therapy is still in animal testing: fighting arthritis. Paul D. Robbins at the University of Pittsburgh showed it is possible to block the effects of interleukin-1, an immune system signal shown to trigger the painful inflammations of rheumatoid arthritis. By genetically transferring a gene for IL-1 receptor antagonist protein [IRAP] into the synovial cells that line the knee joints of test animals, Robbins has been able to block 70% to 80% of IL-1's inflammatory effect. The Pittsburgh team, however, ran into an old problem: The current vectors make IRAP for only 2 weeks and then shut down. Robbins and colleagues are looking for a new system to keep IRAP production turned on.

Two years ago, as the first gene transfer experiments were getting under way, few would have predicted that researchers would even be contemplating treating diseases like arthritis with gene therapy. But rapid progress on several fronts—especially the successful treatment of the two children with ADA deficiency—means that "it is intellectually permissible to think about gene therapy now," says Blaese. Even more important, he adds, "now, you can get funding."

–Larry Thompson

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