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## HIV's Deep Pocket May Reveal Vulnerability

Using three-dimensional images of a key AIDS virus protein as their guide, a team of Howard Hughes Medical Institute (HHMI) investigators has found several chemical compounds capable of preventing the human immunodeficiency virus (HIV) from fusing with human cells. Without the ability to fuse to human cells, HIV cannot cause infection.

"Seeing the structure of this protein, gp41, suggested the whole experimental design," said Stephen Harrison, an HHMI investigator at Children's Hospital in Boston and Harvard University. In 1997, independent research teams led by HHMI investigators Don Wiley, also of Children's Hospital and Harvard University, and Peter Kim at the Whitehead Institute for Biomedical Research determined the three-dimensional structure of gp41 using x-ray crystallography.

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HIV infects immune system cells that bear the CD4 molecule on their surface. A viral glycoprotein - a complex of protein and carbohydrate - called gp120 recognizes and binds to CD4, which triggers a change in the shape of the associated viral glycoprotein, gp41. These subsequent structural changes in gp41 must occur in order for HIV to enter a human cell.

In the October 1999 issue of *Nature Structural Biology*, Harrison, Wiley, fellow HHMI investigator Stuart Schreiber, and members of their laboratories at Harvard University, describe the creation of a library of more than 61,000 chemical compounds that could potentially fit into a deep pocket within the gp41 structure. Harrison and his colleagues screened each of the compounds and identified several that could prevent HIV infection by interfering with gp41.

Rather than develop drugs that interfere with viral replication, which occurs after the virus enters the host cell, the researchers are trying to identify chemical compounds that can stop the virus before it enters host cells. Thus far, most antiviral drugs designed to thwart HIV attack viral enzymes and prevent the virus from reproducing only after HIV has already entered a host

cell. HIV is also adept at developing resistance to these enzyme-attacking drugs, making them effective for only a limited time.

The researchers say that gp41 is an attractive target because the protein does not seem to vary much between strains of HIV. If there is strong evolutionary pressure for the virus to conserve the gp41 protein, Harrison said, then it might be reasonable to presume that it won't be a hotspot for drug resistance.

To attack gp41, the team turned to Wiley's earlier research on the influenza virus, which suggested how gp41 might work. "First, one end of gp41 flips outwards and upwards to bury itself in the membrane of the cell to be infected," Harrison said. "Then gp41, which has its other end anchored in the viral surface, jackknives, folding in the middle and bringing the host cell membrane and the viral membrane together." The resulting juxtaposition of the two membranes leads to their fusion and opens the host cell to the virus.

In searching for compounds that could prevent gp41 from jackknifing and bringing the two membranes together, the researchers first created a "library" of more than 61,000 molecules that seemed likely to interfere with gp41 activity. The starting point for the library was a fragment of gp41 itself, which earlier studies had shown could prevent gp41 folding. While interesting from a research perspective, this fragment of gp41, or peptide, is not a suitable drug candidate because it breaks down too quickly in the blood stream, Harrison said.

Instead, the researchers coupled that small fragment of gp41 to each of the 61,000 small molecules, reasoning that the small fragment might "lead" the rest of the molecule to the ultimate target — the gp41 pocket. Sure enough, the investigators found several compounds capable of binding tightly to gp41 more tightly than the small fragment alone and interfering with the change in gp41's shape that is needed for infection to occur.

The next phase of the research, says Harrison, will be to revise the screening process to narrow the list of candidate compounds. "We feel the screening process wasn't tough enough," he explained.

The scientists also plan to create additional libraries based on the information gained from this study in an attempt to identify compounds lacking the protein fragment entirely. Such compounds would, in theory, be still easier to turn into drugs.

Though this research involved HIV, it could have application to a number of other viruses, including influenza and Ebola. "We do believe this is a pretty general mechanism used by a whole class of viruses," Harrison said.