

## Perspective

# Viral Binding and Fusion—The Next Targets in Antiretroviral Therapy

*At the International AIDS Society–USA course in New York in March 2002, Eric Hunter, PhD, discussed steps in HIV binding and entry to host cells that present targets for drug development.*

Currently available antiretroviral agents exert their anti-HIV effects at 2 post-entry stages of viral replication. The nucleoside and nonnucleoside reverse transcriptase inhibitors act to block viral DNA synthesis, whereas the protease inhibitors inhibit a late step in the process of viral budding from the host cell. Viral binding and fusion to the host cell is a multistep process that offers a number of potential targets for intervention. Agents that target viral entry to the host cell might complement the effects of those inhibiting post-entry events.

### Characteristics of HIV Binding and Fusion

It has long been recognized that HIV entry into susceptible cells involves the interaction of CD4 molecules on the host cell surface with the HIV envelope glycoproteins gp120 and gp41. More recently, it was found that interaction of the virus with coreceptors on the cell surface is crucial to entry, with the predominant coreceptors being the chemokine receptors CCR5 and CXCR4. In general, viral tropism is determined by which receptors are used by a virus, how well the receptor is used and with what affinity, and the degree of tissue expression of receptors. Late in HIV disease, a shift in viral tropism from the CCR5 to the CXCR4 coreceptor is often observed, probably in association with relatively greater availability of target cells expressing this coreceptor.

Molecular studies have greatly facili-

tated the understanding of the process of HIV entry into host cells. Nonspecific attachment of the virus to the target cell (eg, through interaction of gp120 carbohydrates with cell receptors) permits specific binding of the viral envelope with the CD4 molecule. The binding of CD4 induces a conformational change in both gp120 and gp41, so that the CD4-modified gp120 can bind chemokine coreceptors with a 100- to 1000-fold greater affinity. Coreceptor binding is

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necessary for the completion of conformational changes in the viral glycoproteins (Env) that are initiated by CD4. New antigenic epitopes in gp120 and gp41 are exposed after CD4 binding, consistent with the proteins having undergone an alteration in conformation.

These changes in the viral envelope proteins recapitulate those induced in the influenza virus hemagglutinin (HA) during low pH activation in the endosome. The similarities suggest that the mechanism by which viral-cell membrane fusion occurs is similar for the 2 viruses. In the case of the trimeric influenza virus HA, the HA2 domain, corresponding to the HIV gp41, undergoes a structural rearrangement in the acid pH of the endosome that results in

the formation of a trimeric coiled-coil structure. The formation of this structure enables the fusion peptide to be extended approximately 100 angstroms from its original position and allows its interaction with the target cell membrane. It is believed that a similar process is triggered by CD4 and coreceptor binding in HIV fusion. The extracellular domain of the gp41 contains a fusion peptide region and 2 potentially helical regions, the N-terminal heptad region (HR1) and the C-terminal heptad region (HR2). The HR1 regions of the Env trimer appear to form a trimeric coiled-coil structure similar to that of HA following interaction of the glycoprotein with cell receptors, forcing the fusion peptides into the target cell membrane (Figure 1). The second heptad-repeat regions then bind to the outside of the HR1 coiled-coil structure to form a 6-helix bundle, or hairpin, that mechanically brings the viral and target cell membranes together.

Rational drug discovery is based on an understanding at the molecular level of the process to be inhibited. Since the induction of membrane fusion by HIV is a complex process, there are discrete steps at which interference with the process can be attempted. Since there are several possible targets, there is also potential for the development of drugs with synergistic effects in inhibiting viral entry. The precedence for developing compounds that block viral entry is provided by the observation that naturally occurring neutralizing antibodies can be very effective in inhibiting this process. Three major steps in viral entry have been targeted for drug development: inhibition of CD4 binding, inhibition of coreceptor binding, and blocking of the gp41 conformational changes that permit viral fusion.

### Inhibition of CD4 Binding

The binding of HIV to CD4 is an attractive drug target, both because the CD4

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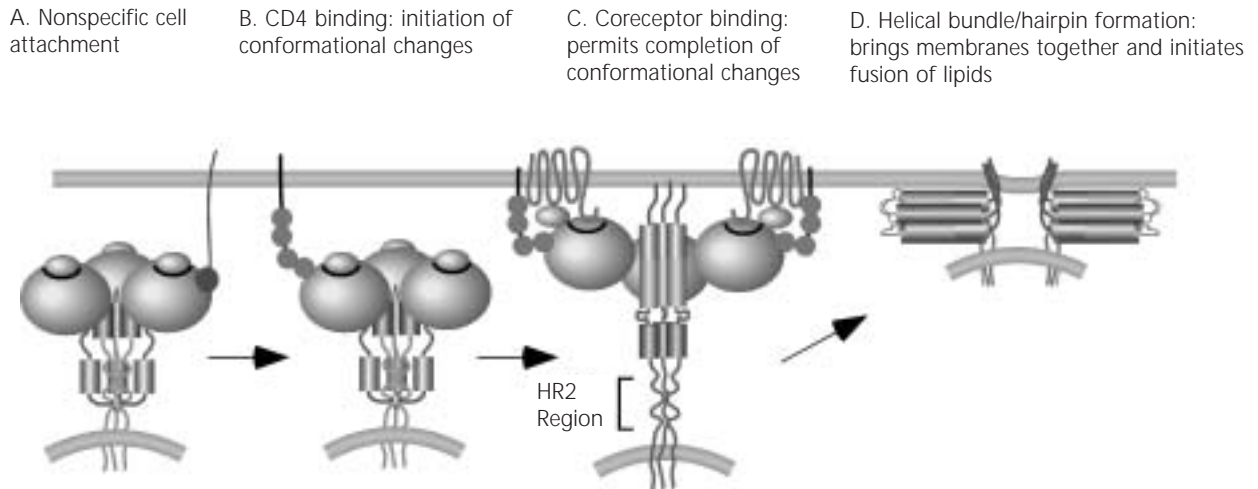


Figure 1. General schema of HIV binding and fusion with target cell. HR2 indicates C-terminal heptad region.

binding site is highly conserved and because it is known that neutralizing antibodies can effectively block this step. The first attempts to block CD4 binding in the clinical setting involved the use of soluble CD4. From 1988 to 1990, several investigators showed that soluble CD4 could block entry of laboratory-adapted strains of HIV into target cells and prevent Env-mediated syncytium formation in vitro. Initial clinical investigations indicated that soluble CD4 was associated with minimal toxicity and dose-dependent reductions in viral load, but only at very high doses (5-10 mg/kg) and in patients in which virus was susceptible to soluble CD4 neutralization (Schacker et al, *J Acquir Immune Defic Syndr Hum Retrovirol*, 1995). This apparent contradiction between laboratory and clinical results was in part explained when it was shown that CD4 binding triggered the very conformational changes in the HIV envelope glycoproteins that induce fusion, and that soluble CD4 could potentiate infection by some primary HIV isolates (Sullivan et al, *J Virol*, 1998).

Ongoing study of this approach has resulted in the development of a novel chimeric molecule consisting of domains of CD4 critical to gp120 binding combined with an immunoglobulin molecule. This tetravalent CD4-IgG, Pro 542, binds to gp120 with nanomolar affinity and neutralizes primary HIV isolates. Pro 542 has been shown to reduce

viral infectivity by 90% in vitro at a concentration of 20 µg/mL and to protect against HIV infection in the hu-PBL-SCID mouse model (Franti et al, 9th CROI, 2002). Initial clinical investigations in HIV-infected adults and children indicate that it is well tolerated and produces modest reductions in plasma HIV-1 RNA levels of approximately 0.5 to 0.7 log<sub>10</sub> copies/mL (Jacobson et al, *J Infect Dis*, 2000; Shearer et al, *J Infect Dis*, 2000). There is hope that this agent may prove useful in combination antiretroviral therapy.

Attempts have been ongoing to develop inhibitors of the CD4 binding pocket that do not induce the same conformational changes in the HIV envelope glycoproteins induced by CD4. Small-molecule inhibitors have recently been described that bind to gp120 and competitively inhibit CD4 binding; these formulations are orally available and appear to be safe in the animal studies conducted thus far (Lin et al, Abstracts 9 and 10, 9th CROI, 2002).

### Inhibition of Coreceptor Binding

Approaches to inhibiting viral entry by interfering with viral binding to the CCR5 and CXCR4 coreceptors include the development of inhibitors that bind the coreceptor and block binding to gp120 through steric or allosteric hindrance or by inducing receptor down-

regulation, and also the development of inhibitors to block the coreceptor binding site on gp120.

Points in favor of the approach of inhibiting the coreceptor (Table 1) include: first, proof of concept, since chemokines and chemokine derivatives that use the CCR5 receptor can effectively block HIV infection via this receptor; second, the considerable experience with receptors of this type (7-transmembrane receptors) in pharmaceutical development; third, the potential to inhibit the receptors without triggering functional activity; and fourth, the fact that individuals homozygous for deletions in the gene for CCR5 ( $\Delta 32$ ) are highly resistant to HIV infection and exhibit no adverse consequences of this deletion, which suggests that inhibition of CCR5 might not be associated with adverse effects on normal cellular function. On the other hand, potential disadvantages (Table 1) include the possibility that coreceptor inhibition will drive the virus to use alternative coreceptors, and the fact that the consequences of blocking chemokine pathways remain unknown (notwithstanding the absence of adverse effects in individuals with CCR5 deletions). With regard to the latter issue, it is known, for example, that CXCR4 is important in vascularization and organogenesis, and it is unclear what effects in this regard might be produced by inhibition of the receptor.

A number of CXCR4 antagonists have

**Table 1.** Selected Pros and Cons of Inhibition of CCR5 and CXCR4 Coreceptor Binding

Pro	Con
<ul style="list-style-type: none"> <li>• Proof of concept</li> <li>• Considerable experience with 7-transmembrane receptors in pharmaceutical development</li> <li>• Potential to inhibit receptors without triggering functional activity</li> <li>• Inhibition of CCR5 may not be associated with adverse effects on cellular function</li> </ul>	<ul style="list-style-type: none"> <li>• Coreceptor inhibition may cause virus to use alternative coreceptors</li> <li>• Consequences of blocking chemokine pathways are not known</li> </ul>

been developed (ALX40-4C, AMD-3100, T22), but none is currently being pursued in the clinical setting. Although one of these compounds, AMD-3100, proved to be a potent inhibitor of CXCR4-using HIV in vitro, an unpublished phase 1a/2b trial of the agent was discontinued as a result of observation of abnormal cardiac activity in 2 patients at higher doses and absence of significant anti-HIV activity at lower doses (Hendrix et al, 9th CROI, 2002). CCR5 inhibitors that have been developed include TAK-779 and the SCH-C and SCH-D compounds; the former is not orally bioavailable and is not being pursued in the clinical setting. The SCH-C compound has shown potent inhibition of CCR5-using virus in vitro and is orally available (Strizki et al, *Proc Natl Acad Sci USA*, 2001). In early-phase clinical testing, some cardiac abnormalities involving prolongation of the QTc interval have been noted at higher doses of 400 to 600 mg per day; a 0.7- $\log_{10}$  reduction in viral load was observed at lower doses of 50 mg per day (Reynes et al, 9th CROI, 2002), and phase 2 trials are proceeding. A second generation of this type of molecule, SCH-D, has already entered clinical evaluation (Chen et al, 9th CROI, 2002).

The approach of targeting the coreceptor binding site on viral gp120 is attractive, since it would involve targeting of a viral component rather than a cellular molecule. However, the coreceptor binding site on gp120 is a broad area consisting of a bridging sheet between 2 gp120 domains, and it is unclear how efficiently this region can be targeted for inhibitor development.

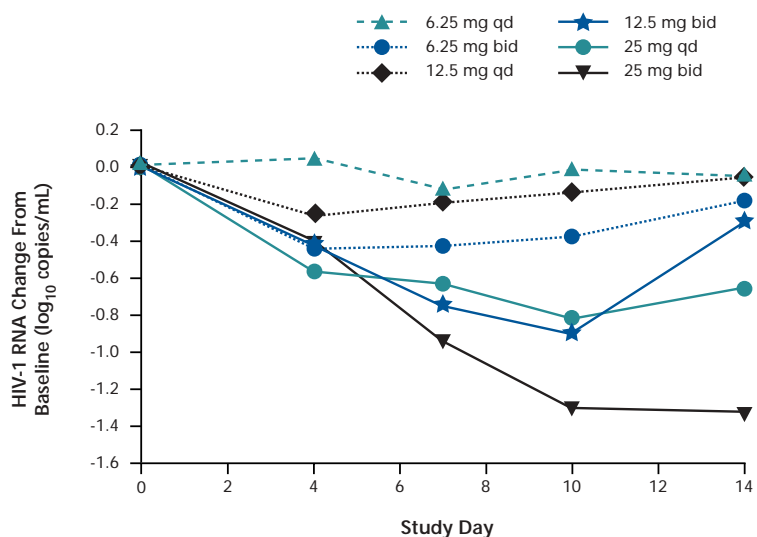
### Inhibition of Conformational Changes in Membrane Fusion

Initial studies on the potential for blocking conformational changes that mediate HIV fusion with susceptible cells showed that mutations in the HR1 region of gp41 resulted in loss of viral fusion and that peptides corresponding to the HR1 and HR2 regions of gp41 (T-21 and enfuvirtide [T-20], respectively) inhibited fusion and infectivity of both cell culture-adapted and primary HIV isolates in vitro. Enfuvirtide was shown to have very low viral inhibitory concentrations in vitro and has undergone extensive clinical investigation. It is

believed that enfuvirtide exerts its effect by competitively inhibiting the HR2 binding site on the gp41 trimeric coiled-coil structure, thereby preventing the folding of the molecule that brings the viral and cell membranes together.

In a phase 1/2 trial, intravenous enfuvirtide produced a 1.5- $\log_{10}$  decrease in plasma HIV-1 RNA level at the highest dose examined (100 mg twice daily) after 15 days of treatment in patients who had undergone a drug washout period (Kilby et al, *Nat Med*, 1998). Subsequent phase 2 studies have included a chronic administration safety study and assessment of an improved formulation that allows twice-daily subcutaneous administration. Pivotal phase 3 studies were initiated in July 2001 and are fully enrolled.

T-1249 represents the second generation of peptide inhibitors; this agent is a synthetic compound utilizing non-native sequences corresponding to the HR2 region. T-1249 has a longer plasma half-life than enfuvirtide and has been shown to inhibit enfuvirtide-resistant virus. The effects of this agent given subcutaneously have been assessed in a 14-day, dose-escalation study in 63 patients with a mean plasma HIV-1 RNA level of 5.3  $\log_{10}$  copies/mL and mean CD4+ cell count of 121/ $\mu$ L. As shown in Figure 2 (Eron et al, 8th CROI, 2001), a dose of 25 mg twice daily produced a mean reduc-



**Figure 2.** Mean changes in plasma HIV-1 RNA level according to dosage of subcutaneously administered T-1249. Adapted with permission from Eron et al, 8th CROI, 2001.

tion in plasma HIV-1 RNA level of 1.3 log<sub>10</sub> copies/mL.

### Influence of Coreceptor Binding on Sensitivity to Peptide Inhibitors

Anecdotal observations from the initial clinical studies of enfuvirtide suggested that a majority of HIV that utilized the CXCR4 coreceptor (X4 viruses) were more susceptible to this agent than HIV that utilized CCR5 (R5 viruses). Testing of a panel of R5, X4/R5, and X4 viruses showed that the enfuvirtide 50% inhibitory concentration for the X4 viruses was 67 ng/mL, compared with 400 ng/mL for R5 viruses. Testing of chimeric viruses (produced by substitutions in the V3 loop of gp120 that switched binding tropism from CXCR4 to CCR5) also showed coreceptor modulation of enfuvirtide sensitivity, with susceptibility being reduced by 4- to 6-fold (Derdeyn, *J Virol*, 2000; Derdeyn, *J Virol*, 2001).

In addition to what they indicate about possible effects on enfuvirtide susceptibility, these findings also suggest differences in the fusion process based on HIV coreceptor affinity. It is known that HIV binds to CXCR4 with lower affinity than to CCR5. It can be hypothesized that HIV with low coreceptor affinity binds to CD4 normally, with the pre-hairpin structure of gp41 (the enfuvirtide target) being triggered normally. However, because gp120 would take longer to engage the coreceptors, formation of the hairpin structure would be delayed, with fusion occurring more slowly; since the enfuvirtide target is longer-lived in this case, the virus would be predicted to be more sensitive to enfuvirtide. In contrast, HIV strains with high coreceptor affinity would quickly engage coreceptors, with fusion occurring more rapidly; in this case, the enfuvirtide target is shorter-lived, and the virus would be less sensitive to enfuvirtide. Preliminary evidence supports this hypothesis (Reeves et al, 9th CROI, 2002).

However, it should be noted that other investigators have not found differences in sensitivity between X4 and R5 viruses, and the clinical significance of such differences is not clear.

### Conclusions

HIV entry to susceptible cells is a multi-step process offering many opportunities for therapeutic intervention. The effects of first- and second-generation peptide inhibitors in blocking conformational changes necessary for viral fusion provide proof of principle that the entry process is a viable target for therapeutic intervention. Inhibitors of CD4 and coreceptor interactions with gp120 have been identified that exhibit potent effects in vitro and are strong candidates for continued clinical development. It is of considerable interest that studies in vitro suggest the potential for synergistic interactions between drugs targeting different steps in viral entry. For example, synergistic effects have been reported for coreceptor inhibitors and enfuvirtide (Tremblay et al, *J Acquir Immune Defic Syndr*, 2000) and for enfuvirtide and Pro 542 (Nagashima et al, *J Infect Dis*, 2001). It is therefore hoped that combination therapies targeting viral entry steps can be developed to maximize viral suppression at this stage of viral replication. Finally, it is likely that continued study of agents designed to inhibit viral entry will provide new insights into the mechanisms of the fusion process itself.

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*Financial Disclosure: Dr Hunter has served as a scientific advisor to Trimeris.*

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## Updated Recommendations for Antiretroviral Therapy

Updated recommendations for antiretroviral therapy in adult HIV-1 infection, written by an International AIDS Society–USA panel of experts, appear in the July 10, 2002 issue of the *Journal of the American Medical Association*.

To access the recommendations online, please visit:

[www.jama.com](http://www.jama.com) or  
[www.iasusa.org](http://www.iasusa.org)