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# Immune cells engineered in lab to resist HIV infection

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Researchers at the Stanford University School of Medicine have found a novel way to engineer key cells of the immune system so they remain resistant to infection with HIV, the virus that causes AIDS.

A new study describes the use of a kind of molecular scissors to cut and paste a series of HIV-resistant genes into T cells, specialized immune cells targeted by the AIDS virus. The genome editing was made in a gene that the virus uses to gain entry into the cell. By inactivating a receptor gene and inserting additional anti-HIV genes, the virus was blocked from entering the cells, thus preventing it from destroying the immune system, said Matthew Porteus, MD, an associate professor of pediatrics at Stanford and a pediatric hematologist/oncologist at Lucile Packard Children's Hospital.

"We inactivated one of the receptors that HIV uses to gain entry and added new genes to protect against HIV, so we have multiple layers of protection—what we call stacking," said Porteus, the study's principal investigator. "We can use this strategy to make cells that are resistant to both major types of HIV."

He said the new approach, a form of tailored gene therapy, could ultimately replace drug treatment, in which patients have to take multiple medications daily to keep the virus in check and prevent the potentially fatal infections wrought by AIDS. The work was done in the laboratory, and clinical trials would still be needed to determine whether the approach would work as a therapy.

"Providing an infected person with resistant T cells would not cure their viral infection," said Sara Sawyer, PhD, assistant professor of molecular genetics and microbiology at the University of Texas-Austin and a co-author of the study. "However, it would provide them with a protected set of T cells that would ward off the immune collapse that typically gives rise to AIDS."

The study will be published in the Jan. 22 issue of *Molecular Therapy*.

One of the big challenges in treating AIDS is that the virus is notorious for mutating, so patients must be treated with a cocktail of drugs—known as highly active antiretroviral therapy or HAART—which hit it at various stages of the replication process. The researchers were able to get around that problem with a new, multi-pronged genetic attack that blocks HIV on several fronts. Essentially, they hope to mimic HAART through genetic manipulation.

The technique hinges on the fact that the virus typically enters T cells by latching onto one of two surface proteins known as CCR5 and CXCR4. Some of the latest drugs now used in treatment work by interfering with these receptors' activity. A small number of people carry a mutation in CCR5 that makes them naturally resistant to HIV. One AIDS patient with leukemia, now famously known as the Berlin patient, was cured of HIV when he received a bone marrow transplant from a donor who had the resistant CCR5 gene.

Scientists at Sangamo BioSciences in Richmond, Calif., have developed a technique using a protein that recognizes and binds to the CCR5 receptor gene, genetically modifying it to mimic the naturally resistant version. The technique uses a zinc finger nuclease, a protein that can break up pieces of DNA, to effectively inactivate the receptor gene. The company is now testing its CCR5-resistant genes in phase-1 and -2 trials with AIDS patients at the University of Pennsylvania.

The Stanford scientists used a similar approach but with an added twist. They used the same nuclease to zero in on an undamaged section of the CCR5 receptor's DNA. They created a break in the sequence and, in a feat of genetic editing, pasted in three genes known to confer resistance to HIV, Porteus said. This technique of placing several useful genes at a particular site is known as "stacking."

Incorporating the three resistant genes helped shield the cells from HIV entry via both the CCR5 and CXCR4 receptors. The disabling of the CCR5 gene by the nuclease, as well as the addition of the anti-HIV genes, created multiple layers of protection.

Blocking HIV infection through both the CCR5 and CXCR4 receptors is important, Porteus said, as it hasn't been achieved before by genome editing. To test the T cells' protective abilities, the scientists created versions in which they inserted one, two and all three of the genes and then exposed the T cells to HIV.

Though the T cells with the single- and double-gene modifications were somewhat protected against an onslaught of HIV, the triplets were by far the most resistant to infection. These triplet cells had more than 1,200-fold protection against HIV carrying the CCR5 receptor and more than 1,700-fold protection against those with the CXCR4 receptor, the researchers reported. The T cells that hadn't been altered succumbed to infection with 25 days.

Porteus said he views the work as an important step forward in developing a gene therapy for HIV.

"I'm very excited about what's happened already," he said. "This is a significant improvement in that first-generation application."

He said a potential drawback of the strategy is that while the nuclease is

designed to create a break in one spot, it could possibly cause a break elsewhere, leading to cancer or other cell aberration. He said it's also possible the cells may not tolerate the genetic change.

"It's possible the cells won't like the proteins they're asked to express, so they won't grow," he said.

But he said he believes both problems are technically surmountable. He said the researchers' next step is to test the strategy in T cells taken from AIDS patients, and then move on to animal testing. He said he hopes to begin clinical trials within three to five years.

Though the method is labor-intensive, requiring a tailored approach for each patient, it would save patients from a lifelong dependence on antiretroviral drugs, which have adverse side effects, Porteus noted.

He said he also hopes to adapt these techniques for use against other diseases, such as sickle cell anemia, one of his areas of interest. Porteus works with patients in the Pediatric Bone Marrow Transplant service at Packard Children's.

In addition to Sawyer, he collaborated with Richard Voit, a former Stanford graduate student who is now an MD/PhD candidate at the University of Texas Southwestern Medical Center, and Moira McMahon, PhD, a former postdoctoral scholar at Stanford who is now at the University of California-San Diego.

Provided by Stanford University Medical Center