Inner Strength

Gene therapy aims to build cells that thwart HIV replication

By JOHN TRAVIS

Like an attacking army that has bridged a castle's moat and scaled its walls, the AIDS virus is free to enjoy the spoils of war once it has invaded an immune cell. Infected cells may attempt suicide or try to alert other cells to HIV's presence, but the virus seems to stymie these ploys. Secure in its new home, HIV hijacks the cell's internal machinery and begins to make copies of itself.

While recent combinations of antiviral medications have shown a dramatic ability to curtail HIV replication, they must be taken several times a day, are expensive, can produce undesirable side effects, and don't work for everyone.

"There are now increasing numbers of HIV-infected patients that are failing combination therapies," says Wayne A. Marasco of the Dana Farber Cancer Institute in Boston. One recent study suggested that more than half of all AIDS patients may not benefit from the drug treatments, he notes.

Consequently, investigators like Marasco continue to explore the possibility of building a better immune system, one whose cells safely tolerate the normally deadly intrusion of HIV. In particular, they hope to equip immune cells with various genes that thwart the virus' ability to reproduce.

A decade ago, Nobel prize-winning virologist David Baltimore bestowed the name "intracellular immunization" on this idea of using gene therapy to create HIV-resistant immune cells (SN: 10/1/88, p. 213). He and other investigators imagined periodically infusing people with the hardier cells or even genetically engineering immune cell precursors, so-called stem cells, to permanently reconstitute a person's immune system with cells that check HIV.

"The advantage of intracellular immunization, if it works right, is that it wouldn't require continually taking pills," says Baltimore, now president of the California Institute of Technology in Pasadena and head of the national effort to develop an AIDS vaccine.

Today, with physicians employing the strategy in a number of safety trials with HIV-infected people, intracellular immunization is moving beyond the test-tube proving ground. Furthermore, investigators have been buoyed up by news that one version of intracellular immunization has apparently protected a few macaque monkeys from the ravages of SIV, a monkey virus that closely resembles HIV.

"Intracellular immunization works in the most relevant animal model we have for HIV infection," says study leader Richard A. Morgan of the National Human Genome Research Institute in Bethesda, Md.

The origins of intracellular immunization date to 1988, when investigators first blocked the replication of a virus by providing cells with genes that encode a mutant version of a protein naturally made by the virus. In that study—of a herpesvirus, not HIV—the mutant proteins interfered with the actions of the normal viral protein, apparently by competing for the same cellular targets.

With that result in hand, scientists immediately began speculating about trying the same strategy against HIV. Two obvious targets were Tat and Rev, proteins that are used early in the infection process and are essential to the replication of the AIDS virus. "You shut those two down, you shut down the virus," says Larry A. Couture of Ribozyme Pharmaceuticals in Boulder, Colo.

Several research groups have made progress with a mutant version of Rev that disrupts HIV's attempts at replication without seeming to harm the infected cell. Mutant versions of Tat are also under study.

Other intracellular immunization strategies have also drawn a bead on Tat. Several years ago, inspired by antibodies' talent for binding proteins of bacteria or viruses floating in the bloodstream, Marasco and his colleagues wondered if they could engineer antibodies to perform similar duties inside cells. These so-called intrabodies might home in on bacterial or viral proteins or even on mutant cellular proteins that drive the uncontrolled growth of cancer cells, they proposed.

Normally, immune cells secrete antibodies or display these proteins on their surface. Recently, scientists have grown increasingly adept at shaping antibodies to their needs by mixing and matching the many genes that encode the proteins. Indeed, Marasco and his colleagues found that they could design antibodies which would remain in a cell and even move to specific cellular regions.

Initially, the group created intrabodies that fuse to gp160, a precursor of the protein that makes up HIV's outer envelope. By binding gp160, the intrabodies deprived any new viruses of a protein that plays a crucial role in HIV's ability to attach to cells and infect them. Marasco's group found that the normal AIDS virus is 1,000 times more infectious than the viruses manufactured in cells with the added intrabody gene.

Because those first intrabodies did not actually stem the production of HIV, the scientists shifted their attention to Tat. They have since developed intrabodies that zero in on that crucial protein and virtually arrest HIV replication. Later this year, Marasco plans to start testing this Tat intrabody on HIV-infected people for whom current drug therapies have failed.

Another HIV protein, integrase, faces attack from intrabodies. Roger J. Pomerantz of Thomas Jefferson University in Philadelphia and his colleagues recently created intracellular antibody fragments that bind integrase, thereby preventing HIV from incorporating its genes into the genome of an infected cell. "Integrase is something that is obviously vital to the virus' life cycle, but no therapies currently target it," says Pomerantz.

Not all intracellular immunization depends on the production of antiviral proteins. Many researchers in the field have turned to ribonucleic acid (RNA).

The single strands of RNA often serve as cellular couriers, conveying the protein-making instructions encoded in a gene's DNA sequence to the factories in the cell where such molecules are built. Yet RNA strands can be put to many other uses. One intracellular immunization strategy employs strands of RNA to distract RNA-binding viral proteins from their assigned duties. Rev, for example, binds to an RNA sequence in the HIV genome that is known as a Rev-

response element, or RRE. After arming cells with a gene that encodes RRE, investigators can flood these cells with the decoy and dramatically curtail HIV replication.

Intracellular immunization can target HIV's RNA in two other ways. Investigators have created so-called antisense genes, which synthesize strands of RNA that recognize and bind to specific sequences of the virus' RNA. This bond halts production of HIV because a cell can no longer read the protein-building instructions of the viral RNA.

In an even more destructive approach, genes for ribozymes—RNA strands that bind to other RNA strands and chop them to pieces—can enable cells to destroy the instructions for building HIV proteins such as Tat and Rev (SN: 9/18/93, p. 182).

At this point, it's impossible to know which intracellular immunization strategy, if any, will prove useful in treating patients. Fans of intrabodies laud the ability to direct their proteins to specific sites in a cell. On the other hand, advocates of antisense and ribozymes caution that the body's immune cells may view an intrabody as foreign and attack it.

"The potential for an immune response to a protein-based therapeutic is much greater than to an RNA therapeutic. There's absolutely no evidence of an immune response to RNA," says Couture.

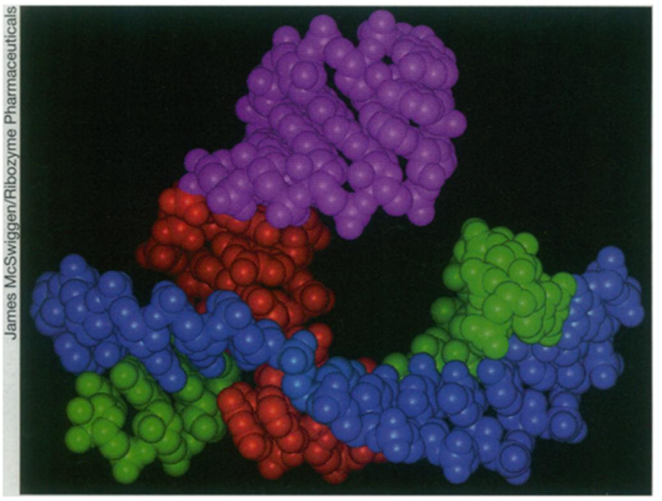
Another major issue concerns the kind of cells with which investigators should work. Some scientists harvest mature immune cells in the bloodstream and slip the antiviral genes into them. Others favor isolating and genetically engineering the much rarer blood stem cells.

While the former strategy is less daunting technically, the latter offers the possibility of creating an immune system that is permanently resistant to HIV. A physician would infuse the modified stem cells into a patient in hopes that they would make themselves at home in bone marrow, where the body generates new immune cells.

John A. Zaia of the City of Hope National Medical Center in Duarte, Calif., expects that physicians will ultimately make use of both strategies, although he is currently working with stem cells to which he has added a gene for an antiviral ribozyme. He and his colleagues have already infused such cells into five HIV-positive people and plan next to treat AIDS patients suffering from lymphomas. Those patients are scheduled to receive bone marrow transplants to treat their cancer, which may make it easier for the altered stem cells to take up permanent residence, says Zaia

Since all of the intracellular immunization methods work to some degree on HIV-infected immune cells grown in the laboratory, the only way to settle the various debates is by experiments such as Zaia's. Morgan and his colleagues have begun studying identical twins, one already infected with HIV and one not. By harvesting immune cells from the uninfected twin, engineering them to make antiviral proteins or RNA, and infusing those cells into the infected twin, the investigators plan to compare the effectiveness of various intracellular immunization strategies.

The researchers have already treated eight people with infusions of cells engineered to make the Rev mutant and with cells engineered to make both that mutant and an antisense RNA strand.



James McSwiggen/Ribozyme Pharmaceuticals

Ribozymes, such as the one depicted above in a computer model, can chop up the protein-making instructions produced by HIV, thereby halting the reproduction of the AIDS virus.

"For the majority of our patients, we do see a survival advantage for cells with these therapeutic genes," Morgan told SCIENCE NEWS.

Like most other intracellular immunization studies so far, designed only to test the safety of the strategies, Morgan's involves HIV-positive people who have kept the virus in check with drugs and therefore aren't suffering from AIDS. In such cases, scientists cannot really determine whether the gene therapy bolsters the health of the volunteers; they can only measure whether the manipulated immune cells survive longer than normal cells.

While nearly a dozen small-scale tests of these gene therapy strategies are in progress or poised to begin, published reports on them are still few and far between.

Gary J. Nabel of the Howard Hughes Medical Institute at the University of Michigan in Ann Arbor and his colleagues described their works with a mutant version of Rev in the Feb. 3 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. In three HIV-positive people, the immune cells engineered to make the mutant Rev persisted in the bloodstream in larger numbers and for a longer time than did immune cells to which a non-protein-producing gene had been added.

The most compelling testament to the promise of intracellular immunization appears in the February NATURE MEDICINE, where Morgan's team details gene therapy efforts designed to protect rhesus macaque monkeys from SIV, a virus that usually kills the animals within a year or two.

The investigators harvested immune cells from three macaques and added a gene for an antisense RNA strand that binds to the RNA used by SIV to build its versions of Tat and Rev. The researchers then returned the cells to the animals and infected the macaques with SIV.

Although the altered cells accounted for only a tiny percentage of the animals' immune cells, they had a dramatic impact. Macaques that received unaltered immune cells suffered a massive initial wave of viral reproduction, slowly lost significant numbers of immune cells, and showed considerable damage to their lymph nodes. In contrast, the three treated animals didn't experience nearly as large an initial viral outburst and for the first year have largely maintained the original number of immune cells. "Two of the animals showed no signs of disease in the lymph nodes," adds Morgan.

These results are "remarkable" and "almost too good to be true," says Pomerantz.

While the macaque study has raised the hopes of some AIDS researchers, other scientists caution that gene therapy always sounds easy but usually proves challenging. Investigators have found it difficult, for example, to ensure that the genes they add to cells remain active for extended periods of time, let alone forever. "The jury is still out" on intracellular immunization, cautions an openly skeptical Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases in Bethesda, Md.

Are researchers frustrated that intracellular immunization hasn't progressed further in a decade? Seemingly not. "When we first conceived of this, it was clear it would take a long time to develop," notes Baltimore.

Pomerantz concludes, "This field has matured. It's shown its problems and its pluses. It's now moved into animals and the first studies in people. I think that's pretty good in 10 years." □