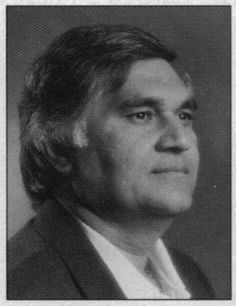
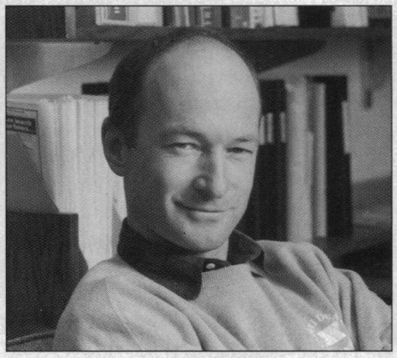
GENE THERAPY

New Role for HIV: A Vehicle For Moving Genes Into Cells

Like proponents of the ill-starred Strategic Defense Initiative of the 1980s, gene therapists have been accused of overselling their technology and rushing to deploy it. Critics charge that clinicians have attempted some crude treatments, rather than waiting until the techniques to repair defective genes catch up with the theory of how best to do it (*Science*, 25 August 1995, p. 1050). None of those treatments has yet shown convincing clinical benefits, in part because the vectors—the viruses used to deliver genes into target cells—don't work very well. One big problem: Most current vectors can't get into many types of cells, rendering them as powerless as Trojan horses that can't get past the city gates. Now a high-powered team of gene therapy researchers—including some leading critics of the field—has taken a step forward with a vector famous for causing, not solving, major problems: the AIDS virus.





PHOTOS BY THE SALK INSTITUTE

Gene engineers. Inder Verma (*above*) and Didier Trono hope HIV may point the way to solving one of gene therapy's most intractable problems.

Building on the work of several other groups, a team led by Didier Trono and Inder Verma of the Salk Institute for Biological Studies detail on page 263 how they have constructed what they hope is a harmless form of the retrovirus known as HIV that can deliver genes to a wide range of cells. "It's an important and useful new addition to retrovirus technology," says pediatrician Theodore Friedmann, a pioneer in the field who is at the University of California, San Diego (UCSD). Arthur Nienhuis, director of St. Jude's Children's Hospital and one of the first to experiment with an HIV vector, calls the work "extremely interesting." Says Nienhuis: "The outcome is quite encouraging."

The vector most often used in human experiments to date is murine leukemia virus (MLV), a mouse retrovirus that can only infect cells if they are dividing. But, as Trono explains, "most of the targets one thinks about with gene therapy rarely, if ever, divide"—an obstacle for modifying neurons and the stem cells that form the foundation of the immune system. That limitation has forced researchers to harvest these cells from the body, treat them with chemicals to make them divide, transfect them with a vector, and then reintroduce them to the body—a process that by itself can harm the cells. "Why do we need to do all this circus?" asks Trono. "It would be ideal to have a vector that could be directly injected."

Trono and co-workers show in this paper that HIV—or one of its close relatives—could be just such a vector. Michael Emerman and co-workers at the Fred Hutchinson Cancer Research Center in Seattle first showed in 1992 that HIV, like others in the lentivirus family, can infect nondividing cells. The labs of Emerman, the University of Nebraska's Mario Stevenson, and Trono have played key roles in identifying how the HIV proteins Vpr and matrix make this trick possible. Essentially, these proteins help shuttle HIV into the nucleus of cells, whether they are dividing or not.

The Salk group, working with Richard Mulligan and Daniel Ory of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, built on that work by constructing an HIV vector that contains the Vpr and the matrix proteins. For safety reasons, they deleted all of the genetic elements that the virus needs to copy itself. In one vector, they also swapped the gene for HIV's surface protein, the primary factor that limits the range of cells HIV can infect, with a gene for MLV's surface protein, which is much less discriminating. In another experiment, they stitched in the gene for a surface protein from a different source, vesicular stomatitis virus (VSV), to make the vector more stable and able to produce more of the protein encoded by the therapeutic gene.

Researchers who closely follow gene-therapy vector development say that what really distinguishes this study is the demonstration that the vector works in vivo. Specifically, Salk's Fred Gage and Ulrike Blömer injected the vector carrying a gene for β-galactosidase into the brains of rats and showed by immunocytochemistry that the vector infected nondividing neurons and produced the β-galactosidase protein. A similar MLV vector used as a control did not. "That's what really shows that these vectors act as we expect them to act," says Emerman.

As intriguing as these results are, researchers caution that there is still a large divide between this work and the clinic. "There still are a lot of practical hurdles," says virologist Joseph Sodroski, who works with HIV at the Dana-Farber Cancer Institute in Boston. The biggest one, he says, is to improve on the system used to make the vectors. Currently, the researchers engineer the vectors by transiently transfecting human kidney cells with the different HIV, MLV, and VSV genes. A more practical approach would be to develop what are known as "packaging cells." These are cell lines that are engineered to make copies, ad infinitum, of the replication-defective virus used as the vector. By using this system, the vector can be reliably reproduced and, theoretically, any gene can be added to it.

But developing packaging cell lines for an HIV vector has eluded others. "Packaging lines will be very difficult with HIV," says Emerman, who notes that HIV kills cells because Vpr prevents them from going through mitosis. Trono acknowledges the difficulty, but says they are working on developing a packaging cell line and "it's not very far [off]."

Another serious limitation of an HIV vector is the remote possibility that it could recombine into a virulent form and cause AIDS. Although some researchers, such as Nienhuis, think the risk of using HIV components could be reduced to negligible, others, including the authors of the paper, are more wary. "I'd move as far away as possible from HIV," says Trono, who with his co-authors suggests using the techniques they have developed for HIV to modify lentiviruses known to cause disease in other species.

UCSD's Friedmann sees this work as one step on a long journey, ultimately leading to vectors that can be injected into a person, target specific types of genetically damaged cells, stitch themselves into precise spots in the cells' genomes, and produce large amounts of the given corrective protein. "Slowly but surely these things are beginning to look soluble," says Friedmann. Yet, in keeping with the sober attitude of many basic researchers in the field, he stresses that this work is just one of many potential approaches to achieve these ends. "Gene therapy is not a technique," says Friedmann. "It's a goal." And an HIV vector, or one like it, is just another possible way to kick the ball toward it.

–Jon Cohen