Cell, Vol. 93, 681–684, May 29, 1998, Copyright ©1998 by Cell Press

HIV Entry and Its Inhibition

Minireview

David C. Chan and Peter S. Kim
Whitehead Institute for Biomedical Research
Howard Hughes Medical Institute
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142

The human immunodeficiency virus type 1 (HIV-1) is an enveloped virus, and its envelope protein complex controls the key process of viral entry. This envelope protein determines viral tropism and facilitates the membrane fusion process that allows invasion of the viral genome. The envelope protein can also promote the fusion of infected cells with uninfected neighboring cells, a phenomenon called syncytium formation that is readily observed in cultured cells and may be responsible for some of the cytopathic effects of advanced HIV infection. Here we review recent insights in HIV envelope protein structure and function and present our current understanding of the entry process. We also review how these findings lead to new approaches for inhibiting HIV entry and may provide insights into the design of better HIV vaccines.

The HIV-1 envelope protein complex is initially produced as the precursor gp160, which is extensively glycosylated and proteolytically cleaved into two subunits by a cellular convertase (Luciw, 1996). The resulting surface subunit (gp120) and transmembrane subunit (gp41) remain noncovalently associated and oligomerize, most likely as trimers, on the surface of the virion. gp120 binds to CD4 and a coreceptor (a seven-transmembrane protein of the chemokine receptor family), which are present on susceptible cells such as T lymphocytes and macrophages. Subsequently, gp41 undergoes conformational changes that mediate fusion of the viral membrane with the target cell membrane. HIV entry is therefore a complex process involving multiple protein interactions, each of which is a potential target for the development of antiviral compounds.

gp41 Structure

The gp41 molecule is a transmembrane protein with several important features within its ectodomain (Figure 1A). First, the amino terminus of gp41, created by proteolytic cleavage of the gp160 precursor, contains a hydrophobic, glycine-rich "fusion" peptide that is essential for membrane fusion. Second, there are two regions with a 4,3 hydrophobic (heptad) repeat, a sequence motif characteristic of coiled coils. Synthetic peptides (see below) derived from these two regions are termed N (amino-terminal) and C (carboxy-terminal) peptides. Between these two heptad repeat regions is a loop region containing two cysteines.

A large number of studies support the notion that the envelope complex exists in at least two major conformations (see references in Chan et al., 1997). The native, or nonfusogenic, conformation exists on the surface of free virions after budding from infected cells. Upon binding of gp120 to target cell receptors, gp41 undergoes a conformational change to a fusion-active state. The details of this conformational change are only beginning to be understood, but at least include exposure of the "fusion" peptide at the amino terminus of gp41. By analogy with the spring-loaded model of influenza virus (Carr and Kim, 1993), this fusion-peptide region is thought to insert into the target membrane at an early step of the fusion process. The observation that the HIV envelope complex readily undergoes receptor-activated conformational change suggests that its native state is metastable, again similar to the pH-activated envelope protein of influenza virus (Carr et al., 1997). That is, the labile native state of the HIV envelope complex is transformed by receptor binding to an energetically more stable, fusion-active conformation.

Protein dissection studies demonstrated that the two 4,3 hydrophobic repeat regions within gp41 form a helical trimer of antiparallel dimers (Lu et al., 1995). Crystallographic analysis confirmed that this gp41 core is a six-helix bundle in which the N and C helices are arranged into three hairpins (Figures 1B and 1C) (Chan et al., 1997; Weissenhorn et al., 1997). The N peptides form three central helices arranged in a trimeric coiled coil. The C peptides form three outer helices that pack in an antiparallel manner into highly conserved, hydrophobic grooves on the surface of this coiled coil (Figure 1D). This structure likely represents the fusion-active conformation of gp41 (see discussion in Chan et al., 1997), and resembles the proposed fusion-active conformations of the transmembrane envelope proteins from influenza virus and Moloney murine leukemia virus.

Fusion Mechanism

Any model for HIV entry must account for the remarkable observation that synthetic C peptides inhibit HIV infection and syncytia formation at nanomolar concentrations (Jiang et al., 1993; Wild et al., 1994). Preincubation of virus with C peptide, followed by its removal, does not block HIV entry; this feature suggests that these peptides do not act on the native conformation of gp41. Based on the structural features of the fusion-active gp41 core, these peptide inhibitors likely work by binding to the trimeric coiled coil of gp41, thereby acting through a dominant-negative mechanism (Lu et al., 1995; Chan et al., 1997; Weissenhorn et al., 1997).

A simple dominant-negative model, however, fails to explain adequately one puzzling feature of C peptide inhibition: How can C peptides act at such low concentrations, given that the N and C peptide regions are within a single gp41 molecule? That is, how can the C peptides so effectively inhibit an intramolecular association reaction, in which the two regions of gp41 are present in a high effective concentration? It seems likely that the solution to this puzzle is that C peptides must bind to gp41 *prior* to formation of the six-helix complex because, once this gp41 core is assembled, it is extremely stable (the melting temperature of the gp41 core is in excess of 90°C) and is unlikely to be disrupted by exogenous peptides. These observations suggest the existence of a transiently populated, third state of gp41. C peptides act at this intermediate stage, after the native

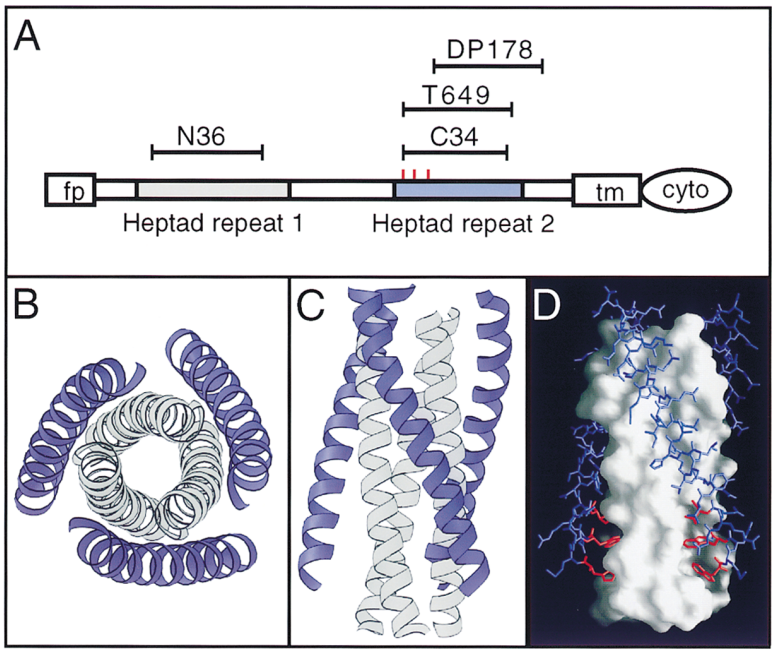

Figure 1. HIV gp41 Structure

(A) A schematic view of gp41, showing the location of the fusion peptide (fp), the two hydrophobic heptad repeats, the transmembrane segment (tm), and the cytoplasmic region (cyto). The location of various gp41 peptides are shown above. The locations of the three residues that bind the cavity in the central coiled coil (D) are indicated by red bars. (B and C) A ribbon diagram of the N36/C34 complex (which forms the core of the gp41 ectodomain) viewed looking down the 3-fold axis (B) and from the side (C). The N36 helices (gray) form a central, trimeric coiled coil, and three antiparallel C34 helices (blue) wrap obliquely around this coiled coil. (D) Representation of the N36 coiled coil as a molecular surface (gray), with the three C34 helices (blue and red) depicted as rods. The C34 helices pack against a conserved groove on the surface of the coiled coil. The bottom of the coiled coil has a large cavity that provides a binding pocket for three C34 residues (red).

state has undergone a conformational transition but before formation of the hairpin structure. In this "prehairpin" intermediate, the N and C peptide regions are not associated, allowing synthetic C peptides to bind to the N peptide region of gp41 and inhibit transition to the fusion-active conformation (Figure 2).

The prehairpin intermediate appears to be induced rapidly upon interaction of gp120 with cellular receptors and is then relatively stable, with a lifetime of many minutes. Video microscopy and the fluorescent dye bis-ANS have been used to monitor the kinetics of receptor-activated conformational changes (Jones et al., 1998). These studies indicate that conformational changes are initiated within 1–4 min of receptor binding and are complete within 20 min, well before any evidence of lipid mixing. Significantly, C peptides retain much of their

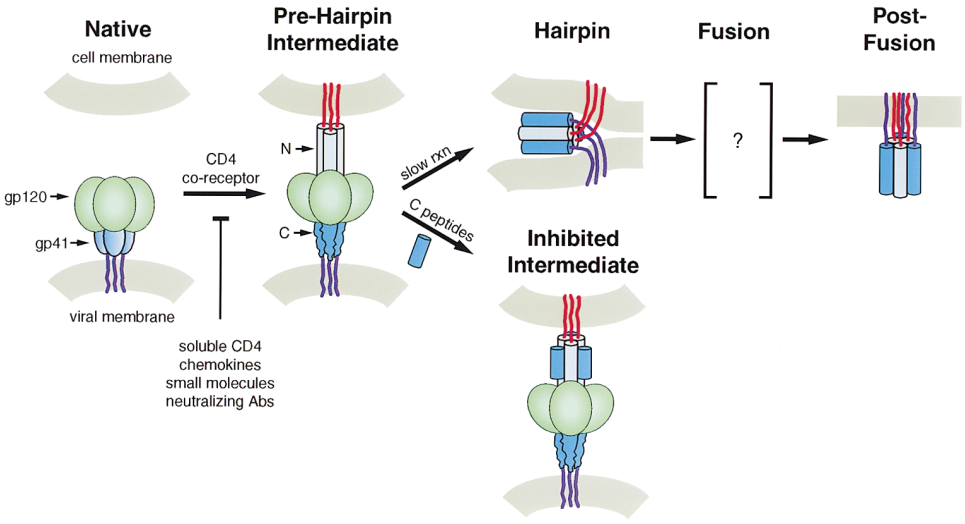

Figure 2. Model of HIV Membrane Fusion

In the native state of the trimeric gp120 (green)/gp41 (blue/gray shading) complex, the fusion peptide (not shown) is buried. Upon interaction of gp120 with cellular receptors, the envelope complex undergoes a conformational change to the prehairpin intermediate, in which the fusion peptide (red lines) is inserted into the target membrane and the N peptide region (gray) is a trimeric coiled coil. The C peptide region (blue) has not yet associated with the N peptide coiled coil because of a kinetic block due to association with either another portion of gp41, or more likely, gp120. This intermediate is relatively long-lived (many minutes) and is vulnerable to C peptide inhibition (bottom). The prehairpin intermediate resolves to the fusion-active hairpin structure when the C peptide region binds to the N peptide coiled coil and adopts a helical conformation. This rearrangement results in membrane apposition. The interactions necessary for fusion are unknown (as indicated by "?"), but may involve aggregation of gp41 trimers to form fusion pores. After fusion is completed, the fusion peptide and the transmembrane segment (purple lines) of gp41 lie within the same membrane. The steps at which various HIV entry inhibitors act are shown.

inhibitory activity against membrane fusion when added *after* these rapid conformational changes (Munoz-Barroso et al., 1998).

Taken together, these observations suggest the following model for gp41-mediated membrane fusion (Figure 2) (for related models, see Hughson, 1997; Weissenhorn et al., 1997; Furuta et al., 1998). In free virions, gp41 exists in a native, nonfusogenic conformation in which the fusion peptides are buried within the envelope complex. This gp41 structure is stabilized by its interactions with gp120. When gp120 binds to cell-surface CD4 and a chemokine receptor, a conformational change occurs in gp120 that alters gp120–gp41 interactions. This perturbation of the gp120–gp41 interaction triggers gp41 to undergo its transition to the prehairpin intermediate. These changes in gp41 include exposure of the fusion peptide and its insertion into the target membrane. We imagine that the prehairpin intermediate penetrates the target membrane with its fusion peptide, which lies on top of the N peptide coiled coil. The C peptide region is not associated with this coiled coil, perhaps because it is constrained by an interaction with another region of gp41, or more likely, gp120. This prehairpin intermediate represents the vulnerable target of C peptide inhibition. Consistent with this view, the C peptide DP178 binds to gp41 only after interaction of the envelope complex with cellular receptors (Furuta et al., 1998).

The resolution of this intermediate to the six-helix complex may be slow because the constraints holding the C peptide region are slow to dissociate. Moreover, there are topological constraints that prevent a simple, symmetric transition from the prehairpin intermediate to the six-helix structure. Once these constraints are overcome, association of the C peptide region with the N peptide coiled coil leads to formation of the fusion-active hairpin structure and the local apposition of viral and cellular membranes. How membrane apposition leads to complete fusion is unclear but may involve clustering of envelope protein trimers to form fusion pores. After fusion is complete, the fusion peptide and the transmembrane segment of gp41 lie in the same membrane (Figure 2). Recent work suggests that, in a manner analogous to viral entry, fusion between cellular membranes mediated by t-SNARE and v-SNARE protein complexes also involves helical structures to appose two membranes for fusion (Weber et al., 1998).

gp41-Derived Peptides Are Potent Fusion Inhibitors

In the gp41 core, each of the outer C helices binds along a conserved groove on the surface of the central coiled coil. The broad inhibitory activity of C peptides against diverse HIV isolates is explained by the highly conserved nature of the hydrophobic groove to which these peptides bind. Toward the bottom of the groove is a deep cavity that is filled by three hydrophobic residues from the C helix (Figure 1D). This pocket is potentially a good target for inhibiting HIV invasion (Chan et al., 1997), although peptides lacking the cavity-occupying residues (e.g., DP-178; see Figure 1A) can still be potent inhibitors. As may be expected, mutant viruses that escape DP178 inhibition bear mutations in the N peptide region of gp41 (Rimsky et al., 1998). Interestingly, it is much more difficult to select for viruses resistant to T649 (Figure 1A), a C peptide that contains the cavity binding region (Rimsky et al., 1998). Steps toward the development of more potent versions of C peptides have begun (Judice et al., 1997).

Targeting Cellular Receptors

Early work targeting cellular receptors focused on using soluble versions of CD4 as HIV entry inhibitors. This approach, while effective against laboratory-adapted HIV strains in vitro, has proved ineffective on primary HIV isolates. The identification of the chemokine receptors as essential cofactors has led to new targets for inhibition of viral entry. The chemokines themselves, or variants thereof, form a potent class of inhibitors (reviewed in Littman, 1998). HIV strains that use the CCR5 coreceptors are blocked by the CCR5 ligands RANTES, MIP-1α, and MIP-1β; likewise, strains that use the CXCR4 coreceptor are blocked by the CXCR4 ligand SDF (stromal-derived factor). These factors appear to work through two mechanisms. First, they competitively block interaction of gp120 with the chemokine receptors. Second, they down-regulate chemokine receptor expression in the target cells, thereby limiting the number of HIV entry points.

Progress has already been made in identifying small-molecule inhibitors of the chemokine receptors (see references in Littman, 1998). Two such compounds, ALX40-4C and T22, are short, positively charged peptides. A third, called AMD3100, is a bicyclam compound. All three of these compounds inhibit entry of HIV strains that utilize CXCR4 as a coreceptor and likely work by binding directly to CXCR4, thereby blocking its interaction with gp120.

Eliciting Neutralizing Antibodies

The need for an HIV vaccine remains urgent, and many past efforts have focused on eliciting a neutralizing antibody response against the envelope complex (reviewed in Burton, 1997; Haynes, 1996). Passive transfer of high levels of neutralizing monoclonal antibodies has been shown in animal models to protect against a virus challenge. The ability of live-attenuated SIV vaccines to protect macaques from a challenge virus in the absence of a strong neutralizing antibody response suggests that other mechanisms, including cell-mediated immunity, are capable of providing protection. The importance of generating T cell responses to HIV is well recognized, but we restrict our discussion to approaches for eliciting neutralizing antibodies to HIV.

Human vaccine trials using monomeric gp120 as an immunogen have yielded disappointing results (VanCott et al., 1995; Haynes, 1996; Bolognesi and Matthews, 1998; Connor et al., 1998). Individuals immunized with monomeric gp120 show low levels of neutralizing antibodies that are effective only against the HIV strains to which they were immunized, and not to primary isolates. Preparations of gp120 used in early vaccination trials were derived from laboratory-adapted HIV strains, which are now known to be substantially more sensitive to neutralization than primary clinical isolates (Mascola et al., 1996). Perhaps most telling, antibody responses in gp120-vaccinated individuals are often directed against linear epitopes exposed on denatured gp120 but not correctly folded gp120 (VanCott et al., 1995). In marked

contrast, the majority of anti-envelope neutralizing antibodies in the sera of HIV-infected individuals recognize conformation-specific, discontinuous epitopes (VanCott et al., 1995).

It is questionable whether even natural HIV infection elicits a sufficient neutralizing antibody response, since passive transfer of pooled Ig from seropositive donors fails to lower viral titers in infected humans. Even in cases where vaccinees show reactivity to folded, monomeric gp120 (Connor et al., 1998), there is as yet no direct evidence for binding to the oligomeric envelope protein on the surface of clinical HIV isolates. Therefore, the challenge at hand for vaccine developers is formidable: to elicit an antibody response that is more effective than that obtained through a natural HIV infection.

Unfortunately, broad-spectrum neutralization epitopes on the envelope complex appear to be rare and poorly immunogenic, perhaps because they are efficiently masked by numerous carbohydrates. Only three human monoclonal antibodies capable of efficient, broad-spectrum neutralization of HIV infectivity have been isolated. Two of these monoclonal antibodies (b12 and 2G12) bind to distinct epitopes on gp120, whereas the third (2F5) recognizes a region of gp41 close to the transmembrane region. These epitopes are poorly immunogenic, since monoclonal antibodies to them have been isolated only once from individual donors. Clearly, the envelope proteins of HIV have evolved to evade the human immune system. Indeed, most of the humoral response to a natural HIV infection appears to be directed against forms of the envelope protein that are not present on the native virus; the reasons for this are unknown, but it has been suggested that viral debris is a dominant immunogen that may serve as a decoy (Burton, 1997).

Looking ahead, there is a clear need to develop envelope protein preparations, based on primary isolates, that have a correctly folded conformation and oligomerization state. Since the unprocessed envelope precursor is different from the mature protein, these preparations should be processed to gp120/gp41. However, this maturation-cleavage step presents another problem: gp120 is readily "shed" from gp41. Shedding was also a problem with early whole-killed (inactivated) HIV preparations, and methods for cross-linking envelope protein subunits may prove crucial. Similar considerations apply to pseudotyped-virus preparations, such as the canarypox virus vaccine candidate (see Bolognesi and Matthews, 1998).

Finally, it remains possible that, even with optimized envelope protein preparations, these vaccines will generate only strain-restricted responses because broadly neutralizing epitopes are rare and poorly immunogenic. In this case, it may be necessary to resort to cocktails of envelope proteins from selected primary isolates. It seems almost certain, however, that the challenge of eliciting broadly neutralizing antibodies to HIV will require close attention to issues of protein structure and conformation in vaccine candidates.

Selected Reading

Bolognesi, D.P., and Matthews, T.J. (1998). Nature *391*, 638–639.

Burton, D.R. (1997). Proc. Natl. Acad. Sci. USA *94*, 10018–10023.

Carr, C.M., and Kim, P.S. (1993). Cell *73*, 823–832.

Carr, C.M., Chaudhry, C., and Kim, P.S. (1997). Proc. Natl. Acad. Sci. USA *94*, 14306–14313.

Chan, D.C., Fass, D., Berger, J.M., and Kim, P.S. (1997). Cell *89*, 263–273.

Connor, R.I., Korber, B.T.M., Graham, B.S., Hahn, B.H., Ho, D.D., Walker, B.D., Neumann, A.U., Vermund, S.H., Mestecky, J., Jackson, S., et al. (1998). J. Virol. *72*, 1552–1576.

Furuta, R.A., Wild, C.T., Weng Y., and Weiss, C.D. (1998). Nat. Struct. Biol. *5*, 276–279.

Haynes, B.F. (1996). Lancet *348*, 933–937.

Hughson, F.M. (1997). Curr. Biol. *7*, R565–R569.

Jiang, S., Lin, K., Strick, N., and Neurath, A.R. (1993). Nature *365*, 113.

Jones, P.L., Korte, T., and Blumenthal, R. (1998). J. Biol. Chem. *273*, 404–409.

Judice, J.K., Tom, J.Y., Huang, W., Wrin, T., Vennari, J., Petropoulos, C.J., and McDowell, R.S. (1997). Proc. Natl. Acad. Sci. USA *94*, 13426–13430.

Littman, D.R. (1998). Cell *93*, this issue, 677–680.

Lu, M., Blacklow, S.C., and Kim, P.S. (1995). Nat. Struct. Biol. *2*, 1075–1082.

Luciw, P.A. (1996). In Fields Virology (Philadelphia: Lippincott-Raven Publishers), pp. 1881–1952.

Mascola, J.R., Snyder, S.W., Weislow, O.S., Belay, S. M., Belshe, R.B., Schwartz, D.H., Clements, M.L., Dolin, R., Graham, B.S., Gorse, G.J., et al. (1996). J. Infect. Dis. *173*, 340–348.

Munoz-Barroso, I., Durell, S., Sakaguchi, K., Appella, E., and Blumenthal, R. (1998). J. Cell Biol. *140*, 315–323.

Rimsky, L.T., Shugars, D.C., and Matthews, T.J. (1998). J. Virol. *72*, 986–993.

VanCott, T.C., Bethke, F.R., Burke, D.S., Redfield, R. R., and Birx, D.L. (1995). J. Immunol. *155*, 4100–4110.

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H., and Rothman, J.E. (1998). Cell *92*, 759–772.

Weissenhorn, W., Dessen, A., Harrison, S.C., Skehel, J.J., and Wiley, D.C. (1997). Nature *387*, 426–430.

Wild, C.T., Shugars, D.C., Greenwell, T.K., McDanal, C.B., and Matthews, T.J. (1994). Proc. Natl. Acad. Sci. USA *91*, 9770–9774.