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New Protein Thwarts HIV Attachment

Researchers have synthesized a protein that jams the "grappling hook" that HIV uses to attach to target cells. The synthetic protein prevents a spring-loaded component of the grappling hook from snapping shut and drawing the virus to its target—one of the key steps in HIV infection. If HIV cannot fuse with the membrane on its target cell, infection cannot occur.

The researchers believe that the new protein may be useful in treating patients with drug-resistant HIV or those patients who experience side effects when taking antiviral medications. Howard Hughes Medical Institute (HHMI) investigator Peter S. Kim and his colleagues at the Whitehead Institute for Biomedical Research at MIT reported on the new anti-HIV protein in a research article published online on January 12, 2001, by the journal *Science*.

Development of the HIV-inhibiting protein, called 5-Helix, is based on earlier studies that showed that HIV uses a spring-loaded mechanism to attach to T cells, its primary targets in the immune system. In attaching to T cells, HIV first uses a protein called gp120 to recognize the CD4 receptor on the surface of a T cell. Once gp120 recognizes CD4, an HIV attachment protein called gp41 launches a harpoon-like component into the T cell's membrane. Next, gp41's spring mechanism snaps shut—forming what is known as the "trimer of hairpins," (named for its triple helical, u-shaped protein structure)—and draws the virus to the T cell like a grappling hook. The virus can then fuse its membrane with the T cell's membrane.

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- Peter Kim

In previous studies, a research team led by Kim and a second that included HHMI investigators Stephen C Harrison and Don Wiley at Harvard independently solved the structure of gp41. Kim's group focused on deep

pockets in the molecule that looked to be excellent targets for inhibitory molecules. Last year, Kim's team identified D-peptide inhibitors of HIV infection that bound solely to the pocket, laying the groundwork for the potential development of "orally bioavailable inhibitors of HIV entry," said Kim. In addition, Harrison and HHMI investigator Stuart L. Schreiber created a library of compounds that fit into the pocket, when attached to a longer peptide, to block the action of gp41.

Other research on gp41 inhibitors has also shown that synthetic peptides could be created to bind to the grappling hook end of gp41 nearest the target cell—called the N-terminal end. In fact, such peptides have proven to be potent inhibitors of HIV, and one is currently in clinical trials.

Kim and his colleagues wanted to test the idea that protein inhibitors that bind to the C-terminal end of gp41—the end nearest the virus—could also be potent inhibitors of HIV infection by stopping the closing of the trimer-of-hairpins. To test this hypothesis, they synthesized 5-Helix, a small protein designed to bind to the C-terminal region, and looked at whether it could jam gp41 in cell culture.

"We really didn't know whether binding to the C-terminal region would actually stop the virus," said Kim. "So, we were pleasantly surprised to find that not only did it inhibit the virus, but it did so quite effectively. What's more, we were very pleased to find that 5-Helix was capable of inhibiting a wide range of HIV isolates."

Analyzing the structure of the C-terminal region of gp41 in many versions of HIV, Kim and his colleagues found that, although there is variability in the region, the "face" of the C-terminal helix that actually interacts with 5-Helix is highly conserved among the different viral strains.

According to Kim, the 5-Helix molecule is stable, and is therefore likely to be resistant to degradation by the body's enzymes. This is one reason why Kim believes that 5-Helix may be a good candidate for becoming an injectable anti-HIV therapy. Also, he said, the molecule can be altered—for instance, making it larger to reduce its elimination in the kidneys—without diminishing its ability to jam gp41. And carbohydrate molecules could also be added to the molecule to help shield it from the immune system, he added.

Kim foresees 5-Helix or its molecular cousins proving to be effective as "salvage therapies" to treat HIV patients who are suffering side effects from other drugs, or in whom HIV has mutated to become drug resistant. The durability of 5-Helix and its effectiveness bode well for rapid progress toward a therapy, he said.

"I think we are only a few steps away from testing in monkeys—for which good models of HIV infection exist—to determine whether 5-Helix or a derivative can reduce the viral load in the bloodstream," Kim said.

Other clinically important viruses also use similar trimer-of-hairpins proteins in infection, and a similar approach to inhibiting attachment might also work against them. "Work from our lab and from Don Wiley's lab has shown that the influenza and Ebola viruses use a similar mechanism, and we have recently shown that the human respiratory syncytial virus also uses the trimer-of-hairpins motif. All of these viruses are significant threats to human health, and we are hopeful that our approach to HIV inhibition can be broadly effective against them as well," said Kim.