

### NEWS

# Building an HIV-Proof Immune System

Despite past setbacks in the field of gene therapy, several research teams are testing whether that strategy can provide people with immune cells that are more resistant to HIV or that can cripple the virus

Except for a lucky few, everyone infected by the AIDS virus suffers a prolonged and inexorable attack that obliterates the immune system. Yes, anti-HIV drugs can stave off this destruction. But drugs can have serious toxicities, they're costly and difficult to take every day, and the virus can develop resistance to all of them. So a small group of researchers has long explored an ambitious alternative to drug therapy: introducing new genes into the bodies of HIV-infected people to help fortify their immune systems. Some are even pursuing the option of destroying the remaining immune cells in an infected person so that new, better ones can take their place. Fanciful as it sounds, scientists are hoping to reboot people's immune systems with HIV-proof cells.

Researchers haven't made much headway with this approach, which some call "intracellular immunization." But more effective gene-therapy techniques recently have begun to advance a parade of novel strategies. They include disabling a critical receptor that HIV uses to infect immune cells, silencing the regulators that turn on

HIV genes, and incapacitating HIV with lab-designed antibodies. In an ironic twist, some scientists are even using forms of HIV to deliver the genes for these strategies. "These are exciting times now because the technologies are there," says immunologist Carl June, who develops HIV gene therapies at the University of Pennsylvania.

The National Institutes of Health invested more than \$10 million last year in three dozen labs pursuing this pioneering HIV gene-therapy research. And as part of its Grand Challenges in Global Health initiative, the Bill and Melinda Gates Foundation has committed nearly \$14 million to a project headed by Nobel laureate David Baltimore, who wants to engineer the immune system to produce an ultrapotent antibody against HIV.

For the most part, industry has left the exploration of these radical ideas to academics. "One has to be somewhat optimistic to go after this type of therapy because it is labor-intensive, and we don't know all the rules yet," says Jerome Zack, an immunologist at the University of California, Los

Angeles (UCLA). "The field is still waiting to be cultivated."

### On trial

HIV gene therapies all adhere to the same basic principles: Researchers remove blood or bone marrow from an HIV-infected patient, isolate the immune cells that HIV targets or the blood-forming stem cells that spawn them, introduce new genes, and reinfuse the cells (see table, p. 613). Key distinctions between different labs include the viral "vectors" they design to shuttle in genes (a process called transduction) and how they culture cells to expand their number. Success depends critically on how many cells the vectors actually transduce and how long the modified cells survive once back in the body.

Gary Nabel, a leading HIV gene-therapy investigator in the 1990s who subsequently moved into AIDS vaccine research, says the field's rejuvenation owes much to improvements in the gene-carrying vectors and discoveries about the molecular weak points of HIV. "We just know so much more now than we did then," says Nabel, who heads the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

One of the earliest gene-therapy attempts to build an HIV-resistant immune system employs a ribozyme, a catalytic bit of RNA. Developed by Zack and other UCLA researchers in association with a Johnson & Johnson (J&J) division in Sydney, Australia, the ribozyme clips an HIV gene, *tat*, that the virus needs for replication, rendering it incapable of producing new copies. Nearly a decade ago, a clinician at UCLA who collaborates with Zack began a study of this ribozyme approach in 10 patients, all also receiving anti-HIV drugs. To deliver the ribozyme into each patient's cells, the researchers used a mouse retrovirus.

At the time, the most unusual aspect of the experiment was the target cell. HIV preferentially infects white blood cells that have CD4<sup>+</sup> surface receptors, and researchers had typically focused on transducing these cells. The UCLA group decided that because the CD4<sup>+</sup> cells are mature and have a finite life span, it made better sense to add the ribozyme gene to stem cells, marked by a cell surface protein called CD34<sup>+</sup>, that theoretically can produce CD4<sup>+</sup> cells indefinitely. As Zack and co-workers reported in the March 2004 issue of *Human Gene Therapy*, they found that the ribozyme-containing vector was still inside CD4<sup>+</sup> cells as long as 3 years after an infusion

of the modified stem cells, presumably because they produced progeny that survived. The strategy looked promising enough that J&J moved ahead with a larger study, which began in August 2002 and has 74 patients enrolled at UCLA and other sites.

The only other HIV gene-therapy strategy now in clinical trials pioneered the use of HIV itself as the vector. Whereas the mouse retrovirus employed by Zack and his colleagues only transduces dividing cells, HIV has no such limitation. Many in the field also contend that because lentiviruses such as HIV are not known to directly cause cancer, they are inherently safer than mouse retroviruses, one of which triggered leukemia in some children in a gene-therapy trial (*Science*, 17 June 2005, p. 1735).

The taming of HIV begins by stripping the virus down to its bare bones so that it can insert the genetic material it carries into human chromosomes but not make dangerous new copies of itself. VIRxSYS Corp. in Gaithersburg, Maryland, spliced into such a vector an “antisense” gene that stops HIV from making its crucial envelope protein. (The RNA strand made by this gene complements the messenger RNA for the protein and prevents its translation.) Once integrated into an immune cell’s DNA, the antisense gene should prevent any normal HIV that gets into the cell from making new copies.

In 2003, June and co-workers used this vector to transduce CD4<sup>+</sup> T cells taken from five people who were failing on their anti-HIV drugs. Subsequently, they gave the patients a single infusion of the modified cells. In the 14 November 2006 *Proceedings of the National Academy of Sciences*, the researchers report that the HIV vector, as expected, far more efficiently transduced cells than did mouse retroviruses. Although the study was meant to address only safety and not whether the therapy worked, one patient had a dramatic drop in HIV levels. And whereas the transduced CD4<sup>+</sup> cells had a half-life of less than 1 month, the researchers unexpectedly found signs of the antisense-totting vector in two patients’ CD4<sup>+</sup> cells more than a year after the infusions. Two separate clinical trials in HIV-infected people, including one in which participants will stop taking

their antiviral drugs, are now evaluating multiple infusions of the VIRxSYS vector.

June says it may turn out that multiple infusions aren’t necessary. In the first trial, they found evidence that an infected person’s own “wild-type” HIV could “package” the vector and carry it to uninfected CD4<sup>+</sup> cells, possibly expanding the number of protected cells and extending the durability of the therapy. “Potentially, you could infuse a limited number of transduced cells that could infect their neighboring cells in vivo,” says June. In most gene-therapy studies, mobilizing a

To infect immune cells, HIV must first bind to chemokine receptors. Researchers discovered in 1996 that people who had a naturally occurring mutation in their genes for one of these, CCR5, were strongly protected from developing AIDS—or even becoming infected in the first place—and suffered no ill effects from lacking the receptor.

Sangamo specializes in developing enzymes called zinc finger nucleases that can bind to genes, clip their DNA, and repair mutations (*Science*, 23 December 2005, p. 1894). But for the HIV gene therapy, they’ve created a nuclease to specifically disrupt the CCR5 gene in the same manner as the natural mutation. In the new trial, researchers will put the gene for this zinc finger nuclease into an adenovirus vector, transduce harvested CD4<sup>+</sup> T cells of HIV-infected people, and infuse those cells back. June says this is the first gene-therapy experiment that aims to create a phenotype that’s known to confer disease resistance.

A single infusion of these transduced cells will, at best, only protect a small fraction of the body’s CD4<sup>+</sup> cells. But a gene-therapy approach could have a much greater impact if scientists instead transduce the stem cells that make CD4<sup>+</sup> cells and “condition” the existing immune system to make “space” for those stem cells.

Toward that end, Donald Kohn at Children’s Hospital Los Angeles will use a chemotherapeutic agent to partially ablate the immune systems of children who are failing on anti-HIV drugs. Building on earlier work he did with mouse-based retroviral vectors, Kohn will infuse the children with their own CD34<sup>+</sup> cells that he has transduced with an HIV-based vector to carry a gene known as *RevM10*, which produces a mutant form of the critical HIV protein Rev. When the virus infects such a transduced cell, it uses the wrong *Rev*, disrupting its replication.

Kohn says a “home run” from this conditioning would lead the vast majority of cells to express the protective gene. Still, even a much smaller percentage of protected stem cells could powerfully bolster the immune system. “Those cells over time could amplify in number because they’re resistant to HIV,” says Kohn. He notes, too, that the transduced

## HIV GENE THERAPY TRIALS

Investigators	Sponsors	Vector	Transduced Genes Encode	Protocol	Development Stage
Jerome Zack and Ronald Mitsuasu, UCLA	Johnson & Johnson Research, Sydney, Australia, NIH	Moloney murine leukemia virus	Ribozyme that targets HIV <i>tat</i>	CD34 <sup>+</sup> cells, no conditioning	74 patients in randomized, controlled phase II trial. Results expected early 2008
Carl June, University of Pennsylvania	VIRxSYS, Gaithersburg, Maryland, NIH	Modified HIV	Antisense that targets HIV <i>env</i>	CD4 <sup>+</sup> cells, no conditioning	Two studies with 65 patients. First results expected in fall
Carl June	Sangamo BioSciences, Richmond, California, NIH	Adenovirus	Zinc finger nucleases that target CCR5	CD4 <sup>+</sup> cells, no conditioning	12 patients, expected to start later this year
Donald Kohn, Children’s Hospital Los Angeles	NIH	Modified HIV	RevM10 that overrides HIV <i>rev</i>	CD34 <sup>+</sup> cells, partial ablation with busulfan	12 patients failing therapy expected to start in early 2008
John Rossi and John Zaia, City of Hope, Duarte, California	NIH	Modified HIV	Short RNA against HIV <i>rev</i> and <i>tat</i> , ribozyme against CCR5, and TAR decoy against HIV <i>tat</i>	CD34 <sup>+</sup> cells, myeloablation	5 patients with AIDS lymphoma, enrolling
Dorothee von Laer, Georg-Speyer-Haus, Frankfurt, Germany	Fresenius Biotech, Vision7 GmbH, EU	Moloney murine leukemia virus	Peptide that disrupts HIV’s gp41	CD34 <sup>+</sup> cells, partial ablation with chemotherapy	5 patients with AIDS lymphoma, start later this year
David Baltimore and Pamela Björkman, Caltech, Pasadena	Bill and Melinda Gates Foundation	Modified HIV	Lab-designed anti-HIV antibody	CD34 <sup>+</sup> cells	Mouse studies
Irvin Chen, UCLA	NIH	Modified HIV	Short interfering RNA that targets CCR5	CD34 <sup>+</sup> cells	Monkey studies

retroviral vector like this would raise staggering safety concerns, but for whatever reason, and unlike other retroviral vectors, HIV integrates its genes at spots on human chromosomes unlikely to trigger cancers. (The lymphomas often seen in AIDS patients stem from general immune suppression.)

### Tomorrow’s front burners

A new generation of sophisticated therapies designed to HIV-proof the immune system promises to enter the clinic soon. For example, June, working with Sangamo BioSciences in Richmond, California, later this year plans to start trials in 12 HIV-infected people of a gene therapy designed to endow immune cells with a genetic mutation that protects them from HIV.

## Challenges in Immunology

cells could at a minimum leave people with enough of an immune system to ward off serious disease.

At the nearby City of Hope in Duarte, California, John Rossi heads a study that's recently started enrolling patients in the most aggressive HIV gene therapy yet. In five people with AIDS lymphoma, a cancer of the lymph nodes, Rossi, John Zaia, and colleagues will use various chemotherapies or radiation to completely destroy each person's immune system—a dangerous procedure that is the standard of care for that highly lethal condition. The researchers will then infuse the patients with their own previously harvested immune stem cells that an HIV-based vector has transduced with three genes. The therapeutic genes encode a ribozyme that knocks down CCR5, a short RNA that interferes with the virus's ability to copy itself, and a decoy that codes for an essential HIV protein and throws a wrench in the viral replication machinery. "The nice thing is, the targets are multiple," says Rossi, who hopes this will overcome a risk in all these strategies—namely, that HIV will develop resistance to the gene therapy.

### Instructive immunotherapy

At the California Institute of Technology in Pasadena, David Baltimore has teamed up

with immunologist Pamela Björkman on an HIV gene-therapy project that he calls "instructive immunotherapy." Rather than bolstering the natural immune response, Baltimore says, "we're instructing the immune system [about] what to make."

This 5-year experiment lives up to its Grand Challenges billing with its focus on inventing virus-fighting antibodies. Gene therapists have paid antibodies little heed because HIV notoriously remains impervious to their attack. "I didn't think we should be giving up on the historically most powerful part of the immune system," says Baltimore. So he and Björkman are attempting to construct an antibody against HIV that's far more powerful than anything naturally produced by the immune system. Baltimore and co-workers then want to use an HIV-based vector to transduce the gene for this antibody into immune stem cells.

Baltimore originally explored intracellular immunization strategies—he even coined the term—but his work now on instructive immunotherapy reflects a belief that multiple forms of gene therapies may be needed to defeat HIV. "I'm hedging my bets," says Baltimore.

Two years into the project, Baltimore says his team is making steady progress, but

they have an added hurdle to overcome. They need to craft antibody genes that will continue to function as the CD34<sup>+</sup> stem cells mature into the B cells that ultimately secrete the antibody. Within 3 years, the scientists hope to show that this can work in chimeric mice that have humanlike immune systems. "We're very aware that this is complicated and expensive and difficult to imagine using in the less developed world," says Baltimore, noting that the Gates initiative demands that researchers work on projects applicable to the world's poor. With that in mind, Baltimore says they've been testing another strategy in mice: injecting the vector directly into the body to see if it will home in on CD34<sup>+</sup> cells.

In the end, Baltimore and other researchers in the field imagine that different gene therapies and anti-HIV drugs will complement each other. And many anticipate that in wealthy countries, demand for a gene-therapy approach will grow as ever more people become resistant to the best anti-HIV drugs available. "With the right techniques and vectors, I think this can be just like what the Red Cross does with blood transfusions," predicts the University of Pennsylvania's June. "Unfortunately, it's going to take time."

—JON COHEN

## NEWS

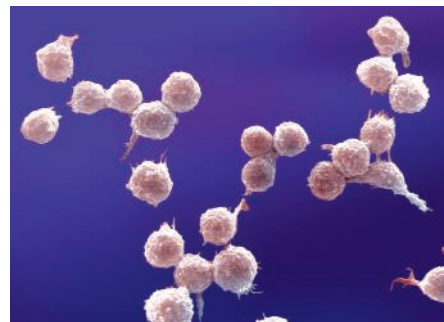
# Mast Cells Show Their Might

They are the most reviled cells in the body. Their meddling makes our skin itch, our eyes swell, and our noses stream; the cells even provoke suffocating asthma attacks that kill thousands of people every year. In fact, these villains, known as mast cells, are responsible for so much suffering that some researchers have proposed eradicating them.

That could be a big mistake. Over the past decade or so, the reputation of these immune cells has been turned around. Researchers have learned that mast cells are vital sentinels that orchestrate counterattacks on invading bacteria and viruses. The cells link the innate immune system, which deploys a standard set of defenses, with the adaptive immune system, which customizes the body's weapons to a specific attacker. Mast cells even neu-

tralize toxins from snakebites and bee stings (*Science*, 28 July 2006, p. 427).

However, mast cells turn out to be fickle allies. Extending the cells' disease connections far beyond allergic reactions, recent studies put them at the center of multiple



**Standing guard.** Mast cells from the umbilical cord.

Once dismissed as "allergy cells," mast cells have proven crucial for immunity. But they've also shown a dark side

sclerosis, rheumatoid arthritis, cancer, and atherosclerosis. "What this research tells you is that mast cells are key to a lot of biological processes," says immunologist Dean Metcalfe of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland.

The catalyst for many of these discoveries was the identification of mutant mice that lack mast cells. A white-spotted coat on one of these rodents first attracted geneticists' attention in 1937. But it wasn't until the late 1970s that Yukihiko Kitamura of Osaka University Medical School in Japan and colleagues determined that the genetic defect responsible for the color change also short-circuits mast-cell development. Led by Kitamura and pathologist Stephen Galli of Stanford University in Palo Alto, California,