Novel Targets for Antiretroviral Therapy: Pushing the *ENV*elope

Eric Hunter, PhD, discussed prospects for preventing HIV-1 fusion with and entry into target cells. Such prospects are based on the current understanding of the interaction of components of the viral envelope (Env) glycoprotein complex with CD4 and chemokine receptors on the target cell. The following article is a summary of Dr Hunter's presentation.

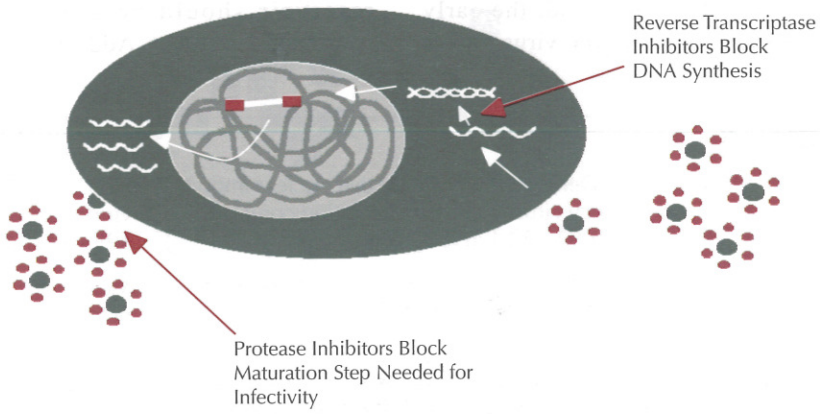

Figure 1. *Actions of currently available antiretroviral drugs in preventing viral replication.*

Currently available antiretroviral drugs act at one of 2 distinct phases of the life cycle of HIV-1 by inhibiting the viral reverse transcriptase or the protease enzymes (Figure 1). The reverse transcriptase inhibitors (RTIs) act to block viral DNA synthesis carried out by the enzyme. Nucleoside RTIs (nRTIs) act as analogues of the nucleosides used in viral DNA synthesis, whereas nonnucleoside RTIs (NNRTIs) act as allosteric inhibitors that modify the structure of the enzyme to render it inactive. The second stage of the life cycle shown to be particularly sensitive to inhibition is a stage in the maturation of the virion when the long precursor proteins that function to assemble virions at the plasma membrane are converted into protein shells that encase the viral genetic information and replicative enzymes. This process is accomplished by proteolytic cleaving of the individual precursor proteins to activated individual protein domains by the HIV-1 protease. Protease inhibitors act to inhibit this enzyme and block the virus from maturing into an infectious form. Both basic types of drugs currently used must thus enter the host cell in order to be functional against virus that has already entered the host cell; this requirement also results in cellular side effects due to cross-reactivity of the compounds with other enzymes within the cell and through other poorly defined mechanisms. The prospect of preventing viral entry is thus inviting as a means of more directly blocking the viral replicative cycle. Increased understanding of the mechanisms of viral entry into host cells has allowed the formulation of a number of approaches by which this end might be achieved.

INTERACTION OF HIV-1 ENV COMPLEX WITH CD4 AND CHEMOKINE RECEPTORS

It is now known that HIV-1 interacts with 2 molecules encoded by the target cell to gain cell entry: the high-affinity receptor CD4, which is essential for both viral entry and cell-cell fusion in syncytium formation, and 1 of a group of 7-transmembrane protein molecules that function as receptors for cellular chemokines. Interaction of the HIV-1 surface envelope glycoprotein with both of these surface molecules appears to be required for the process of fusion of the viral membrane and cell membrane that permits viral entry (Figure 2).

The role of the CD4 molecule in viral entry was recognized many years ago on the basis of the observation that the virus primarily infected cells with significant levels of surface CD4 molecules; it was subsequently shown that monoclonal antibodies to CD4 blocked infection of susceptible cells. After the gene encoding CD4 was isolated, it was further demonstrated that transfection of human cells with the gene rendered the cells susceptible to infection. Initial evidence for a second receptor for viral entry was provided by studies showing that transfection of mouse cells with CD4 genes did not render the cells susceptible to infection. Studies also showed that heterokaryons formed by fusion of the CD4-expressing mouse cells with human

Increased understanding of the mechanisms of viral entry into host cells has allowed the formulation of a number of approaches for preventing entry

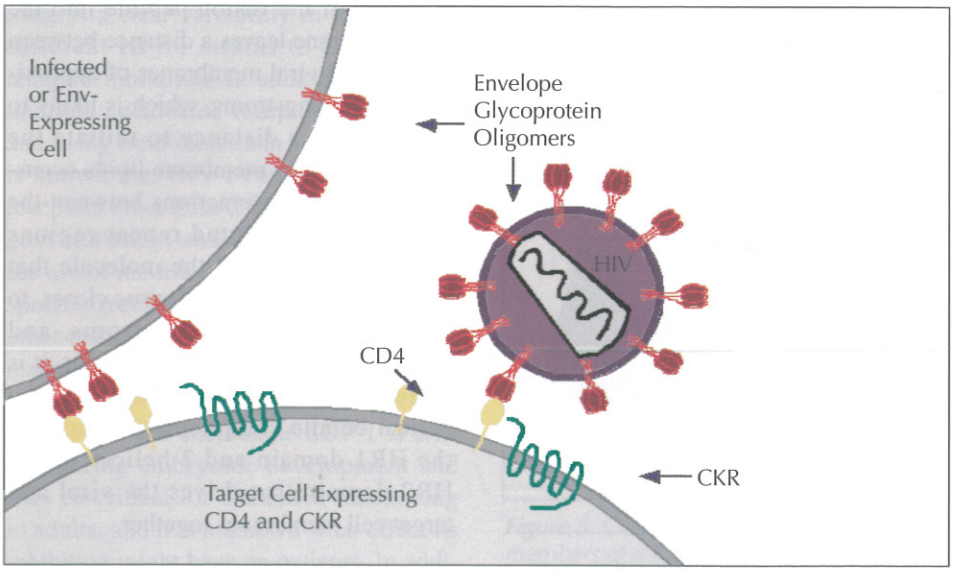

Figure 2. *Interaction of HIV-1 envelope (Env) complex with CD4 receptors and chemokine receptors (CKR).*

cells lacking CD4 were susceptible to viral infection. This finding indicated that a component of the human cells in addition to CD4 was active as a viral receptor. Prior to the identification of this second receptor, it was found that HIV-1 comprised macrophage-tropic and T-cell-tropic variants; although both types of virus efficiently bind CD4, it was found that neither infected the alternative target-cell type in culture.

Investigators at the National Institutes of Health subsequently identified a human gene that in conjunction with CD4 permitted entry of T-cell-tropic virus into mouse cells and did not permit entry of macrophage-tropic virus into the same cell. The gene encoded a previously cloned 7-transmembrane G-protein-binding chemokine-receptor-like molecule that was subsequently identified as the chemokine receptor CXCR4, a receptor that binds the chemokine SDF-1 and is involved in lymphocyte response to antigen stimulation. SDF-1 is a potent chemoattractant for lymphocytes and progenitor cells and appears to be constitutively expressed in many tissues.

The recognition that the chemokines RANTES, MIP-1α, and MIP-1β produced by CD8+ cells could block entry of macrophage-tropic but not T-cell-tropic virus led to the demonstration that the gene encoding the receptor (CCR5) for these chemokines conferred susceptibility to the former but not the latter viral variants. Additional evidence that the CCR5 receptor is involved in macrophage entry came from studies showing that peripheral blood cells with homozygous deletions in CCR5 genes are resistant to infection by macrophage-tropic virus.

ROLE OF GP120 AND GP41 IN VIRUS-CELL FUSION

Evidence has accumulated to show that binding with CD4 induces conformational changes in the glycoprotein components of the HIV-1 envelope complex. The changes permit binding to the coreceptor and viral and cell membrane fusion. Studies employing a monoclonal antibody as a pseudoreceptor believed to bind to HIV-1 at the same site as the extracellular portions of the chemokine receptor have permitted the determination of the 3-dimensional structure of the core of HIV-1 gp120, the viral envelope receptor-binding protein (Figure 3). Determination of the structure of the core as a complex with the CD4 molecule and the monoclonal antibody revealed that CD4 was bound specifically into a cleft in gp120, with it being hypothesized that the binding of CD4 resulted in exposure of the chemokine receptor-binding site. It was also shown that soluble CD4 induces conformational changes in both gp120 and gp41, the 2 components of the HIV-1 envelope

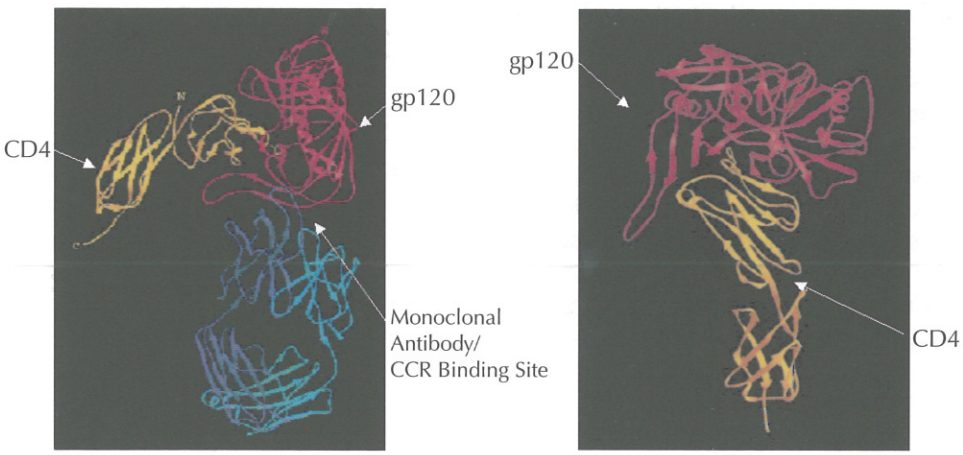

Figure 3. *Left: Overall structure of HIV-1 gp120 (shown in red) determined as a complex with CD4 (yellow) and a monoclonal antibody occupying a site believed to bind the chemokine receptor (light blue indicates light chain; purple, heavy chain). The ribbon diagram lacks many of the variable loop domains. Right: CD4 gp120 interactions. Ribbon diagram shows gp120 (red) binding to CD4 (yellow). Adapted from Kwong PD, et al.* Nature. *1998;393:648–659.*

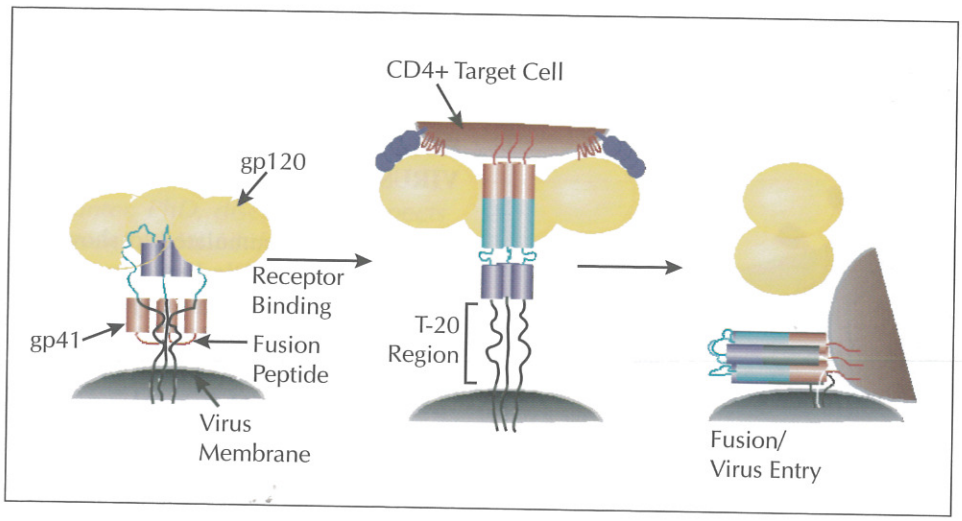

Figure 4. *Model for trimeric HIV-1 Env complex. Left: One gp120 molecule is cut away to show N-terminal heptad repeat constrained in an unstructured form. Middle: Following binding of the receptor complex, the extended-chain heptad repeat region can assemble into a trimeric coiled-coil, thereby allowing the fusion peptide to insert into the target cell membrane. Right: A second step in the conformational change is the formation of the 6-helix, coiled-coil bundle that results in apposition of the viral and target cell membranes. Adapted from Kilby JM, et al.* Nat Med. *1998;4(11):1302–1307.*

glycoprotein complex. As a result of binding with CD4, gp120 binds the chemokine receptor with a 100- to 1000-fold increased affinity, with normally hidden antigenic epitopes in both gp120 and gp41 being exposed.

These findings suggested that the conformational changes in the viral envelope were similar to those observed in the trimeric hemagglutinin molecules on the surface of the influenza virus upon exposure to the acidic environment of the cell endosome. In this case, rearrangement of the hemagglutinin HA2 region results in formation of a helical domain from an unstructured protein chain, with the coiled-coil formation acting as a structured rod to force a hydrophobic peptide, termed the fusion peptide, into the target cell membrane. It is believed that the changes occurring in HIV-1 gp120 and gp41 result in alteration of gp41 structure analogous to that observed with the hemagglutinin HA2 domain. In addition to 3 gp120 molecules, the HIV-1 envelope complex consists of 3 gp41 molecules; the extracellular regions of gp41 consist of an amino terminal hydrophobic peptide and 2 heptad repeat regions (HR1 and HR2).

Receptor binding results in formation of a coiled-coil structure from the unstructured HR1 domain of gp41, similar to that observed in the corresponding influenza hemagglutinin region, that forces the fusion peptide into the host cell while the host CD4 and chemokine receptors remain bound (Figure 4). The insertion of the fusion peptide into the cell membrane leaves a distance between the cell and viral membranes of approximately 100 angstroms, which is likely to be too large a distance to initiate the actual mixing of membrane lipids occurring in fusion. Interactions between the HR1 and HR2 heptad repeat regions result in a folding of the molecule that brings the target cell and virus closer, to within less than 20 angstroms, and allows fusion to occur. In particular, it is believed that it is the formation of a helical bundle comprising 3 helices of the HR1 domain and 3 helices of the HR2 domain that drives the viral and target cell membranes together.

Strategies to block the processes of viral fusion and entry include blockade of CD4-receptor or chemokine-receptor binding and inhibition of gp41-mediated fusion

STRATEGIES FOR BLOCKING FUSION AND ENTRY

Based on current knowledge of the viral fusion and entry processes, a number of strategies to block these processes have been formulated, including blockade of CD4-receptor or chemokine-receptor binding and inhibition of gp41-mediated fusion.

More than a decade ago, it was shown that soluble CD4 molecules could block HIV-1 infection of cells and syncytium formation in vitro, with it being shown that its activity in this regard was synergistic with nRTIs. However, although the use of soluble CD4 was associated with minimal toxicity, clinical investigation revealed an absence of effect on viral replication in vivo. Moreover, it was shown that soluble CD4 did not inactivate uncultured patient isolates of HIV-1, and it has since been demonstrated that it can potentiate entry of infecting strains in vivo.

The prospects for inhibition of chemokine receptors remain uncertain. It is known that chemokines capable of binding these receptors, such as RANTES and the CCR5 macrophage coreceptor, can block entry of virus. Further, the pharmaceutical industry has experience with 7-transmembrane receptors, and it has been shown that variants of RANTES, for example, can compete for the receptor-binding site with gp120 without triggering functional activity of the lymphocyte (ie, without triggering G-protein-coupled stimulation). However,

there is a clear variability in response of different HIV-1 strains to chemokine-receptor inhibition. In addition, there are multiple chemokine receptors (Figure 5), and many bind more than 1 chemokine; it is known that HIV-1 can use chemokine receptors other than CXCR4 and CCR5 to gain cell entry, and it is unclear whether the use of modified chemokines to block a specific receptor might drive virus to use other receptors. Finally, the potential clinical consequences of blocking chemokine pathways are unknown; for example, it is known that the chemokine SDF-1 is critical during embryonic development and may be critical for continued functioning in adults, and it is unknown what effect its inhibition might have on patients. In addition to the RANTES derivatives and other beta chemokines that have been shown to inhibit HIV-1 in vitro, 2 small-molecule inhibitors that block gp120 binding with the CXCR4 receptor for T-cell-tropic virus recently have been described; both ALX40-4C and the bicyclam AMD3100 have been found to be potent inhibitors of HIV-1 replication in vitro. Small-molecule inhibitors of the viral envelope interaction with CCR5 receptors are in preclinical development.

The potential promise of strategies for preventing viral entry by inhibiting gp41-mediated fusion was suggested by initial findings indicating that mutations in the gp41 HR1 region rendered virus noninfectious. Subsequent investigation has shown that peptides corresponding to the HR1 and HR2 regions (DP107 and DP178) can inhibit membrane fusion and virus infectivity if they are present at the time of virus-cell interaction, with a correlation being found between inhibition of viral replication and the ability of the peptides to form helical coiled-coil structures. The DP178 peptide, now known as T-20, is the most potent of these peptides, and has entered early phase clinical study.

It is postulated that T-20 peptide works by blocking the formation of the helical bundle that brings the viral and cellular membranes together. Hence, the peptide would prevent the gp41 HR2 domain from folding into the already formed triple helix coiled-coil structure of the altered HR1 domain. T-20 was shown to be active at a concentration of 1 ng/mL (IC_{50}) and to inhibit infectivity of a variety of primary HIV-1 isolates in in vitro studies and to reduce viral RNA to levels below limits of detection in SCID-Hu mouse models of HIV-1 infection. In an initial dose-escalation trial, T-20 was administered intravenously twice daily at doses of 3, 10, 30, and 100 mg for 15 days, and the effect on viral load was observed. As shown in Figure 6, changes in plasma viral load were dose dependent, with patients receiving the highest dosage of 200 mg/d having viral load below limits of detection by the end of treatment. It is noteworthy that the kinetics of reduction in viral load in the latter patients resemble those observed in patients receiving potent combination antiretroviral therapy. Subsequent early phase studies of subcutaneous administration of T-20, which was shown to result in blood concentrations and trough levels comparable to those observed with intravenous administration, have yielded promising results. Although use of even a relatively small peptide could generate an anti-peptide immunogenic response, anti-

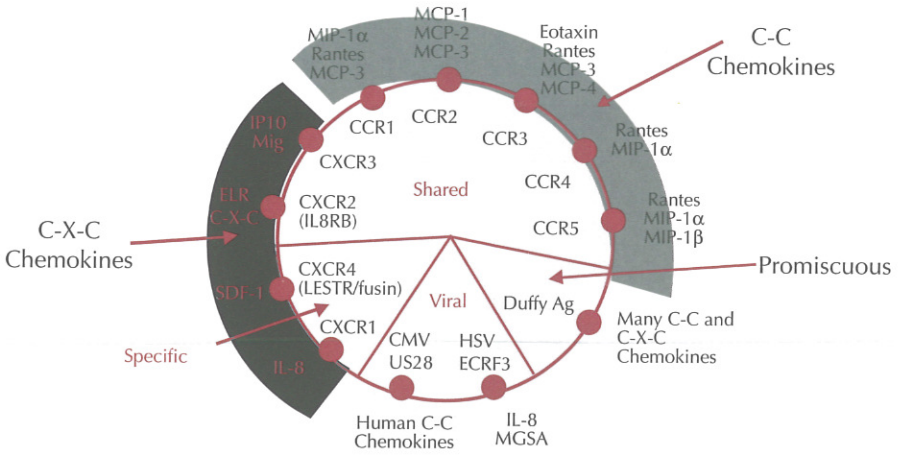

Figure 5. *Classification of chemokines and their receptors. Chemokine receptors are members of a 7-transmembrane G-protein-binding family of proteins. The receptors are broadly classified as C-C and C-X-C receptors based on amino acid structure. Adapted from Premack BA, et al.* Nat Med. *1996; 2(11):1174–1178.*

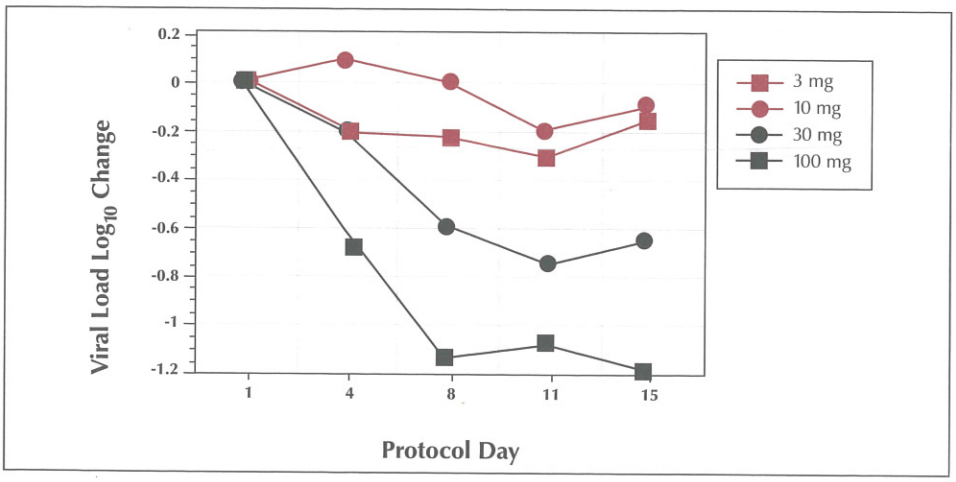

Figure 6. *Median plasma viral load change in patients receiving T-20 3, 10, 30, or 100 mg intravenously twice daily. Adapted from Kilby JM, et al.* Nat Med. *1998;4(11):1302–1307.*

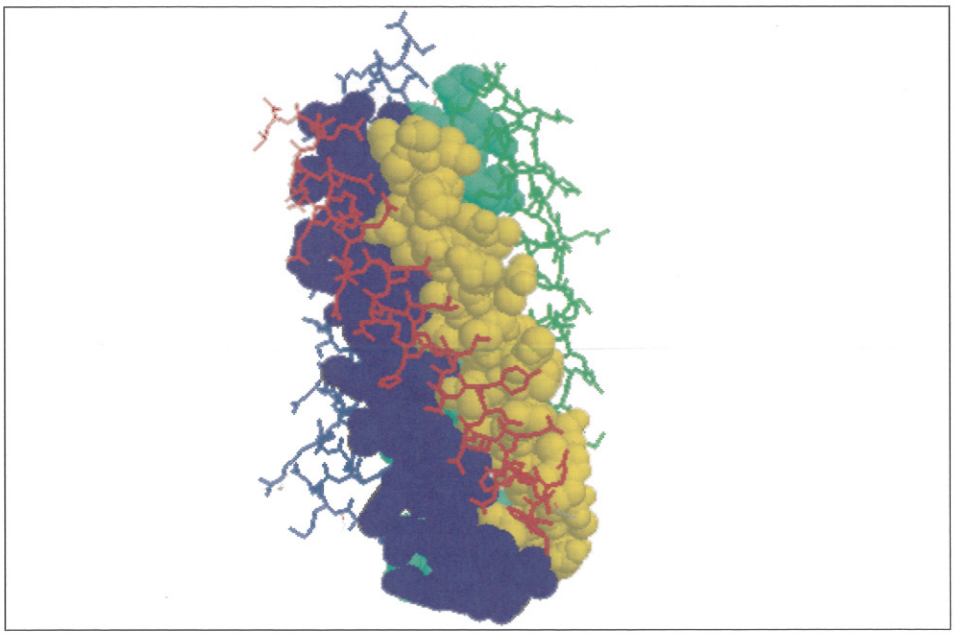

Figure 7. *Three-dimensional structure of the helical bundle formed between the HR1 (N-terminal) and HR2 (C-terminal) domains of gp41 showing HR2 fitting into a binding groove on the HR1 coiled-coil structure.*

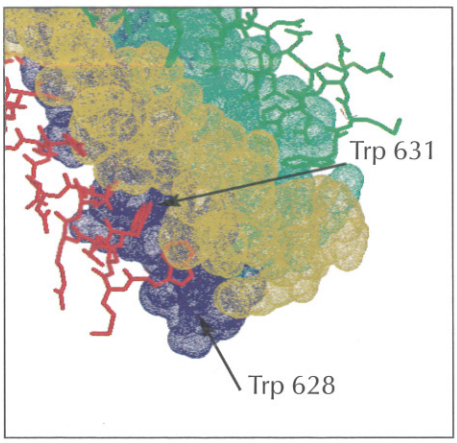

Figure 8. *C-terminal heptad repeat pocket in HR1 structure into which tryptophans (Trp) of the HR2 domain are embedded.*

bodies to T-20 do not appear to inhibit T-20 activity in HIV-1-infected patients or in vitro. Specific antibody responses to T-20 have not been observed thus far in treated patients, including at least 1 patient treated for more than 1 year. Early viral rebound has been observed in some patients in an early phase II study in apparent association with viral resistance to T-20; testing in vitro indicates that mutations can arise in the region of the viral genome encoding the HR1 region of gp41 that forms the groove into which the T-20 molecule fits. These changes presumably prevent contact of T-20 but allow function of the corresponding wild-type glycoprotein sequence. Overall, however, these early findings indicate that fusion inhibition may be a feasible strategy for inhibiting viral replication in vivo and could form part of a divergent treatment strategy.

Given these encouraging findings and the proposed mechanisms of virus-cell fusion, it is plausible that small-molecule inhibitors of fusion might be developed. Analysis of the 3-dimensional structure of the helical bundle formed between the gp41 HR1 and HR2 domains as part of the fusion process indicates that the HR2 peptide fits into a deep binding groove on the surface of the HR1 coiled-coil structure (Figure 7); within this groove is a deep pocket into which the tryptophans of the HR2 domain are embedded (Figure 8). Small molecules that can inhibit the fusion process at this site are now actively being sought.

CONCLUSIONS

HIV-1 entry into host cells requires the presence of both CD4 and a chemokine receptor protein on the host cell. Viral binding with the target cell induces conformational changes in the gp120 and gp41 components of the HIV-1 envelope complex that mediate viral and cell membrane fusion. Receptor binding and the protein-protein interactions involved in the conformational change appear to be suitable targets for therapeutic intervention. The early clinical evaluations of the fusion inhibitor T-20 suggest that targeting interventions at the fusion stage will be a useful strategy. Additional investigation of small-molecule fusion inhibitors is needed.

Eric Hunter, PhD, is Professor of Microbiology and Director of the Center for AIDS Research at The University of Alabama at Birmingham.

Suggested Reading

Kilby JM, Hopkins S, Venetta TM, et al. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat Med.* 1998:4(11):1302–1307.

Kwong PD, Wyatt R, Robinson J, et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature.* 1998;393:648–659.

Premack BA, Schall TJ. Chemokine receptors: Gateways to inflammation and infection. *Nat Med.* 1996;2(11):1174–1178.