Perspective

Unwelcome Guests With Master Keys: How HIV Enters Cells and How It Can Be Stopped

HIV entry to host cells begins with binding of the viral envelope protein to CD4 molecules on the host cell surface. This binding initiates conformational changes in the envelope protein that result in binding to a coreceptor (CCR5 or CXCR4), exposure of a previously hidden domain in the viral protein, insertion of a viral fusion peptide into the host cell membrane and fusing the viral and cell membranes. Each of these steps provides an opportunity for intervention to prevent viral entry, and a number of agents targeting these steps are in development. Studies of coreceptor inhibitors and fusion inhibitors have indicated the presence of host and viral factors that can result in variability of antiretroviral effect. Improved understanding of these factors will help to guide clinical use of these new agents. This article summarizes a presentation by Robert W. Doms, MD, PhD, at the International AIDS Society–USA course in Chicago in May 2004.

The processes of HIV binding, fusion, and entry to host cells provide targets for the development of antiretroviral drugs. Inhibition of this stage of the viral life cycle would complement approaches targeting other aspects of the life cycle, such as blocking viral replication through inhibition of the viral reverse transcriptase, protease, and integrase enzymes.

There are several steps to HIV entry into target cells. The envelope protein of HIV is a trimer, with each of the components consisting of 2 subunits, gp41 and gp120. The gp120 subunit of the viral envelope binds to the cellular CD4 molecule; this receptor binding induces conformational changes in the viral envelope protein that include exposure of a previously hidden, highly conserved domain that binds to a second receptor (coreceptor). The viral coreceptors, CCR5 and CXCR4, are members of the chemokine subfamily of 7 transmembrane domain receptors. Binding with the CCR5 coreceptor typically predominates in initial infection.

As infection progresses, mutations in the viral envelope enable the virus to utilize the CXCR4 coreceptors instead of or in addition to CCR5. The CXCR4 coreceptors are present on approximately 90% of CD4+ cells, but CCR5 coreceptors are present only on approximately 10%; thus the switch from CCR5 to CXCR4 as a coreceptor permits infection of a much greater number of CD4+ cells and is associated with accelerated HIV disease progression. Coreceptor binding induces conformational changes in the gp41 subunit that result in the insertion of a fusion peptide into the cell membrane and the binding of gp41 helical region 1 and helical region 2, which mechanically draws the viral and cell membranes together and permits membrane fusion.

These steps in viral entry present opportunities for intervention. Figure 1 shows some of the therapeutic agents currently in development for blocking steps in the entry process. These include neutralizing monoclonal antibodies directed against the native trimeric structure of the viral envelope; CD4 binding inhibitors, including BMS-806 (which binds in a cleft of gp120 and thus prevents CD4 binding); CCR5 binding inhibitors and CXCR4 binding inhibitors (eg, AMD3100); and fusion inhibitors (eg, the enfuvirtide derivative, T1249).

Enfuvirtide, a fusion inhibitor, is the only entry inhibitor currently approved by the US Food and Drug Administration for use as an antiretroviral agent. Basically, enfuvirtide mimics the structure of helical region 2 of gp41, which binds with helical region 1. By binding with helical region 1, the drug molecule prevents binding to helical region 2 and thus prevents fusion of the viral and cellular membranes. As discussed further below, much has been learned about entry inhibitor drug development from the development and study of enfuvirtide.

There are a number of challenges inherent to the development and use of viral entry inhibitors. Practical implications to HIV receptor antagonist use include the potential for inhibitors to adversely affect normal cell function. This has been found to be the case for CD4 receptor inhibitors that have been

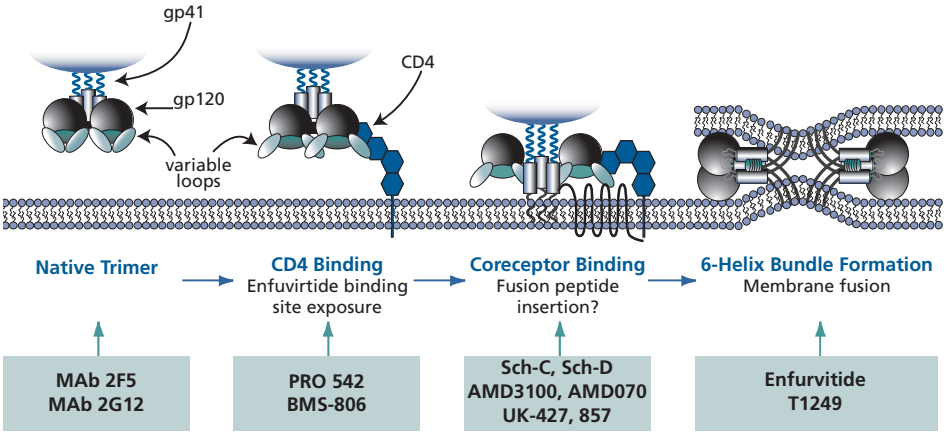

Figure **1.** Steps in HIV entry into target cells and therapeutic agents to inhibit these steps. MAb indicates monoclonal antibody. All of the agents listed are investigational except enfuvirtide. Adapted from Moore and Doms, *Proc Natl Acad Sci U S A*, 2003

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tested, but it appears unlikely to occur with CCR5 inhibitors. That CCR5 is a good drug target is suggested by the fact that individuals who lack CCR5 due to a naturally occurring polymorphism are highly resistant to HIV infection and do not suffer any apparent effects from the loss of CCR5 function. However, one question with regard to coreceptor inhibitors is whether the use of CCR5 inhibitors will select for virus that uses the CXCR4 coreceptor, changing the viral population from the CCR5 phenotype to the CXCR4 phenotype that is associated with more rapid disease progression. Whether this will ever occur cannot be predicted, but it is evident that the use of coreceptor inhibitors will require monitoring of viral load as well as assessment and monitoring of viral phenotype, since, for example, the use of a CCR5 inhibitor in a patient harboring predominantly CXCR4 virus would not be expected to be very effective in reducing viral replication.

There are a number of host and viral factors that may have significant impact on how well entry inhibitors work. Experiments using primary viral isolates from different patients who have never received entry inhibitors have shown that there is a 2- to 3-log (100- to 1000-fold) variation in the amount of entry inhibitor required to block infection in different cell lines. This degree of variability is much greater than the 2- to 4-fold variation in inhibitory concentrations of HIV reverse transcriptase, protease, or integrase inhibitors in similar studies. Although these findings suggest differences in viral factors that affect the activity of entry inhibitors, others indicate a similarly important effect of host factors. In experiments in which a single viral strain is used to infect peripheral blood mononuclear cells or T cells from a number of HIV-uninfected human donors, there is also a 2- to 3-log difference in the inhibitory concentration of entry inhibitors, again compared with the smaller several-fold difference in inhibitory concentrations of reverse transcriptase, protease, or integrase inhibitors. It will thus be important to determine what host and viral factors influence the activity of entry inhibitors and to ascertain how such information might be useful in guiding therapy. It will also be important to determine how HIV might acquire resistance to entry inhibitors and the potential consequences of such resistance for viral replicative fitness (the ability of mutant virus to replicate in the presence of a drug, for example) and susceptibility or resistance to other entry inhibitors. Answers to these questions will help determine how to use these new drugs most effectively.

A number of specific factors that might influence the activity of entry inhibitors have been examined. For example, because enfuvirtide operates within a "kinetic window" that opens with CD4 binding and closes with coreceptor engagement (in the span of approximately 10 minutes under normal conditions), it is likely that faster or more efficient viral fusion would reduce the opportunity for enfuvirtide to bind to its viral target. Also, the level of expression of the CCR5 coreceptor on target cells, which varies fairly widely among individuals, may affect the concentration of enfuvirtide required to inhibit cell infection. An increased number of receptors on the cell surface would allow the virus to locate and engage a receptor more rapidly and thus decrease the activity of enfuvirtide. In studies using peripheral blood mononuclear cells from HIV-uninfected human donors, HIV inhibitory concentrations of enfuvirtide varied by 300-fold (0.004-1.2 µg/mL) and inhibitory concentrations of the CCR5 inhibitor TAK-779 varied by nearly 3000-fold (1.3-3800 nM). That this variability is due at least in part to differences in CCR5 receptor expression was suggested by the finding of a greater than 12.6-fold difference in CCR5 expression on the donor cells as determined by monoclonal antibody binding studies (< 500-6300 binding sites).

Similarly, differences in coreceptor binding affinity among virus strains can affect entry inhibitor activity. Studies in which single amino acid changes were made in the viral protein domain that binds to the CCR5 coreceptor found a number of mutations that affected the strength of binding of the virus to coreceptor to varying degrees but did not affect CD4 binding affinity (Figure 2).

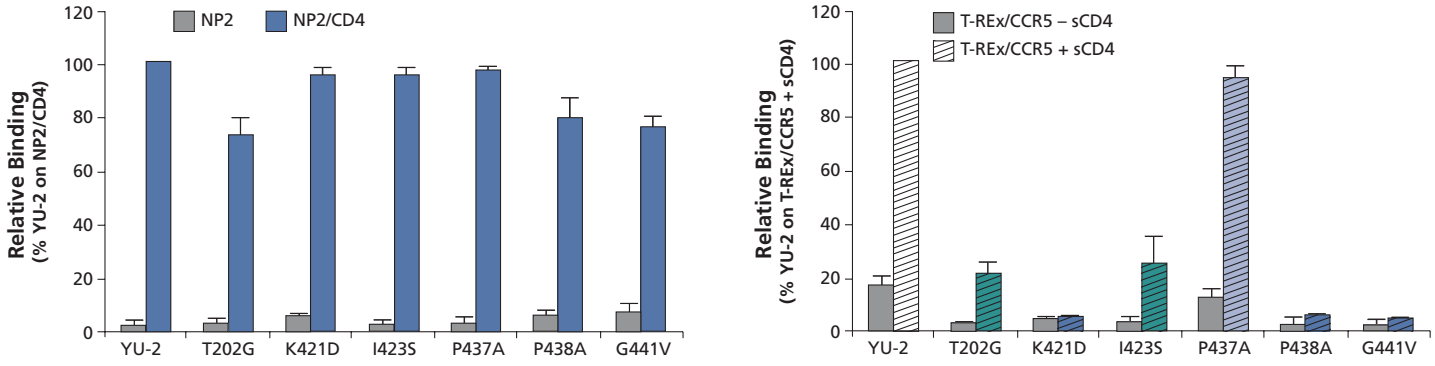

Figure **2**. Effect of single amino acid changes in viral protein domain that binds to CCR5 on CD4 binding (left) and on CCR5 binding (right). In this experiment, single amino acid changes were introduced into the conserved coreceptor binding site of the envelope protein of the R5 HIV-1 strain YU-2. The designation T202G means that the threonine at position 202 has been changed to glycine. The other mutations are similarly designated. The envelope proteins were then tested for their ability to bind to NP2 cells expressing CD4. None of the mutations had significant effect on CD4 binding. The same proteins were then tested for the ability to bind to CCR5. To do this, the proteins were incubated with soluble CD4 (+sCD4) to induce the conformational changes needed for CCR5 binding and then added to cells expressing CCR5 (cell line T-REx/CCR5). Some of the mutations greatly decreased binding to CCR5 (blue bars), some had moderate effects (green bars), and one (light grey bar) had little effect on CCR5 binding. Adapted from Reeves et al, *J Virol*, 2004.

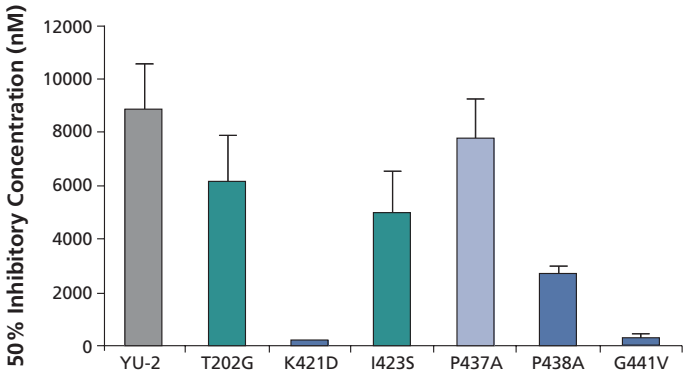

Figure **3**. Relationship between CCR5 binding affinity and CCR5-inhibitor susceptibility. In this experiment, the amount of TAK-779, a CCR5 inhibitor, needed to inhibit virus infection by 50% was determined. The 50% inhibitory concentrations are shown for each virus. Viruses that bound poorly to CCR5 (blue bars) were more easily inhibited by TAK-779 than virus that bound well (light grey bar) and viruses with intermediate binding affinity (green bars). Adapted from Reeves et al, *J Virol*, 2004.

The inhibitory concentrations of CCR5 inhibitors did indeed decrease with reduced viral binding affinity and increase with increased affinity (Figure 3). Similarly, virus with higher CCR5 binding affinity, which thus fused faster, was less susceptible to efuvirtide, and virus with lower affinity was more susceptible (Figure 4).

In summary, HIV entry occurs via initial binding to CD4 receptors and conformational changes that allow coreceptor binding and membrane fusion. There are viral and host factors that have an impact on the rate at which the steps in entry occur. Factors that increase the rate of binding and fusion, such as high viral affinity for coreceptors or high levels of expression of coreceptors, will increase viral resistance to drugs targeting coreceptor binding or fusion. Factors that decrease binding and fusion rates, such as decreased receptor expression or viral binding affinity, will increase viral susceptibility to such drugs.

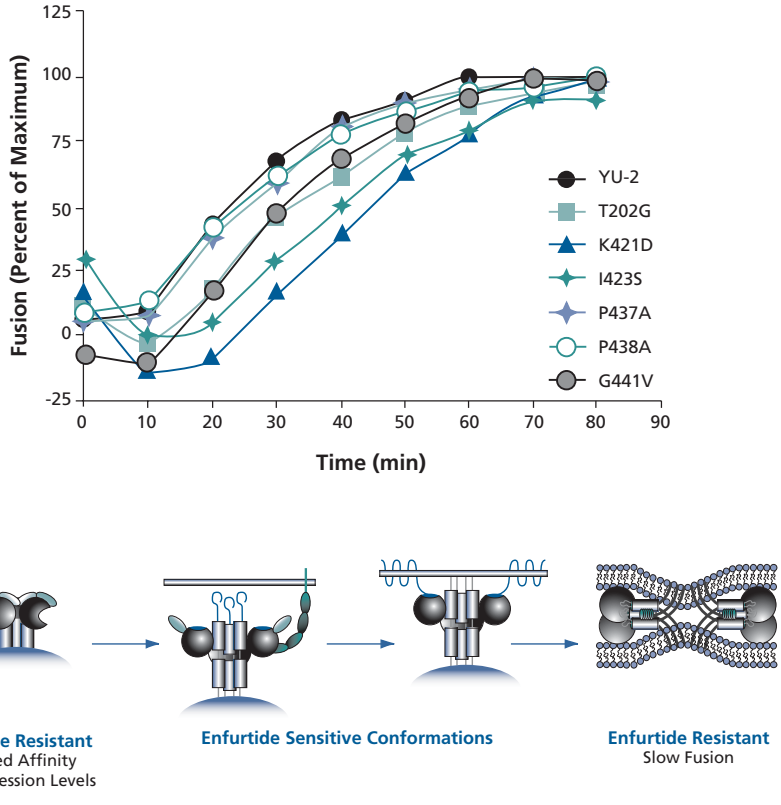

Figure **4**. Correlation between CCR5 binding affinity and fusion kinetics. The rate at which each envelope protein mediated membrane fusion was measured. In general, reduced coreceptor affinity slows fusion kinetics, while high coreceptor affinity results in faster fusion kinetics. Adapted from Reeves et al, *J Virol*, 2004.

These considerations also raise issues regarding the potential combination use of entry inhibitors. For example, since coreceptor inhibitors act to reduce the number of coreceptors available to the virus and thus act to prolong availability of the enfuvirtide binding site by slowing fusion kinetics, the combined use of coreceptor antagonists and enfuvirtide might be expected to have a synergistic effect in inhibiting viral entry. Such an effect has been observed in in vitro studies of CCR5 inhibitors, and clinical development of these latter agents should include studies in combination with enfuvirtide or other candidate fusion inhibitors.

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Suggested Reading

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