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The HIV-1 Envelope Glycoproteins: Fusogens, Antigens, and Immunogens

Richard Wyatt and Joseph Sodroski*

The human immunodeficiency virus–type 1 (HIV-1) envelope glycoproteins interact with receptors on the target cell and mediate virus entry by fusing the viral and cell membranes. The structure of the envelope glycoproteins has evolved to fulfill these functions while evading the neutralizing antibody response. An understanding of the viral strategies for immune evasion should guide attempts to improve the immunogenicity of the HIV-1 envelope glycoproteins and, ultimately, aid in HIV-1 vaccine development.

The human immunodeficiency viruses (HIV-1 and HIV-2) and simian immunodeficiency viruses (SIVs) are the etiologic agents of acquired immunodeficiency syndrome (AIDS) in their respective human and simian hosts (*1*). Typically, infection with primate immunodeficiency viruses is characterized by an initial phase of high-level viremia, followed by a long period of persistent virus replication at a lower level (*2*). Viral persistence occurs despite specific antiviral immune responses, which include the generation of neutralizing antibodies.

The primate immunodeficiency viruses, like all retroviruses, are surrounded by an envelope consisting of a host cell–derived lipid bilayer and virus-encoded envelope glycoproteins (*3*). For the virus to enter target cells, the viral membrane must be fused with the plasma membrane of the cell, a process mediated by the envelope glycoproteins. The exposed location of these proteins on the virus allows them to carry out their function but also renders them uniquely accessible to neutralizing antibodies. Thus, dual selective forces—virus replication and immune pressure—have shaped the evolution of the envelope glycoproteins and continue to do so within each infected host. Here, we summarize our current understanding of the functional features of these proteins.

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Synthesis and Assembly

In the infected cell, the envelope glycoproteins are synthesized as an approximately 845– to 870–amino acid precursor in the rough endoplasmic reticulum. Asparagine-linked, high-mannose sugar chains are added to form the gp160 glycoprotein, which assembles into oligomers (*4*–*6*). The preponderance of evidence suggests that these oligomeric complexes are trimers (*4*, *5*). The gp160 trimers are transported to the Golgi apparatus, where cleavage by a cellular protease generates the mature envelope glycoproteins: gp120, the exterior envelope glycoprotein, and gp41, the transmembrane glycoprotein (*3*). The gp41 glycoprotein has an ectodomain that is largely responsible for trimerization (*7*), a membrane-spanning anchor, and a long cytoplasmic tail. Most of the surface-exposed elements of the mature, oligomeric envelope glycoprotein complex are contained on the gp120 glycoprotein. Selected, presumably well-exposed, carbohydrates on the gp120 glycoprotein are modified in the Golgi apparatus by the addition of complex sugars (*6*). The gp120 and gp41 glycoproteins are maintained in the assembled trimer by noncovalent, somewhat labile, interactions between the gp41 ectodomain and discontinuous structures composed of NH_2- and COOH-terminal gp120 sequences (*8*). When they reach the infected cell surface, a fraction of these envelope glycoprotein complexes are incorporated into budding virus particles. A large number of the complexes disassemble, releasing gp120 and exposing the previously buried gp41 ectodomain. These events contribute to the formation of defective virions, which predominate in any retroviral preparation (*9*).

Binding to the CD4 Receptor

Many cell surface proteins, including adhesion molecules, are incorporated into HIV-1 virions along with the envelope glycoprotein complexes (*10*). These host cell–derived molecules can assist the attachment of viruses to potential target cells. Virus attachment also involves the interaction of the gp120 envelope glycoproteins with specific receptors—the CD4 glycoprotein (*11*) and members of the chemokine receptor family (*12*, *13*) (Fig. 1). The CD4 glycoprotein is expressed on the surface of T lymphocytes, monocytes, dendritic cells, and brain microglia, the main target cells for primate immunodeficiency viruses in vivo. The requirement for CD4 binding exhibited by most primate immunodeficiency viruses for efficient entry is consistent with this observed in vivo tropism.

A major function of CD4 binding is to induce conformational changes in the gp120 glycoprotein that contribute to the formation or exposure of the binding site for

the chemokine receptors (*13*, *14*). Some HIV-1 and HIV-2 isolates cultured in the laboratory, as well as several primary SIV isolates, no longer depend on CD4 for efficient entry, and bind to chemokine receptors without prior CD4 interaction (*15*). These examples and the observation that feline immunodeficiency viruses use chemokine receptors but not CD4 for entry (*16*) raise the possibility that the chemokine receptors represent the primordial, obligate receptors for this retroviral lineage. The use of CD4 as a receptor may have evolved subsequently, allowing the high-affinity chemokine receptor–binding site of primate immunodeficiency viruses to be sequestered from host immune surveillance.

Multiple approaches have yielded insights into the structural basis for CD4 binding by the primate immunodeficiency virus gp120 glycoproteins. Early comparisons of gp120 sequences revealed the existence of five variable (V1 through V5) regions interspersed with five conserved regions (*17*). Intramolecular disulfide bonds in the gp120 glycoprotein result in the incorporation of the first four variable regions into large, looplike structures (6). Antibody binding studies and deletion mutagenesis have indicated that the major variable loops are well exposed on the surface of the gp120 glycoprotein (*18*, *19*). The more conserved regions fold into a gp120 core, which has been recently crystallized in a complex with fragments of CD4 and a neutralizing antibody (*20*).

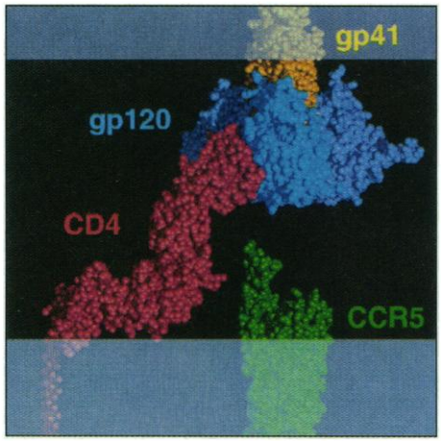

Fig. 1. The HIV-1 entry process. The trimeric HIV-1 envelope glycoproteins, anchored in the viral membrane, are depicted, with gp120 in blue and gp41 in yellow. For simplicity the gp120 variable loops are not shown, but they would extend over the outer surface of the envelope glycoprotein complex. The receptors on the target cell, CD4 (red) and chemokine receptor (green), are also shown. The structures of gp120, gp41, and CD4 are adapted from available x-ray crystallographic studies (5, 20, 21), whereas the chemokine receptor model is hypothetical.

The gp120 core is composed of two domains, an inner and an outer domain, and a β sheet (the "bridging sheet") that does not properly belong to either domain (Fig. 2A). The domain names reflect the likely orientation of gp120 in the assembled envelope glycoprotein trimer: the inner domain faces the trimer axis and, presumably, gp41, whereas the outer domain is mostly exposed on the surface of the trimer. Elements of both domains and the bridging sheet contribute to CD4 binding.

CD4 binds in a recessed pocket on gp120, making extensive contacts over ~800 Å^2 of the gp120 surface. Two cavities are evident in the gp120-CD4 interface. A shallow cavity is filled with water molecules, and a deep cavity extends roughly 10 Å into the interior of gp120. The opening of this deep cavity is occupied by phenylalanine-43 of CD4, which has been shown by mutagenic analysis to be critical for gp120 binding (*21*). Most of the gp120 residues previously identified as important for CD4 binding (*22*, *23*) surround the opening of the deep cavity and contribute to interactions with Phe^{43} of CD4. In addition, Asp^{368} of gp120 forms a salt bridge with Arg^{59} of CD4, also shown by mutagenesis to be important for gp120 binding (*21*). In addition, main-chain atoms on gp120 and CD4 form hydrogen bonds bridging the two proteins. The formation of the deep cavity in gp120 likely contributes to the transmission of CD4-induced conformation-

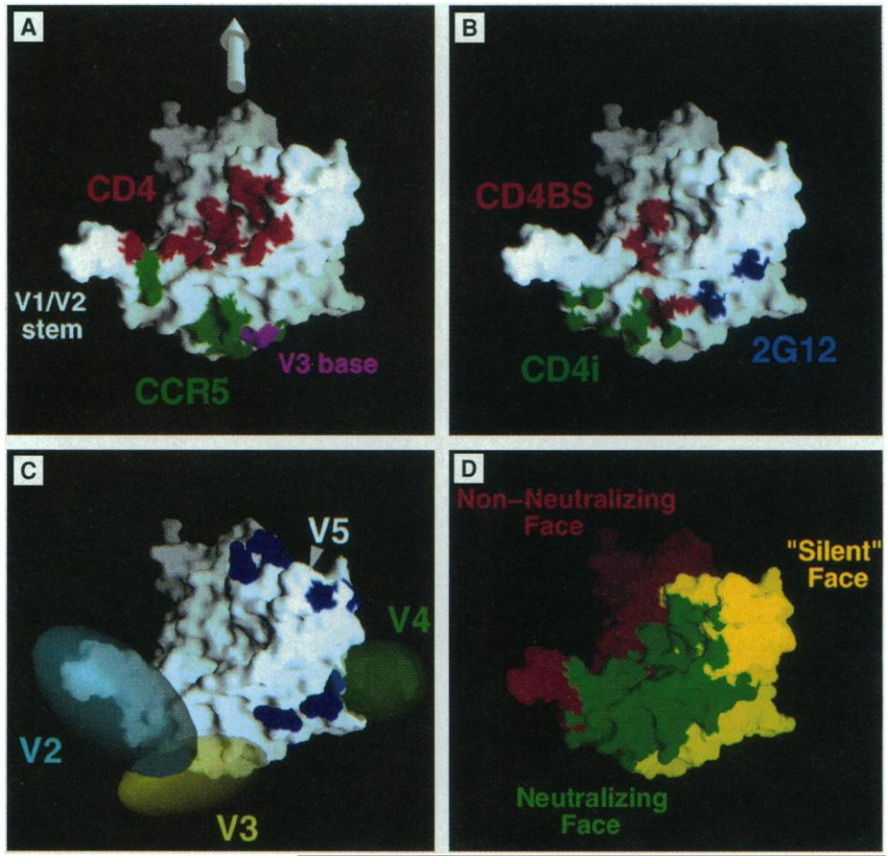

Fig. 2. The HIV-1 gp120 surface. (**A**) The molecular surface of the HIV-1 gp120 core (*20*) is shown, with the arrow pointing toward the viral membrane. The inner domain, believed to interact with gp41, and the outer domain, which is probably exposed on the assembled trimer, are on the left and right, respectively. The gp120 surface interacting with CD4 is shown in red, and the gp120 region thought to be involved in chemokine receptor binding (*27*) is shown in green. The location of the base of the V3 loop is shown in magenta. (**B**) Conserved gp120 neutralization epitopes are shown on the gp120 core, which is oriented identically to that in (A). The location of the epitopes was deduced from mutagenic analyses (*46*–*48*). (**C**) The approximate location of gp120 structures (*20*) that contribute to protection from antibody responses is shown. The major variable loops (V2, V3, and V4), the V5 region, and the sites of N-linked glycosylation (blue) are shown. (**D**) The relation of different surfaces of the gp120 core to the antibody responses generated by the gp120 glycoprotein is depicted. The surface of gp120 that interacts with neutralizing antibodies (*32*) is shown in green, involves both domains and the bridging sheet, and includes the V2 and V3 variable loops (not shown). The surface of gp120 that interacts with nonneutralizing antibodies is depicted in red, is located on the inner domain, and includes gp41-interactive NH_2- and COOH-terminal gp120 regions (not shown). The heavily glycosylated surface of the gp120 outer domain, which appears to be minimally immunogenic, is shown in yellow.

al changes to gp120 elements involved in the interaction with chemokine receptors or gp41, or both. The deep cavity may be a useful target for intervention by small molecular weight compounds.

Chemokine Receptor Binding

Most primary, clinical isolates of primate immunodeficiency viruses use the chemokine receptor CCR5 for entry (*12*). For most HIV-1 isolates that are transmitted and that predominate during the early years of infection, CCR5 is an obligate coreceptor, and rare individuals that are genetically deficient in CCR5 expression are relatively resistant to HIV-1 infection (*24*). HIV-1 isolates arising later in the course of infection often use other chemokine receptors, frequently CXCR4, in addition to CCR5 (*12, 24*). Studies of chimeric envelope glycoproteins demonstrated that the third variable (V3) loop of gp120 is a major determinant of which chemokine receptor is used (*12, 25*). V3-deleted versions of gp120 do not bind CCR5, even though CD4 binding occurs at wild-type levels (*14*). Antibodies to the V3 loop interfere with gp120-CCR5 binding (*14*). These results support an involvement of the V3 loop in chemokine receptor binding.

Other, conserved gp120 structures also appear to play an important role in chemokine receptor binding. The use of CCR5 by a diverse group of immunodeficiency viruses, with divergent V3 sequences, first suggested the involvement of more conserved gp120 elements (*26*). Antibodies that recognize conserved, discontinuous gp120 epitopes that are more exposed after CD4 binding are potent inhibitors of gp120-CCR5 interaction (*14*). These CD4-induced (CD4i) epitopes are discussed further below. Recent mutagenic and structural analyses have revealed the existence of a highly conserved gp120 structure that is important for CCR5 binding (*20, 27*) (Fig. 2, A and B). This structure is adjacent to the V3 loop and the CD4i epitopes and is oriented to face the target cell following gp120-CD4 binding. Whether the conserved gp120 structure directly interacts with the chemokine receptor, influences V3 conformation, or is critical in CD4 induction of the chemokine receptor–binding site requires further investigation.

gp41-Mediated Membrane Fusion

It is likely that the interaction of the gp120-CD4 complex with the appropriate chemokine receptor promotes additional conformational changes in the envelope glycoprotein complex. By analogy with the influenza hemagglutinin, it has been suggested that the HIV-1 gp41 ectodomain undergoes major conformational changes during virus entry (*28*). The proposed result of these changes is the insertion of the hydrophobic gp41 NH_2-terminus (the "fusion peptide") into the membrane of the target cell. Mutagenic analysis (*23, 29*) and the recently determined crystal structures of HIV-1 gp41 ectodomain fragments (*5*) are consistent with this model. The gp41 ectodomain structures reveal an extended, trimeric coiled coil that could potentially bridge the viral and target cell membranes (*5*). Interactions of other gp41 helical segments near the membrane-spanning region with the interhelical grooves of the internal coiled coil are important for fusion-related conformational changes in gp41. This interaction can be inhibited by helical peptides that mimic either of the involved gp41 helices (*30*) and is a potential target for future intervention with small molecular weight compounds.

Envelope Glycoproteins As Antigens and Immunogens

The exposure of the primate immunodeficiency virus envelope glycoproteins on the surface of virions or infected cells makes them prime targets for antibodies that potentially block key functions of these proteins. However, the success of these viruses in achieving persistent infections implies that the viral envelope glycoproteins have evolved to be less-than-ideal immunogens and antigens. Structures on the viral envelope glycoproteins that are conserved among diverse viral strains are, in general, poorly exposed to the humoral immune system. The conserved gp120 surfaces involved in binding to its three minimally polymorphic ligands, gp41, CD4, and chemokine receptors, each exhibit particular problems with respect to the elicitation of or sensitivity to neutralizing antibodies. The moieties involved in gp120-gp41 association are buried in the interior of the functional envelope glycoprotein spike (*18, 31, 32*). The CD4-binding site is recessed, flanked by variable regions exhibiting considerable glycosylation (*19, 20*). The chemokine receptor–binding site is masked by variable loops, probably V3 and V2 (*20, 32, 33*) (Fig. 2C). Even in the relatively conserved HIV-1 gp120 core that has been structurally analyzed, the outer domain exhibits a variable, heavily glycosylated surface (*20*). Because most carbohydrate moieties may appear as "self" to the immune system, this concentrated glycosylation may reduce the potential of a large portion of the gp120 surface to serve as an immunogenic target. Thus, in addition to the neutralizing and nonneutralizing faces of gp120 previously detected by antibody competition analysis (*32*), the crystal structure of the gp120 core reveals a third, immunologically silent face of gp120 (Fig. 2D).

Despite the potential to exert potent antiviral effects, antibodies are not able to suppress virus replication completely in infected hosts. The efficacy of the humoral immune response in limiting the spread of virus in vivo is compromised by at least two factors: the relative resistance of primary virus isolates to neutralization, and the temporal pattern with which neutralizing antibodies are generated.

HIV-1 viruses that have been passaged in immortalized cell lines are typically more sensitive to neutralization by antibodies or soluble CD4 than are primary, clinical isolates (*34*). Although other envelope glycoprotein regions can influence this phenotype, a major determinant is the structure of the gp120 major variable loops, V1 or V2 and V3 (*35, 36*). Thus, replacement of the V1 or V2 and V3 variable loops of a laboratory-adapted virus with those of a neutralization-resistant primary isolate creates a virus similar to the parental primary virus (*35, 36*). The basis for the decreased sensitivity of primary HIV-1 isolates to neutralization appears to involve a decreased exposure of the relevant gp120 epitopes to soluble CD4 or antibody. This decreased exposure is most apparent in the context of the assembled oligomeric complex (*37*). A likely explanation for this neutralization resistance is that the major variable loops of primary viruses assume tightly interfacing, "closed" conformations that decrease the accessibility of many gp120 epitopes to antibodies.

Temporal Pattern of the Antibody Response to HIV-1 Infection

The noncovalent nature of the association between gp120 and gp41 contributes to the lability of the functional envelope glycoprotein trimer (*8, 9*). During natural infections, disassembled envelope glycoproteins apparently elicit most of the antibodies directed to these viral components. The interactive regions of gp120 and gp41 are particularly immunogenic (*38*). However, because the cognate antibodies cannot bind the assembled, functional envelope glycoprotein complex, they do not exhibit neutralizing activity. Thus, although antibodies to the envelope glycoproteins typically can be detected in the sera of HIV-1–infected individuals by 2 to 3 weeks after infection, most of these antibodies lack the ability to inhibit virus infection. By the time that neutralizing antibodies are efficiently elicited, HIV-1 is firmly established in the host.

Several weeks after virus infection, usually after the initial high level of viremia has subsided, neutralizing antibodies can be detected in the sera of infected animals or

humans (39). These antibodies neutralize the infecting virus, but often exhibit little or no activity against other strains of virus. A subset of these strain-restricted antibodies recognizes, the HIV-1 V3 loop (39). These antibodies can block chemokine receptor binding (14). Other variable gp120 elements can contribute to the epitopes recognized by the strain-restricted neutralizing antibodies. It is known, for example, that antibodies to the gp120 V2 loop can also exhibit neutralizing activity (40). The V2 loop–associated neutralization epitopes are typically conformation dependent. The ability of some V2- or V3-directed antibodies to recognize more than one HIV-1 strain (40, 41) suggests that these major variable loops assume a finite number of conformations. This observation is consistent with the functional consequences on virus entry of some changes in these variable structures (42), and with the observation that amino acid substitutions in the variable loops are not random (43). The requirement for chemokine receptor binding probably constrains V3 loop variation. The V2 loop, although dispensible for the replication of some HIV-1 viruses in culture (33), helps protect the V3 loop and the conserved epitopes near the chemokine receptor–binding site from neutralizing antibodies. Thus, the V2 and V3 loops reside proximal to the chemokine receptor–binding site (Fig. 2), masking more conserved gp120 elements and presenting potentially variable epitopes to the immune system.

Later in the course of HIV-1 infection of humans, antibodies capable of neutralizing a wider range of HIV-1 isolates appear (44). A subset of the broadly reactive antibodies, found in most HIV-1–infected individuals, interferes with the binding of gp120 and CD4 (44). Human monoclonal antibodies derived from HIV-1–infected individuals have been identified that recognize the gp120 glycoproteins from a diverse range of HIV-1 isolates, that block gp120-CD4 binding, and that neutralize virus infection (45). The discontinuous epitopes (the so-called CD4BS epitopes) recognized by many of these human monoclonal antibodies have been characterized by mutagenic analysis (46). The gp120 residues important for antibody binding are all located within the CD4-binding pocket on gp120 (Fig. 2B), and several of the most important residues are near the opening of the deep cavity (20). Therefore, some broadly neutralizing antibodies apparently can access the more recessed elements of the CD4-binding pocket. This is consistent with the observation that the gp120-CD4 interface is as large as that of a typical antibody-antigen complex (20).

A second group of neutralizing antibodies found in a smaller number of HIV-1–infected humans is directed to the CD4i epitopes (47). The CD4i epitopes are located near conserved gp120 structures important for chemokine receptor interaction (14) (Fig. 2B). CD4 binding has been shown to cause a change in the V2 loop conformation that allows better CD4i epitope exposure (33). In the absence of CD4, the antibodies recognizing the CD4i epitopes must bypass the overlapping V2 and V3 loops (33). Indeed, as is evident in the current crystal structure (20), this is accomplished by the protrusion of the CDR3 loop of the antibody heavy chain. Antibodies to CD4i epitopes need to bind viruses before CD4 binding occurs to achieve neutralization (36). The reason for this is that, once the envelope glycoprotein complex binds cell surface CD4, there are severe steric constraints on the binding of an antibody to the gp120 surface facing the target cell (Fig. 1).

Another fairly conserved gp120 neutralization epitope is recognized by the 2G12 antibody (48). Unlike the other characterized HIV-1 neutralizing antibodies, which recognize gp120 structures near or within the receptor-binding sites, the 2G12 antibody apparently binds an epitope in the outer domain (Fig. 2B). Given the variability in this outer domain, the ability of 2G12 to neutralize a fair number of HIV-1 strains (48) seems paradoxical. The marked sensitivity of 2G12 binding to alterations in gp120 glycosylation provides a clue to this puzzle. Despite the variability of the underlying primary amino acid sequence, the 2G12 antibody may recognize more conserved carbohydrate structures formed as a result of the heavy concentration of N-linked glycosylation in the gp120 outer domain. The apparent rarity with which 2G12-like antibodies are elicited attests to the success of the viral strategy of using a heavily glycosylated outer domain surface in immune evasion.

Envelope Glycoproteins As Vaccine Components

That the human and simian immunodeficiency virus envelope glycoproteins are not ideal immunogens is an expected consequence of the immunological selective forces that drive the evolution of these viruses. The same features of the envelope glycoproteins that dictate poor immunogenicity in natural infections have hampered vaccine development. The lability of the envelope glycoprotein complex has frustrated attempts to present oligomers mimicking the functional spike to the immune system. As discussed above, the disintegration of envelope glycoprotein oligomers contributes to the preferential elicitation of nonneutralizing antibodies by the newly exposed gp120 NH_2- and COOH-termini. Regardless of the context in which the envelope glycoproteins are presented, the gp120 variable loops elicit the majority of neutralizing antibodies, probably because of the exposed nature of these epitopes. It is still unclear whether conserved features in the V2 and V3 variable loops exist that can be exploited in vaccine design, or whether all possible functional configurations of these variable structures need to be represented in a cocktail of immunogens.

The discontinuous gp120 structures surrounding the receptor-binding sites exhibit a relatively high degree of conservation (20), in keeping with the minimal polymorphism in the host cell receptors. The CD4-binding site constitutes a particularly attractive target. It appears to be accessible to antibodies, more so than the conserved elements of the chemokine receptor–binding region. A large fraction of the broadly neutralizing antibodies that eventually appear in HIV-1–infected individuals is directed to the CD4-binding site (44), indicating the ability of the human immune system to recognize this gp120 region and to generate an appropriate response. Nonetheless, these antibodies have been difficult to elicit in animals and vaccinated humans (49). The reasons for the relatively poor immunogenicity of the CD4-binding site are not yet understood, although several possibilities can be envisioned. Interdomain flexibility may disrupt the CD4BS epitopes and decrease their representation in the pool of immunogens. Masking by variable loops (19, 33) and glycosylation probably contribute to the recessed nature of the CD4BS epitopes, which, even on the crystallized gp120 core, occupy a 20 Å–deep canyon (20). Within the CD4-binding pocket, not all of the gp120 surface is conserved among HIV-1 strains. Therefore, even when elicited, some CD4BS-directed antibodies may lack the breadth and affinity to be optimal neutralizing agents. Although many monoclonal antibodies to the CD4-binding site exhibit reasonable potency and breadth (45), whether a polyclonal response to the envelope glycoproteins can be focused to preferentially contain these types of antibodies remains to be seen.

The conserved elements near the chemokine receptor–binding site will be difficult targets for vaccine-elicited antibodies. Known monoclonal antibodies to the CD4i epitopes must interact with virus before CD4 binding if neutralization is to be achieved (36). Yet these gp120 structures are poorly exposed in the absence of CD4, in large part because of the overlying V2 loop (33). This is consistent with the relative rarity with which these antibodies appear to be elicited in HIV-1–infected humans (47). Attempts to expose these structures better on gp120-based antigens seem warranted.

Concluding Remarks

The HIV-1 envelope glycoproteins have evolved to be inefficient at eliciting effective antiviral antibody responses. The availability of structural information on the conserved HIV-1 gp120 neutralization epitopes should facilitate the modification of this important antigen and allow the rational testing of hypotheses regarding its poor immunogenic properties. These efforts will complement ongoing efforts to improve antigen presentation to the immune system and to create suitable animal models for the screening of vaccine candidates.

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