

EARLY STEPS IN HIV-1 INFECTION: POSSIBLE TARGETS FOR INTERVENTION

Initial events in the interaction of HIV-1 with target cells and strategies for inhibiting these steps in the infection process were discussed at the Boston course by Joseph G. Sodroski, MD.

Successful entry of HIV-1 into target cells requires membrane fusion mediated by interaction of viral envelope glycoproteins and cell surface receptors. Continued elucidation of the elements of binding and fusion has suggested a number of potential strategies for inhibiting the entry process and thereby preventing productive infection.

MECHANISMS OF HIV-1 ENTRY INTO TARGET CELLS

The exterior of the HIV-1 virion consists of a lipid bilayer membrane in which are embedded trimeric spikes consisting of 3 gp120 molecules surrounding 3 gp41 molecules, with the transmembrane region of the gp41 molecule serving as an anchor in the viral membrane (Figure 1). The interaction between gp120 and gp41 is noncovalent, allowing gp120 to dissociate from the complex under certain conditions. It is currently believed that this event, which inactivates the function of a subset of the viral spikes, allows dissociated gp120 and gp41 to elicit non-neutralizing antibodies, thus serving as an immunologic decoy for the virus. The gp120 glycoprotein has conserved regions and variable (V1-V5) regions that differ among viral strains. The variable regions generally form surface-exposed disulfide-linked loops, whereas the conserved regions are folded into a core composed of an inner domain, an outer domain, and a bridging sheet. The gp41 glycoprotein consists of an exterior domain, the membrane-spanning domain, and an intracytoplasmic tail. The gp120

For a related article on novel targets for antiretroviral therapy, please see the July 1999 issue of *Improving the Management of HIV Disease*.

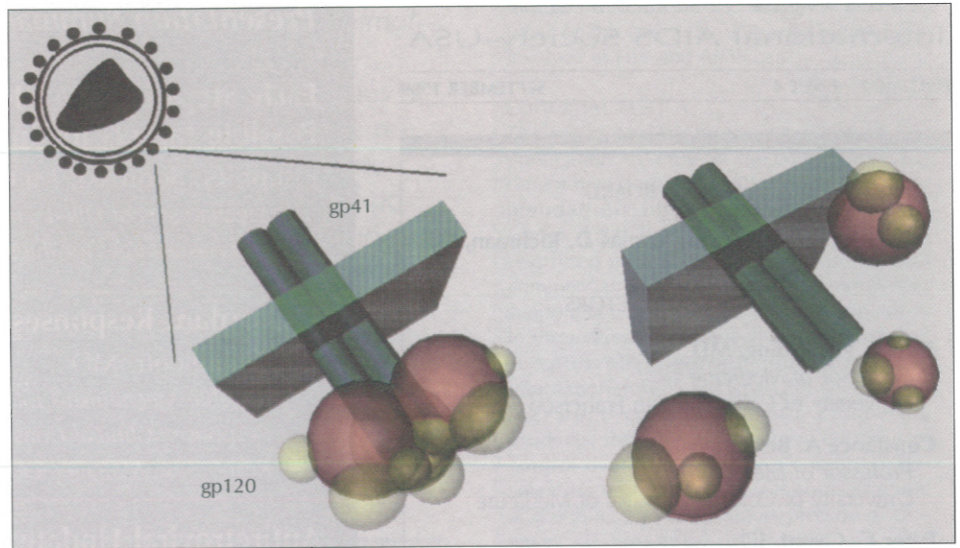


Figure 1. Schematic representation of the HIV-1 envelope glycoproteins gp120 and gp41. Shown at left is the trimeric spike of 3 gp120 molecules consisting of the conserved core region (red balls) and variable loops (yellow bubbles) attached to the transmembrane gp41 (dark green dowels). The complex is anchored in the viral membrane (cross-hatched light green) by the membrane-spanning domain of gp41. On the right is shown the dissociation of gp120 from the complex, an event that appears to serve as an immunologic decoy. Courtesy of JG Sodroski, MD.

surface and the exterior domain of gp41 are modified by the addition of N-linked carbohydrates.

Binding and fusion involve the interaction of viral gp120, gp41, CD4 receptors, and secondary receptors on the target cell. The secondary receptors are members of a class of 7-transmembrane G-coupled proteins that serve as chemokine receptors. The 2 primary HIV-1 coreceptors are CCR5 and CXCR4. Primary monocytes and macrophages express CXCR4 and generally not CCR5, whereas primary T-lymphocytes express both receptors (immortalized T-cell lines used to propagate HIV-1 in tissue culture express only CXCR4). During primary and early infection, CCR5-using virus predominates, with a switch to syncytium-inducing virus that utilizes both coreceptors typically occurring over time. The natural ligands for these chemokine receptors have been shown to inhibit viral entry in vitro by blocking their respective receptors. Natural ligands for CCR5 consist of the β -chemokines

RANTES, MIP-1 α , and MIP-1 β , whereas that for CXCR4 is the α -chemokine stromal cell-derived factor-1 (SDF-1).

Binding of the virion to the target cell is initiated by binding of gp120 to the CD4 receptor; this binding induces conformational changes in gp120 that enable it to bind with high affinity to the coreceptor (Figure 2). X-ray crystallographic studies have enabled the interaction of the viral and cellular components to be visualized. These studies utilized the Fab fragment of a neutralizing antibody that binds to the chemokine-receptor-binding region of gp120, thereby serving as a surrogate chemokine receptor. The gp120-CD4 binding involves a large surface of gp120 spanning the 3 domains of the protein. Figure 3 shows the binding of CD4 in a pocket of gp120, and the 3 domains of the gp120 core; the inner domain interacts with gp41, the outer domain faces outward in the trimeric spike, and the bridging sheet is important for interac-

tion with the chemokine receptor. A feature of the gp120-CD4 interface is an interdomain cavity extending into the interior of gp120 (Figure 4) into which is projected a single phenylalanine 43 (Phe 43) residue of CD4. This component is important for gp120 binding, and the Phe 43 cavity is thus an attractive target for development of drugs for blocking gp120-CD4 binding.

Conformational changes in gp120 upon binding with CD4 allow conserved

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regions of the core and the variable region V3 to come into contact with the chemokine receptor on the target cell surface (Figure 4). The V3 loop determines which cellular coreceptor—CCR5

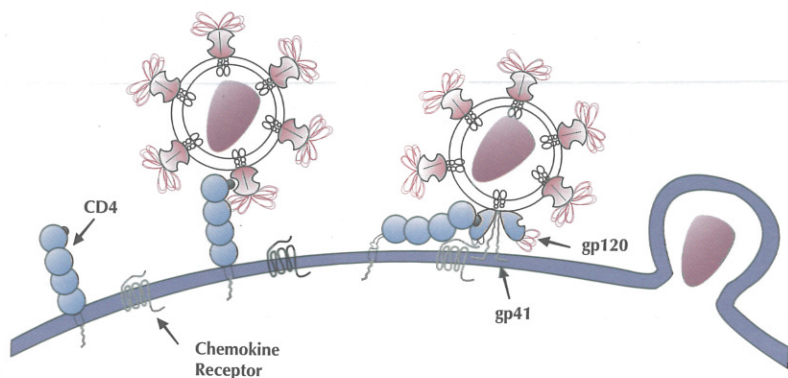


Figure 2. Sequence of binding and entry of the HIV-1 virion into the target cell showing, from left to right, unbound CD4 and chemokine receptors on the target cell surface, binding of gp120 to CD4, binding of gp120 to the chemokine receptor and fusion with the cell membrane via conformational changes in gp120 and gp41, and entry of the virion into the cell. Courtesy of JG Sodroski, MD.

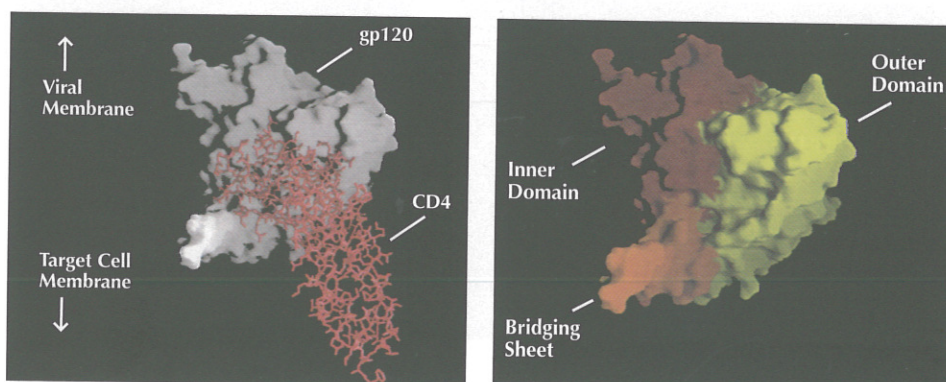


Figure 3. Left: Binding of gp120 and CD4. Right: gp120 in same orientation as at left showing the inner domain, outer domain, and bridging sheet regions of the core. Courtesy of JG Sodroski, MD.

or CXCR4—is to be used. The gp120 region that interacts with chemokine receptors is highly positively charged (ie, basic), which abets interaction with the highly negatively charged (acidic) N-terminal domain of the chemokine receptor. (It has recently been demonstrated that the CCR5 receptor is more highly negatively charged than initially believed, with charge resulting from both the presence of several acidic amino acids as well as post-translational sulfation of several tyrosine residues.)

Binding of gp120 to the chemokine receptor is believed to induce further conformational changes in the viral envelope glycoproteins that enable the gp41 glycoprotein to mediate virus-cell

membrane fusion. Prior to fusion, α helices in the gp41 N-terminus form a coiled-coil structure that initially plays a role in holding the trimer together and in interacting with the gp120 glycoprotein (Figure 5). Binding of gp120 to the chemokine receptor is believed to enable this hydrophobic N-terminus, termed the fusion peptide, to be guided into the cell membrane. The gp41 glycoprotein undergoes a conformational change that results in the interaction of α -C-terminal helices with the N-terminal coiled-coil structure. Since the C-terminal helices are anchored in the viral membrane and the N-terminal helices are embedded in the target cell membrane, this interaction, which may be likened to the springing of a trap, serves to bring the membranes together and permits fusion.

HIV-1 ENTRY AS A TARGET FOR INTERVENTION

Based on current knowledge of the binding and entry processes of HIV-1, a number of events could be targeted by antiretroviral drugs, including CD4 binding, chemokine receptor binding, receptor-induced conformational changes, the gp120-gp41 association, and gp41-mediated fusion. A number of drugs have been developed that target some of these processes, including fusion inhibitors, glycosylation inhibitors, soluble CD4, polyanions, and chemokine receptor antagonists.

Peptides that mimic the C-terminal gp41 peptides have been developed and

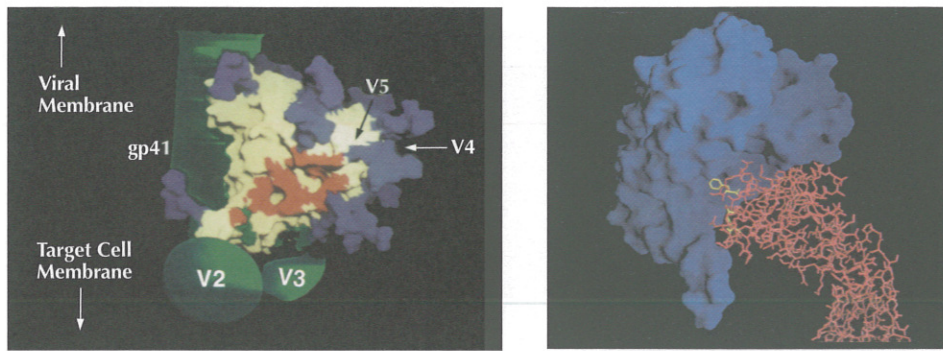


Figure 4. Left: gp120 is shown as the large white area, with the location of the CD4 binding site shown in red. Also shown are the variable loops V2, V3, V4, and V5 and the portions of gp120 that interact with the chemokine receptor (dark green areas at bottom of molecule). The blue structures are sugars marking the surface of gp120 that also appear to protect functional portions of the molecule from immune mechanisms. The gp41 glycoprotein attached to gp120 is shown in dark green at mid- to upper left. Right: Projection of the CD4 Phe 43 residue (yellow) into the cavity on gp120. Courtesy of JG Sodroski, MD.

shown to compete for binding of the α helices to the gp41 N-terminal coiled-coil structure, inhibiting the formation of a fusion-competent conformation. The inhibitor pentafuside (T-20), a large 36-residue peptide, has been evaluated in a Phase I/II study in HIV-1 infected patients. In these patients, who received no antiretroviral treatment for several weeks prior to the study, high intravenous doses of pentafuside resulted in a 99% decrease in plasma HIV-1 RNA level. (Please see accompanying Gulick article, Figure 6, page 16) Although there are drawbacks associated with the compound being a peptide and a large molecule, these findings provide proof of principle that targeting conformational changes and the specific interaction between the C-terminal and N-terminal helices of gp41 can block membrane fusion and viral entry and inhibit viral replication *in vivo*.

The viral envelope glycoproteins are synthesized within the host cell and are extensively glycosylated during the process. Glycosylation inhibitors are directed against the cellular enzymes that modify sugar residues added to the proteins. For the viral glycoproteins gp160, gp120, and gp41, the sugars are initially constructed in elongated form with complex glucose chains at their ends. Under normal conditions, these sugars are

trimmed to achieve appropriate length prior to subsequent processing. Inhibition of cellular glucosidases prevents this trimming, leaving the glycoproteins in elongated form. Inhibitors such as deoxynojirimycin and castanospermine block early steps in the final processing of the proteins (Figure 6) and have been shown to effectively block viral infection of new cells *in vitro*; although viruses produced are still able to bind CD4, they

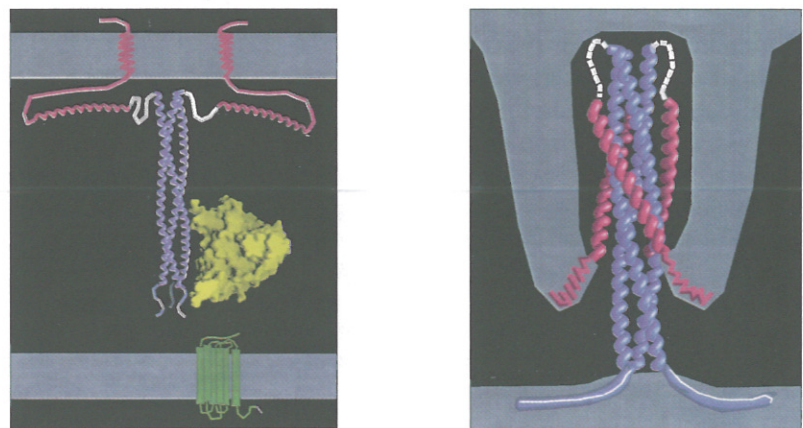


Figure 5. Left: Prefusion state of gp41, showing the coiled-coil structure formed by gp41 N-terminal helices (in blue); C-terminal helices are shown in pink, anchored in the viral membrane. The gp120 molecule is directly above the chemokine receptor (on the cell membrane at bottom). Right: The fusion peptide has been inserted in the cell membrane; the C-terminal helices interact with the coiled-coil structure to bring viral and cell membranes into proximity. Courtesy of JG Sodroski, MD.

are rendered incompetent for subsequent steps in the entry process. Glycosylation inhibitors acting at later steps (eg, swainsonine) have been less effective at inhibition of infection. The primary drawback of these drugs is that by targeting cellular enzymes, they also inhibit processing of normal cellular glycoproteins and are thus associated with toxicity.

Soluble CD4 molecules have been used in the attempt to inhibit viral binding to CD4 receptors on target cells by blocking the gp120-CD4 interaction. Although this approach was actively pursued for some time and appeared to be successful with HIV-1 laboratory strains *in vitro*, enthusiasm for the strategy was dampened by the finding that very high concentrations of soluble CD4 were required to inhibit infection by clinical HIV-1 isolates.

Polyanion compounds bear negative charges that are capable of interfering with the electrostatic interaction of gp120 and chemokine coreceptors, potentially preventing binding with the coreceptors. Most of the polyanion compounds that have been investigated are sulfated compounds. To date, it remains unclear whether the polyanions exhibit sufficient potency or specificity to be effective in a clinical setting, although

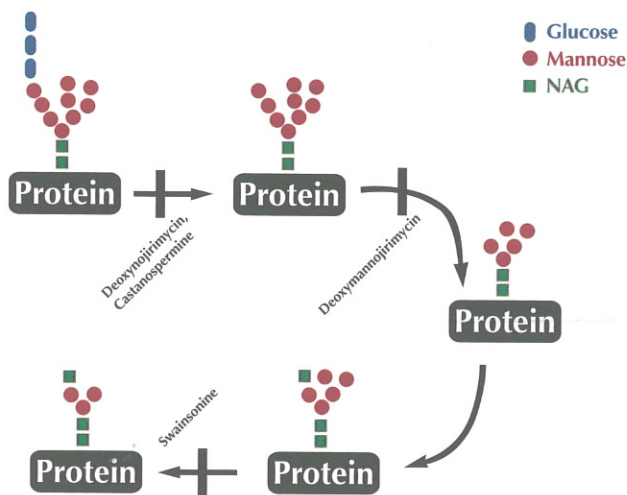


Figure 6. Steps in the final assembly of viral glycoproteins within the host cell at which inhibitors of cellular glucosidases interfere with protein processing. NAG indicates N-acetylglutamate. Courtesy of JG Sodroski, MD.

development of some of these compounds is still being actively pursued.

As noted, the natural ligands for the chemokine receptors used as HIV-1 coreceptors have been found to inhibit infection by blocking receptor binding. AOP-RANTES, a derivative of the natural chemokine, has been shown to inhibit HIV-1 infection *in vitro* by binding the CCR5 coreceptor without signaling through the receptor, but was inactive in a small clinical trial. Other nonchemokine drugs, including AMD 3100, ALX40-4C, and T-22 (an 18-residue polyphemusin analogue), have been reported to block in-

fection by virus that use the CXCR4 receptor. A small-molecule distamycin analogue has been reported to bind both HIV-1 coreceptors and to inhibit infection by viral isolates irrespective of cellular tropism. A particular challenge in the development of such drugs is to identify molecules that do not inhibit the functions of natural ligands. Although humans with CCR5 gene deletions who do not express the protein appear to be healthy and immunocompetent, it is unclear at present how the inhibition of the more ubiquitous CXCR4 coreceptor might affect health. Gene knock-out studies in mice have indi-

cated that such deletions are associated with adverse effects on hematopoiesis and central nervous system function.

CONCLUSIONS

There are a number of conserved features on the assembled envelope glycoprotein complex that appear to be promising as targets for intervention. These glycoproteins, however, have evolved in the *in vivo* milieu of large-molecule neutralizing antibodies. The heavy glycosylation of the surface of these glycoproteins and the masking of the more conserved regions with variable loops appear to be mechanisms that have evolved to protect functional regions of the glycoproteins from the activity of antibodies directed against the proteins. For therapeutic intervention, attempts will thus be focused on producing small-molecule inhibitors capable of binding to specific regions on the envelope glycoprotein complex or interacting with CD4 or coreceptors. These small-molecule inhibitors should be able to evade the viral defenses designed to block large molecules. It is likely that more of the basic research into the viral entry process will come to fruition in clinical trials over the next several years.

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