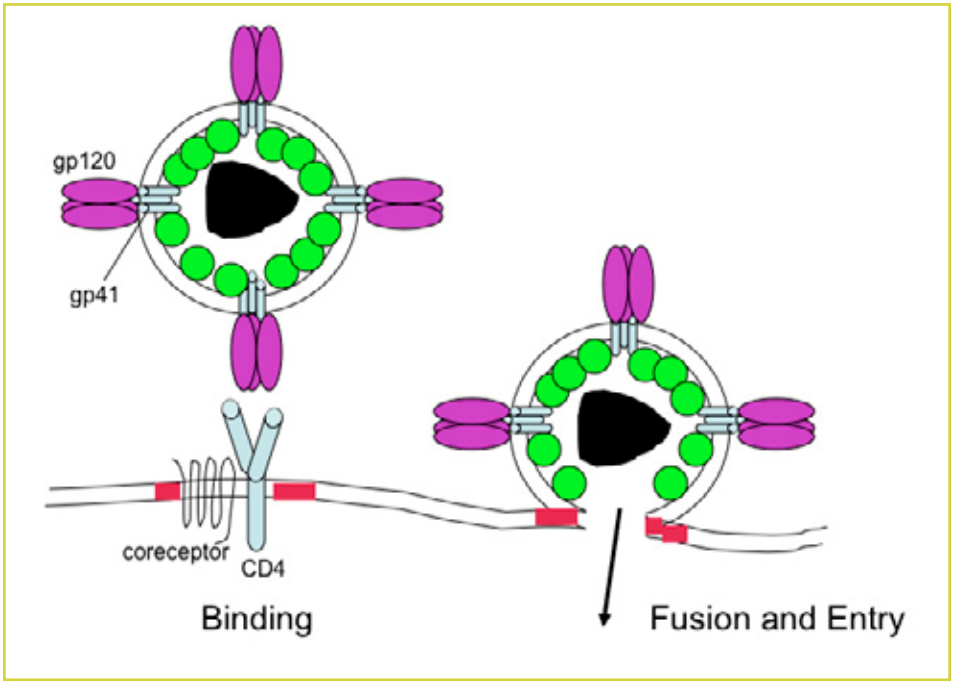
HIV and Drug Resistance:
Hitting a Moving Target

HIV can take many roads to evade the effects of drug therapy. Investigators at CCR and Rutgers University recently identified a novel mechanism by which HIV can circumvent the antiviral activity of a compound called amphotericin B methyl ester (AME), providing new insights into how the virus replicates and evolves into more resistant strains.

Prior research revealed how HIV-1 makes its destructive entry into the target cell by fusing together the cholesterol-rich lipid bilayer of the viral envelope—made with key glycoproteins gp120 and gp41—and the host cell's plasma membrane. Cell-viral interactions begin with the binding of gp120 to the CD4 receptor molecule on the target cell, followed by gp120 binding to coreceptors. These coreceptors likely reside in structures called lipid rafts—areas in the cell plasma membrane that are rich in cholesterol, saturated fatty acids, and certain proteins—that facilitate the entry of viruses into host cells. Finally, sequences in gp41 trigger the fusion of the viral and cellular lipid bilayers. The lipid rafts are then involved in the production of new viral particles.

Drugs that hone in on the close interaction between cell and virus by disrupting lipid rafts would likely slow the virus's spread because they would hinder its ability to enter and leave host cells. AME is such an agent; it acts by binding to cholesterol in the viral membrane, which itself is lipid raft like, potently blocking the virus's entry into immune cells.



Schematic representation of HIV-1 fusion and entry. The infection process begins when the viral surface glycoprotein gp120 binds CD4 and coreceptor on the target cell. Lipid rafts in the target cell membrane are depicted in red. Conformational changes in gp120 and the transmembrane glycoprotein gp41 lead to fusion between the viral and target cell membrane, allowing virus entry. AME likely acts by binding to the viral membrane and blocking fusion between the virus and target cell. Cleavage of the gp41 cytoplasmic tail by the viral protease in AME-resistant mutants reverses the ability of AME to block fusion and entry.

Eric O. Freed, Ph.D., and first author Abdul A. Waheed, Ph.D., both of CCR's HIV Drug Resistance Program, along with other researchers at CCR and Rutgers University, used AME in experimental systems to learn more about how HIV attaches to and infects cells. They found that continual HIV exposure to low levels of AME induced the virus to mutate and become resistant to AME. Normally, the viral transmembrane envelope glycoprotein gp41 bears a long cytoplasmic tail, a domain that directs the protein's incorporation into viral particles during assembly. This glycoprotein plays

a major role in the entry of HIV-1 into target cells.

As reported in the May 15, 2007, issue of the *Proceedings of the National Academy of Sciences U S A*, Freed's team found that HIV-1 became resistant to AME by acquiring mutations in the gene segment encoding the cytoplasmic tail. These changes introduce novel cleavage sites for the viral protease, an enzyme that normally functions to cleave other viral proteins after release of virions from the cell surface. "Because the gp41 cytoplasmic tail is required for incorporation of the envelope glycoprotein into viral particles," Freed said, "in acquiring its resistance to AME, HIV-1 has evolved the ingenious strategy of keeping the cytoplasmic tail intact until gp41 has been incorporated, and then using the viral protease to cleave the tail prior to infection." This might preempt a conformational change associated with AME binding, thus allowing infection to proceed.

Freed and his team identified AME as a useful tool to study the role of lipid rafts in virus replication and the development of resistance to raft-disrupting agents. They also described for the first time activation of HIV-1 envelope glycoprotein function as a consequence of protease-mediated cleavage of the gp41 cytoplasmic tail. These intriguing findings provide a more detailed picture of how HIV travels into and out of cells and demonstrate the diversity of strategies evolved by the virus to develop resistance to antiretroviral compounds.

Reference

Waheed AA, Ablan SD, Roser JD, Sowder RC, Schaffner CP, Chertova E, Freed EO. HIV-1 escape from the entry-inhibiting effects of a cholesterol-binding compound via cleavage of gp41 by the viral protease. *Proc Natl Acad Sci U S A* 104: 8467–71, 2007.