Genetically engineered T cells render HIV's harpoon powerless

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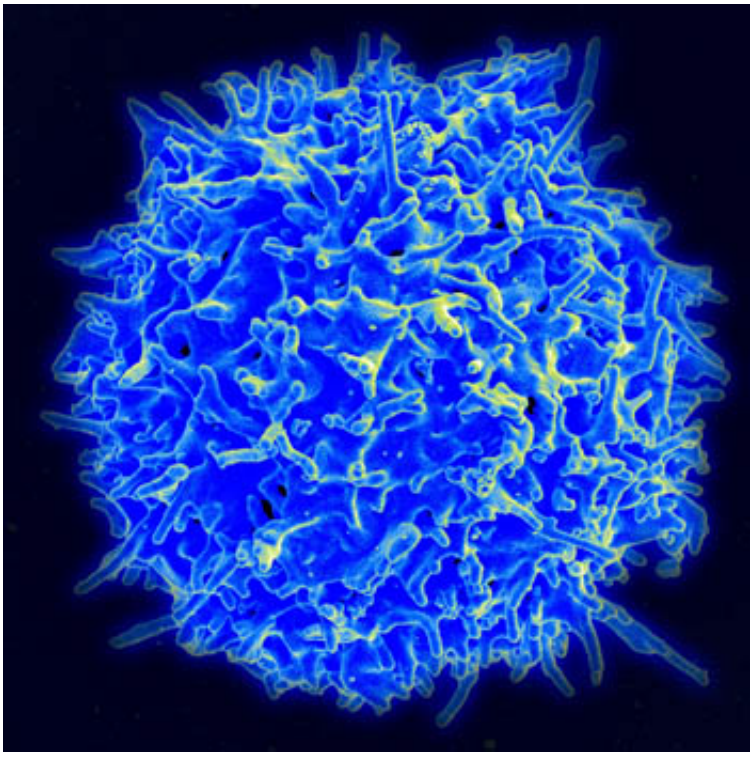

Scanning electron micrograph of human T lymphocyte or T cell. Credit: NIAID/NIH

When HIV attacks a T cell, it attaches itself to the cell's surface and launches a "harpoon" to create an opening to enter and infect the cells. To stop the invasion, researchers from the Penn Center for AIDS Research at the University of Pennsylvania and scientists from Sangamo BioSciences, Inc. have developed genetically engineered T cells armed

with a so-called "fusion inhibitor" to disrupt this critical step and prevent a wide range of HIV viruses from entering and infecting the T cells. The findings were reported today online in a preclinical study in *PLOS Pathogens*.

HIV medicine experienced a breakthrough in the early 2000s with a unique class of drugs known as "fusion inhibitors." Unlike most drugs that block virus replication inside of T cells, these drugs prevent HIV from entering cells in the first place. The drug, enfuvirtide, modeled after a peptide from the viral envelope and used today in combination with other antiretroviral therapies, has been shown to keep the virus at bay. However, patients need to inject enfuvirtide daily under their skin, limiting its utility and acceptability to patients, especially when compared to many other orally available drugs. HIV can also become resistant to enfuvirtide.

Building on this approach with a powerful genetic technique, researchers developed a novel way to deliver the fusion inhibitor peptide precisely to the spot on the cell surface where the virus attaches and launches its envelope, like a harpoon. The team genetically altered T cells by introducing a so-called C34 peptide, modeled after enfuvirtide, directly onto receptors, CXCR4 and CCR5, which are crucial for HIV entry. By using these molecules to deliver the C34 peptide to the site where the virus enters, these investigators showed that HIV was potently inhibited and that this inhibition extended to genetically diverse HIVs, including those that were resistant to the drug, enfuvirtide.

The most impressive results were seen when the C34 peptide was attached to CXCR4, where the Penn investigators showed that T cells expressing this molecule were protected in a mouse model of HIV infection.

"We believe that our approach to precisely target an inhibitory drug to

the site of viral entry creates a new way to engineer human T cells to become resistant to HIV infection," said senior author James Hoxie, MD, a professor of Medicine in the division of Hematology/Oncology in the Perelman School of Medicine at the University of Pennsylvania. "It's potent and very broad. Every strain of HIV we tried was sensitive to it, regardless of whether the virus used CCR5 or CXCR4, which is a big advantage, since HIV typically uses CCR5 to establish infection, but can over time, evolve to use a CXCR4 instead. With this approach, it doesn't matter where the virus came from or what cellular molecule it needs to infect cells."

The findings set the stage for an upcoming phase I clinical trial in HIV-positive patients to determine the safety and appropriate dosage of a patient's own T cells engineered to express the C34-CXCR4 molecule, as well as to demonstrate their ability to resist infection when antiretroviral therapy is interrupted.

The research team also includes James L Riley, PhD, an associate professor of Microbiology, Pablo Tebas, MD, a professor of Medicine and director of the AIDS Clinical Trials Unit at the Penn CFAR, along with co-first authors, George Leslie, PhD, a senior research investigator in Hoxie's lab, Jianbin Wang, PhD and Michael C. Holmes, PhD, of Sangamo Biosciences, Inc. and Max W. Richardson, PhD, a senior research investigator in Riley's Lab.

Peptides derived from the HIV-envelope protein inhibit HIV entry by interfering with the formation of what is termed the 6-helix bundle during fusion of the viral and cellular membranes that occurs during viral entry. This is how enfuvirtide works, although when injected as a drug, enfuvirtide is distributed throughout the entire body. In the work described by the Penn and Sangamo researchers, performed in the laboratory and in a humanized mouse model, the C34 peptide attached to the CXCR4 molecule delivered the peptide to where fusion was actually

occurring.

In the lab, the researchers found that T cells expressing either C34-CCR5 or C34-CXCR4 were enriched in the presence of HIV infection, going from 25 percent of the T cell population to greater than 60 percent after 7-10 days of additional culture. This enrichment was observed against a wide array of HIV strains, suggesting that this approach will be highly effective in a vast majority of individuals. Similar data was obtained using a humanized mouse model of HIV infection. In the experiments, only CD4 T cells expressing C34-CXCR4 were able to resist HIV infection and survive within the mouse. For this reason, C34-CXCR4 was chosen to be used in a phase I clinical trial.

This work builds off past experimental, genetic HIV techniques. In 2014, Penn researchers successfully genetically engineered the immune cells of HIV positive patients to resist infection, and decreased the viral loads of some patients taken off therapy entirely. The group used the zinc finger nuclease (ZFN) technology developed by Sangamo BioSciences to modify the T cells in the patients—a "molecular scissors," of sorts, to eliminate the CCR5 surface proteins. Without it, the virus couldn't enter. However, there are some limitations with this approach: it only addresses viruses that use CCR5 and both CCR5 alleles need to be knocked out for the T cells to be protected from infection.

The clinical trial investigating the work with C34 is slated to start in December 2016. The researchers will infuse C34-CXCR4 expressing T cells into well-controlled HIV infected individuals. It will be a dose-escalation study in which 1, 3, or 10 billion engineered T cells will be infused. After infusion, an "analytical treatment interruption" will occur for about 16 weeks and time to viral rebound and enrichment for the C34-CXCR4 expressing cells will be monitored. At present, patients infected with HIV must continue to take anti-HIV drugs to prevent the virus from replicating and causing disease. Efforts are underway at Penn

and throughout the world to develop strategies that will enable drug therapy for HIV to be discontinued safely.

"This may provide a successful novel strategy to supplement anti-viral immune responses that complement approaches to target or control HIV reservoirs in patients infected with the virus," the authors said.

Provided by Perelman School of Medicine at the University of Pennsylvania