GP120: Target for Neutralizing HIV-1 Antibodies

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Abstract

The glycoprotein (gp) 120 subunit is an important part of the envelope spikes that decorate the surface of HIV-1 and a major target for neutralizing antibodies. However, immunization with recombinant gp120 does not elicit neutralizing antibodies against multiple HIV-1 isolates (broadly neutralizing antibodies), and gp120 failed to demonstrate vaccine efficacy in recent clinical trials. Ongoing crystallographic studies of gp120 molecules from HIV-1 and SIV increasingly reveal how conserved regions, which are the targets of broadly neutralizing antibodies, are concealed from immune recognition. Based on this structural insight and that from studies of antibody structures, a number of strategies are being pursued to design immunogens that can elicit broadly neutralizing antibodies to gp120. These include (*a*) the construction of mimics of the viral envelope spike and (*b*) the design of antigens specifically tailored to induce broadly neutralizing antibodies.

INTRODUCTION

HIV: human immunodeficiency virus

Viral envelope: the membrane surrounding a virus particle, usually containing host cell membrane lipids and proteins as well as virus-encoded (glyco)proteins

gp: glycoprotein

The antibody response to HIV-1 in vivo is directed against several viral proteins. However, essentially all neutralizing antibodies are directed toward the viral envelope spike, in particular the surface unit glycoprotein (gp) 120 (1, 2), which is anchored to the viral surface by gp41, the transmembrane unit (2). Because of its surface-exposed location, gp120 seemed a natural first choice as a subunit vaccine candidate (3). However, it was soon noted that immunization with recombinant monomeric gp120 elicited antibodies that could neutralize viruses that were neutralization-sensitive following extensive passage in immortalized T cell line cultures, but not viruses grown in limited passage in peripheral blood mononuclear cells (PBMCs) (4). The latter are more representative of circulating "primary" viruses. The results from recently completed phase III clinical trials show that antibodies elicited by recombinant-gp120-based vaccine candidates indeed do not protect vaccine recipients against HIV-1 infection or influence disease progression (5, 6).

The general inability of recombinant gp120 to elicit cross-neutralizing (or broadly neutralizing) antibodies to primary viruses has sharpened interest in understanding the differences between the structure of gp120 in its recombinant form and its structure in the context of the envelope spike. Ample evidence suggests that the gp120 conformation in the context of the envelope spike is the crucial structure recognized by neutralizing antibodies (7–13). Here, we summarize recent insights gained in understanding the structural conformation of gp120, particularly as it relates to exposure of antibody epitopes on the viral spike. The recently solved crystal structure of the unliganded gp120 core of simian immunodeficiency virus (SIV) (14) is an important advance in this regard. Although a few caveats are associated with this structure, it nevertheless allows us to appreciate better some of the mechanisms that HIV uses to avoid antibody binding to conserved regions on gp120, which are the targets of broadly neutralizing antibodies. We also highlight two strategies that are currently being pursued to induce broadly neutralizing anti-gp120 antibodies: reconstitution of the viral envelope spike and tailored antigen design. These strategies are based, in part, on our current understanding of interactions between the viral envelope and presently known broadly neutralizing antibodies. This overview represents an update of a previous review on the biological aspects of gp120 structural features (15).

HUMORAL AND CELLULAR IMMUNE RESPONSES IN PROTECTION AGAINST VIRAL INFECTION

A common feature of protective viral vaccines is their ability to elicit neutralizing antibodies. Neutralizing antibodies generally reduce the severity of primary infection and thus prevent the onset of disease. Neutralization can be achieved by the binding of antibodies to free virus particles. However, antibodies may also mediate activities against infected cells that display viral antigens such as antibody-dependent cellular cytotoxicity [the killing of antibody-coated target cells by immune cells that bear Fc receptors, e.g., natural killer (NK) cells] or complement-dependent cytotoxicity (lysis of antibody-coated target cells by complement). Antibodies are not always sufficient, and vaccine-induced T cell–mediated immunity may be required as well for protective efficacy. In such cases, the combination of antibody and T cell may protect at a level that is not achievable with a single component. Some viruses, such as HIV, have evolved elaborate mechanisms by which to protect conserved regions from effective recognition by humoral and cellular immune responses. Understanding and overcoming these barriers represent major scientific challenges for vaccinologists.

STRUCTURAL ORGANIZATION AND TOPOLOGICAL FEATURES OF GP120

The HIV envelope spike is formed as a complex between gp120 and gp41 (2). The gp120 unit mediates attachment of the virus

to the target cell, whereas gp41 is required for the fusion of virus and target cell membranes. During HIV infection, the viral envelope spike is first synthesized as a single polypeptide precursor (2). In the Golgi, the protein subsequently oligomerizes and undergoes extensive glycosylation. The glycosylation process, which is required for proper folding and conformational stability of the envelope glycoprotein (16), mainly involves the attachment of N-linked high-mannose-type oligosaccharides to the protein backbone. As the glycoprotein is transported through the Golgi, accessible glycan moieties are trimmed and modified by various cellular enzymes (2). These modifications generate so-called complex-type oligosaccharides; glycans that are relatively inaccessible to modifying enzymes remain as high-mannose-type glycans (17). The resulting glycoprotein, which has a molecular mass of ~160 kDa, is cleaved in the trans-Golgi network by furin or equivalent endoproteases into gp120 and gp41 (2). The gp120-gp41 complexes, which remain associated through weak noncovalent interactions, are initially expressed at the surface of infected cells. During the HIV budding process, the gp120-gp41 complexes are then incorporated into the virus envelope and displayed on its surface as viral spikes (2).

Organization of gp120 on the Viral Surface

Knowledge of the oligomeric structure of the gp120-gp41 complex is important for vaccine design strategies, as we highlight below. Experimental evidence suggests that the functional unit of the envelope spike is a heterodimeric trimer complex of gp120 and gp41. For example, a recent electron tomography study revealed structures on the surface of negatively stained virions of SIV and HIV-1 that appear to be tri-lobed envelope glycoproteins (18) (**Figure 1**). Furthermore, the HIV core matrix that interacts with gp41 is organized in a trimeric configuration (19), and the crystal structures of HIV-1 gp41 cores resemble the transmembrane proteins of other viruses that have been shown to display trimeric envelope spikes (20–22).

There is, however, also evidence that other envelope species may be present on the surface of HIV-1. For example, atomic force microscopy analyses have failed to reveal any uniform trimeric envelope species on the surface of virions (23). Also, in recent studies, it has been shown that viruses can be captured onto ELISA plate wells using antibodies that are unable to neutralize viral particles in solution (12, 24). Taken together, these observations suggest that, although trimers may likely represent the functional envelope spike, both functional and nonfunctional forms of the envelope may be present on the virion surface. These nonfunctional envelope entities may be monomers, dimers, or tetramers and could possibly arise as the result of (*a*) the dissociation of functional gp120-gp41 complexes, which could perhaps cause gp120 to be shed from the viral surface, or (*b*) inefficient trimerization of the spike in the Golgi (2, 25, 26).

Subunit vaccine: a vaccine that contains only the portion of the pathogen that is considered necessary to induce protection against infection

Neutralization: the loss of infectivity that ensues when antibody molecule(s) bind to a virus particle

PBMC: peripheral blood mononuclear cell

Primary virus: virus that has not been adapted to grow in laboratory culture cell lines

SIV: simian immunodeficiency virus

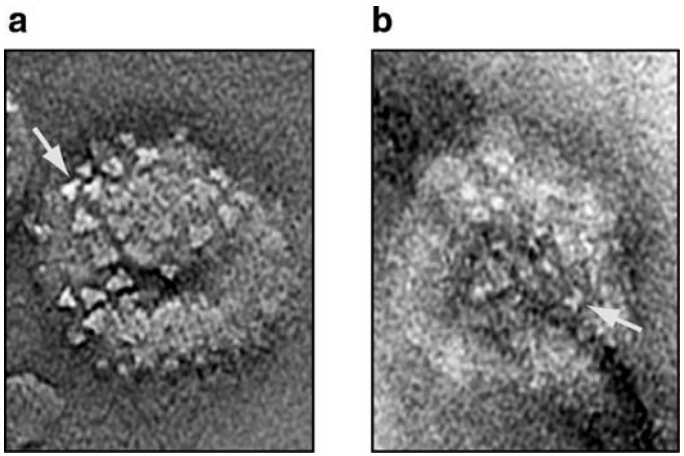

Figure 1

3D tomograms of SIV and HIV pseudovirions. (*a*) Tomogram section from an SIV particle with a truncated cytoplasmic domain and a high level of envelope spike expression on the virion surface. (*b*) Tomogram of an HIV-1 particle expressing the full-length envelope glycoprotein. Representative tri-lobed structures, presumably of the envelope spikes, are indicated (*white arrows*). Tomograms courtesy of Drs. Ping Zhu and Ken Roux (Florida State University, Tallahassee, Florida).

Affinity: the strength of binding of one molecule to another at a single site

Topology of gp120

Based on comparative sequence analyses, gp120 is divided into five conserved (C1–C5) and five variable (V1–V5) segments (27, 28). Prior to obtaining the gp120 core crystal structures, investigators deduced many topological features of monomeric and oligomeric gp120 from antibody binding/competition experiments and mutagenesis studies (29–35). Thus, the C1 and C5 regions were reasoned to be the main areas on gp120 for contact with gp41, as these regions are accessible to antibody on monomeric gp120 but not on gp120-gp41 complexes (29, 31, 35). Major segments of the C2, C3, and C4 regions were suggested to form a buried, relatively hydrophobic core within the gp120 molecule (31, 32). It was proposed that this gp120 core harbors several discontinuous neutralizing antibody epitopes that overlap the binding sites for CD4, the primary HIV receptor, and the coreceptor (31–34). In contrast to the conserved regions, the variable regions (in particular, V1, V2, and V3) were argued to be well exposed on the surface of monomeric gp120 (31). Deletion of V1/V2 and V3 generally increases the binding affinity of antibodies to epitopes that overlap the binding sites for CD4 and the coreceptor, which suggests that these variable regions may shield conserved epitopes from efficient antibody recognition (36–39). For the V4 and V5 variable regions, no definitive role has been ascribed; although deletion of the V4 region has been shown to disrupt gp160 folding (32, 38), V4 also seems to tolerate insertion of foreign antibody epitopes (40). Determination of the structures of gp120 molecules from HIV and SIV in recent years has supported many of the interpretations made from these earlier observations.

MOLECULAR STRUCTURE OF GP120

At present, four crystal structures of HIV-1 gp120 and one of SIV gp120 have been reported (14, 41–43). All five structures are of the gp120 core; i.e., the structures lack the V1/V2 and V3 variable regions, and the N and C termini are truncated. The HIV-1 gp120 structures were determined in complex with the D1D2 fragment of CD4 or CD4 mimics, whereas the SIV gp120 structure was solved unliganded. Because all crystal structures determined so far are of monomeric gp120, they may not adequately represent the structure of oligomeric gp120 on the virus. Despite these and other caveats that we discuss later, the structures do advance insight into the conformational flexibility of monomeric gp120 as well as the locations of receptor-binding sites and putative antibody epitopes on gp120.

Crystal Structures of HIV-1 and SIV gp120 Cores in Complex with CD4

The structure of the gp120 core from the laboratory-adapted virus HXB2 was the first determined in complex with CD4 (**Figure 2**) (42). HXB2 is highly sensitive to antibody neutralization. The second crystal structure was that of CD4 complexed with the gp120 core of the primary virus YU-2 (41), which exhibits a marked resistance to antibody neutralization. The two gp120 core structures are virtually superimposable (41), which is consistent with earlier predictions that the ability of HIV to resist antibody neutralization is likely to be manifested mainly in the context of the gp120 quaternary structure on the viral surface rather than in the monomeric gp120 form (7). Based on these CD4-bound structures, gp120 is organized into three general areas (**Figure 3*a***): (*a*) the inner domain, (*b*) the outer domain, and (*c*) the bridging sheet (44).

Inner domain, outer domain, and bridging sheet. The inner domain is formed mainly by the C1 and C5 regions and is largely devoid of glycans (42, 44), which strongly supports the proposition that C1 and C5 function as the major contact interface with the gp41 transmembrane unit. The outer domain, in contrast, is largely covered by glycans (42,

44). Modeling of the gp120 oligomer suggests that these glycans likely cover large sections of the outer surface of the spike to lower its overall immunogenicity (44, 46). The glycans themselves are poor targets for antibodies because of their heterogeneous expression on the virus and because they are produced by the glycosylation machinery of the host cell and, thus, are self molecules. Comparison of the liganded gp120 structure of HIV-1 (**Figure 3*a***) and the unliganded gp120 structure of SIV (**Figure 3*b***) shows that the respective outer domains are highly similar (14). However, the conformation of the inner domain in the unliganded structure deviates significantly from the conformation in the liganded structures. This observation suggests that the inner domain may have significant conformational flexibility in the absence of CD4. Comparison of the inner domain substructures in the unliganded and liganded core structures suggests that, upon CD4 binding, these substructures are repositioned somewhat independently of each other, rather than a shift of the inner domain as a single unit (14). The large structural rearrangements associated with the repositioning of inner domain substructures seem consistent with the large negative entropy and enthalpy changes measured by isothermal titration microcalorimetry (47). It is noteworthy that the majority of gp120 conformational shifts resulting from CD4 binding are in the portion of gp120 that interacts with gp41. Thus, these conformational changes may be necessary to lock the coreceptor-binding site (CoRbs) into a fixed conformation and also trigger gp41 into initiating the first steps in the fusion process (2).

The conformational changes that occur within the inner domain also affect the formation of the bridging sheet, which links the inner and outer domains. In the CD4-liganded gp120 conformation, the bridging sheet is folded into a compact antiparallel, four-stranded β-sheet (β2-β3 and β20-β21) (42, 44). However, in the unliganded structure, the β-strands that constitute the bridging sheet lie separated in pairs at a distance

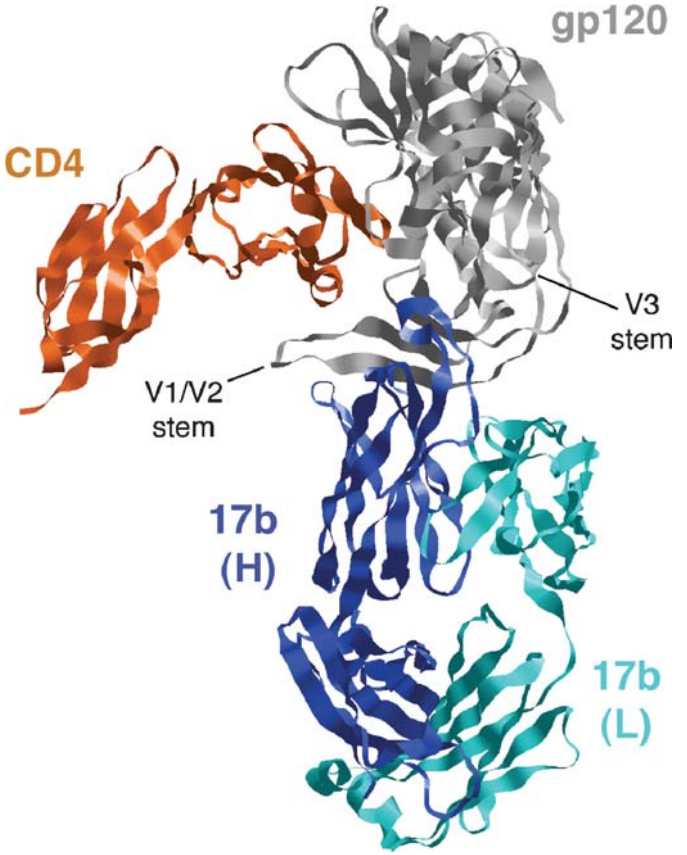

Figure 2

Crystal structure of HIV-1 gp120 complexed to CD4 and an antibody antigen-binding fragment (Fab). The gp120 core of HXB2 (Protein Data Bank ID 1G9M) (*gray*), CD4 (*orange*), the antibody heavy chain (H) (*blue*) and light chain (L) (*green*) are shown. Figure adapted from Kwong et al. (42). All figures were prepared with RasTop (45).

of approximately 20 Å (14); the two β-strands (β2-β3) that constitute the V1/V2 stem are located in the vicinity of the inner domain, whereas the other two strands (β20-β21) are situated near the outer domain in approximately the same location as they are on the liganded structure. Conformational changes that occur within the inner domain upon CD4 binding would result in a 40 Å shift of the V1/V2 stem to form the bridging sheet (14). However, molecular modeling of the liganded and unliganded gp120 structures suggests that the unliganded structure may be one of many conformations that gp120 may adopt in the

CoRbs: coreceptor-binding site

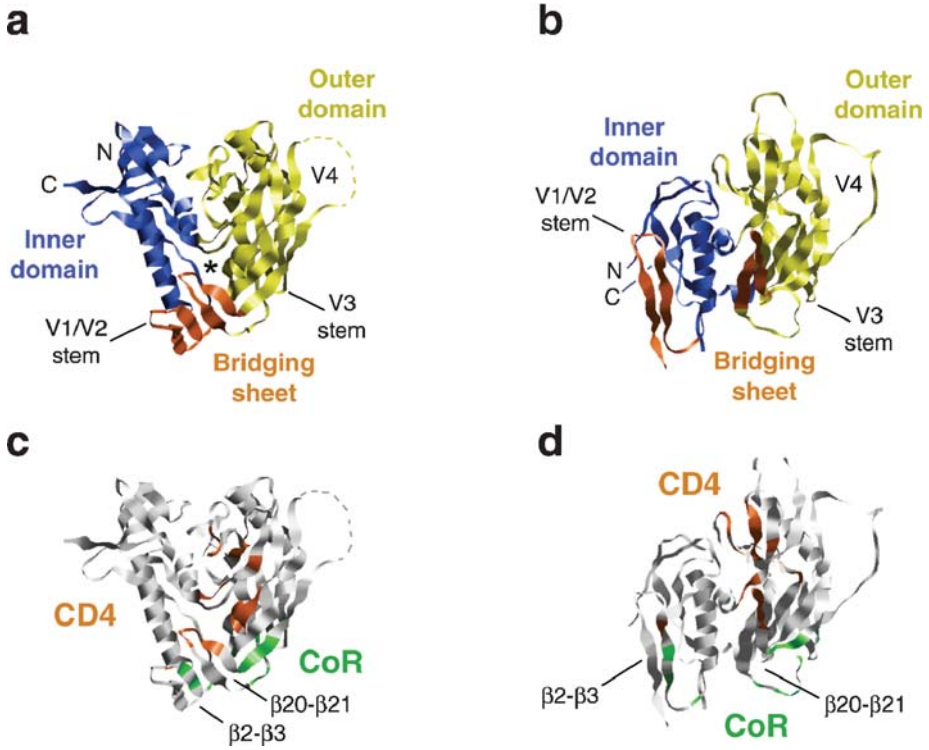

Figure 3

Comparison of the crystal structures of HIV-1 and SIV gp120 core. (*a*) Structure of the CD4-liganded HIV-1 gp120 core (HXB2), viewed from the perspective of CD4. The gp120 inner domain (*blue*), outer domain (*yellow*), and bridging sheet (*orange*) are shown. The locations of various gp120 regions are also denoted. (*b*) Structure of the unliganded SIV gp120 core (Protein Data Bank ID 2BF1), viewed from the perspective of CD4 as in (*a*). (*c*) HIV core gp120 in same orientation as (*a*), depicting CD4 contact residues (*orange*) and residues that influence coreceptor binding (*green*). (*d*) SIV gp120 core in the same orientation as in (*b*), colored according to the scheme for HIV gp120 in (*c*).

CD4bs: CD4-binding site

absence of CD4 (48). In fact, the models suggest that the β2-β3 strands may oscillate from the conformation observed in the unliganded structure to a conformation resembling the CD4-bound structure via a series of intermediate conformers (48). Epitope-mapping studies of antibodies to the CD4-binding site (CD4bs) also suggest that gp120 can adopt a conformation resembling the CD4-bound form relative to the conformation of the unliganded structure. We discuss this in more detail in the subsequent sections.

The CD4-binding site. The binding site for CD4 on the liganded gp120 structure is formed by the interface between the inner domain, bridging sheet, and outer domain (42, 44). At the center of this interface lies a hydrophobic cavity that has been dubbed the Phe43 cavity (**Figure 3*a***) (42). However, most of the CD4 contact residues are located on the outer domain of the liganded HIV-1 gp120 structures and form a contiguous binding region (**Figure 3*c***). On the unliganded SIV gp120 structure no such region is discernible (**Figure 3*d***) (14), assuming that equivalent residues in SIV and HIV-1 contact CD4. On the unliganded SIV gp120 core, many of the residues that are presumed to contact CD4 upon complexation with gp120

are located near or within a long cavity that is formed primarily by portions of the inner and outer domains and the β20-β21 segment of the bridging sheet. The location of these conserved residues likely minimizes their immediate recognition by antibodies, while preserving the ability to contact CD4. In this regard, the curved structure of the D1D2 fragment of CD4 is particularly noteworthy (**Figure 2**); it permits CD4 to curl over the outer domain, so residues located near or within the cavity formed by the inner and outer domains can be reached. Given that the CD4bs is not coherently present on the unliganded structure, it indeed seems likely that gp120 transiently samples conformations that are reflective of the liganded structure; upon interaction with CD4, the gp120 structure is locked in the bound conformation.

The coreceptor-binding site. The region that is important for the interaction with the β-chemokine receptor CCR5 has been mapped to residues in the bridging sheet and near the V3 stem (49, 50). These residues lie close together on the liganded HIV-1 gp120 structure, but the equivalent residues on the unliganded SIV gp120 structure are separated into two areas (**Figure 3*c*,*d***) (14). These differences are consistent with the notion that CD4 binding is required to lock these areas into a contiguous binding site. The fact that the coreceptor site is not presented until after CD4 binding suggests that the site may be susceptible to antibody recognition. Several studies have shown that HIV strains that do not require CD4 for entry are, in fact, highly sensitive to antibody neutralization (51–54). Owing to neutralizing antibody–driven selection pressure in vivo, the prevalence of such viruses during infection is likely low. However, in the absence of circulating neutralizing antibodies, e.g., in the central nervous system, such viruses may occur more frequently (54, 55).

Potential caveats regarding the HIV and SIV gp120 structures are pointed out in the previous section. We note here also that the unliganded SIV gp120 structure is derived from strain SIVmac32H. This simian virus is able to infect CD4-negative target cells in vitro with medium efficiency (56). The gp120 from this strain may thus harbor certain structural features that are not observed in the gp120 of HIV-1 or SIV strains that require CD4 for entry. Crystallization of gp120s from further SIV and HIV strains, including gp120s from viruses that are entirely CD4 independent, will provide further insight into potential differences between the gp120 structures of these viruses.

Protomer: subunits from which a larger structure is built; for example, the tubulin heterodimer is the protomer for microtubule assembly

VIRAL DEFENSE MECHANISMS FOR ANTIBODY EVASION: STRUCTURAL CONSIDERATIONS

The HIV and SIV structures provide valuable insight into the locations of receptor binding sites on gp120, as we summarize above. The structures also allow for a better understanding of some of HIV's defense mechanisms, in particular how structural features may reduce antibody recognition of conserved regions on gp120.

Variable Regions

Previous studies had suggested that the CoRbs is masked by the V1/V2 variable regions (36, 37); anti-CoRbs antibodies typically require CD4 for high-affinity binding to wild-type gp120, but removal of V1/V2 allows these antibodies to bind their epitopes in the absence of CD4 with the same high affinity as when binding to wild-type gp120 in the presence of CD4. The locations of the V1/V2 stem in the CD4-liganded and -unliganded gp120 structures relative to the coreceptor site suggests that V1/V2 may not be close enough to obstruct the CoRbs within the same gp120 molecule on the virus (14, 41). However, oligomeric modeling of the CD4-liganded structure does suggest that V1/V2 from a given gp120 protomer may be in close proximity to the CoRbs on a neighboring

mAb: monoclonal antibody

protomer to mask the coreceptor site (46). Within the gp120 monomer, removal of V1/V2 may allow the conformationally flexible inner domain, in particular the β2-β3 strands, to more easily sample the conformations resembling that of the CD4-liganded structure in which the coreceptor site would be more fully presented (48).

The locations of the variable region stems in the unliganded gp120 structure (**Figure 3*b***) suggest that V1 and V2 may cover portions of the inner domain, including part of the long cavity that harbors the CD4-binding residues, and is formed by the inner and outer domains (14). Based on the unliganded structure, the V3 variable region may occlude the β20-β21 portion of the bridging sheet (14). The positions of the stems of V1, V2, and V3 also support the notion that these variable regions most likely do not interact with each other within a gp120 protomer, but do interact cooperatively with the variable regions of adjacent protomers within the context of the trimer on the viral surface (14, 46). Further support for this notion comes from recent studies involving a human monoclonal antibody (mAb), termed 2909, which binds exclusively to oligomeric gp120 on virus (55). We discuss this antibody in the context of its binding epitope in more detail in a section below.

Glycan Shield

HIV can also use glycans to occlude antibody epitopes on gp120; 50% of the molecule is covered with carbohydrates that render the underlying protein surface invisible to the immune system. This somewhat static glycan shield has been dubbed the gp120 silent face (44). The virus can also shift the locations of its glycans in vivo (58–60). These observations have led to the proposition of a dynamic or so-called evolving glycan-shield model (59) by which, through the continuous repositioning of its glycans, HIV is able to escape type-specific neutralizing antibody responses. Thus, the evolved resistance is not a generalized one but, rather, a specific adaptation to the particular antibody response in each infected individual (59, 60). The repositioning of glycans may also affect local protein folding and, hence, also indirectly affect neutralizing antibody binding. In addition to being involved in blocking neutralizing antibody responses, glycan repositioning also may compensate for conformational changes in the envelope glycoprotein caused by localized amino acid changes directly related to virus escape from neutralizing antibody.

Entropic Masking

It has been postulated that the productive interaction of antibodies with conserved regions on the HIV-1 envelope spike, in particular with the receptor binding sites, may be limited as a result of intrinsic entropic barriers that are imposed upon antibodies within the context of the viral spike (61). Large changes in entropy are generally observed upon binding of nonneutralizing or weakly neutralizing antibodies to receptor sites on monomeric gp120, whereas small changes in binding entropy are typically observed with broadly neutralizing antibodies (47, 61). Consequently, it has been postulated that the failure of nonneutralizing antibodies to bind with high affinity to their respective epitopes on virion-associated oligomeric gp120 may be due, at least in part, to the inability of these antibodies to achieve the necessary entropic changes that are required for their high-affinity interaction (61); neutralizing antibodies, in contrast, do not incur such an entropic penalty because they require relatively less conformational reorganization within the gp120 molecule for efficient binding. Functional oligomeric gp120 is likely more conformationally fixed owing to interaction with neighboring gp120 protomers within the spike (44), which supports the notion of entropic masking. However, whether entropic masking indeed plays a substantial role in limiting efficient binding of nonneutralizing

antibodies to oligomeric gp120 remains to be determined experimentally.

Substitution of Nonessential Residues

Most recently, the structures of two CD4 mimics in complex with the core structure of YU-2 gp120 were solved (43). Although the mimics contact fewer residues on gp120 than does CD4, the conformation of gp120 is essentially the same as when gp120 is bound to CD4. For one mimic in particular, only half of the residues contacted by CD4 are also contacted by the mimic (43). Thus, not all gp120 residues that are in contact with CD4 in the gp120-CD4 complex are required to induce conformational changes in gp120; i.e., the functional CD4-binding epitope is smaller than its structural epitope. Consequently, antibody recognition of the CD4bs may be easily circumvented by the virus if the ensemble of residues that are critical for antibody binding do not closely match those that are essential for CD4 binding, even if the epitope maps of both ligands overlap geometrically (62, 63).

BINDING SITES FOR SMALL MOLECULE INHIBITORS ON GP120

The structure of unliganded gp120 is helpful to vaccinologists, but could also be useful to those who seek to design small molecules that inhibit HIV entry. A small number of such inhibitors have been described, the most prominent of which is compound BMS-378806 (BMS-806) (64). The mechanism by which this compound blocks HIV entry is disputed. Initial studies had indicated that BMS-806 blocks the gp120-CD4 interaction (65, 66) and thus prevents the virus from attaching to target cells. However, other studies have suggested that the compound does not inhibit CD4 binding to gp120 but, instead, blocks steps that are required to initiate the fusion process by gp41 (67, 68). On the unliganded SIV gp120 structure, the putative binding pocket of BMS-806 overlaps partially with the locations of residues that are presumed to contact CD4 upon binding (**Figure 4**), which supports the notion that BMS-806 may indeed prevent CD4 from binding to gp120. It is worth noting here that BMS-806 can be competed off gp120 at (low) micromolar concentrations of CD4 (65). This observation may explain, at least in part, why BMS-806 did not appear to inhibit CD4 binding in select studies (67, 68), in which micromolar concentrations of CD4 were used in some of the assays. Clearly, further studies are required to address the inhibitory mechanism of compound BMS-806 more thoroughly. Nevertheless, the location of the putative BMS-806 binding pocket indicates that the entire long hydrophobic cavity formed by the inner and outer domains is likely to be an attractive target for small-molecule inhibitors that can prevent binding of CD4 to gp120.

ANTIBODY EPITOPES ON HIV-1 GP120

Neutralizing antibody responses to HIV-1 in vivo are generally of limited breadth (2). However, a handful of human mAbs have been isolated that can neutralize broadly (69). Intense biochemical and immunological study of these antibodies, combined with insight gained from their crystal structures, provides important clues as to HIV's vulnerability to antibody. For gp120, two human mAbs exist that can neutralize a wide range of primary viruses (70). The epitopes of these two antibodies, b12 and 2G12, are, therefore, attractive templates for vaccine design.

Conserved, Cross-Neutralizing gp120 Epitopes

CD4bs: the b12 epitope. The antibody b12, which binds to an epitope that overlaps the CD4bs on gp120 (71, 72), has been studied extensively with regard to its antiviral activity in vitro and in vivo. In a recent comprehensive analysis involving a panel of 90 viruses,

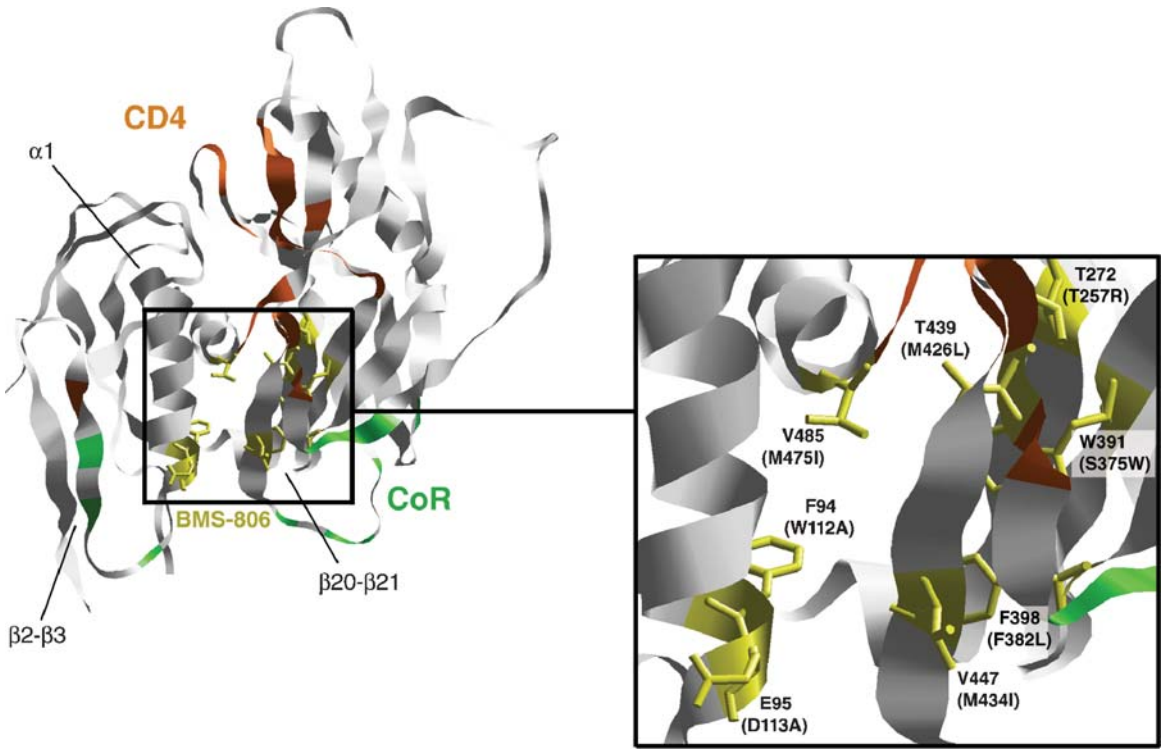

Figure 4

Putative binding site of the small-molecule inhibitor BMS-378806. Map of the sites of mutations (*yellow*) that confer resistance to BMS-806 onto the core structure of SIV gp120. The side chains of residues corresponding to the resistance mutations are shown. The inset shows a close-up of the putative BMS-806 binding pocket. Residues that correspond to resistance mutations are noted, with amino acid substitutions that confer resistance in HIV-1 indicated in parentheses.

the antibody reached an inhibitory concentration [50% (IC_{50})] with approximately half of the viruses within the test panel (70). As such, b12 is currently the most potently and broadly neutralizing anti-gp120 antibody. In vivo studies using macaque models of HIV infection have shown that b12 can also protect animals against viral challenge (73, 74).

IC_{50}: inhibitory concentration (50%)

To gain insight into how b12 is able to neutralize broadly, its structure was determined and, at the time, docked onto the CD4-liganded structure of gp120 (75). Although b12 likely does not interact with the de facto CD4-liganded structure of HIV gp120, the docking model does suggest that b12 may interact with gp120 from an angle that would be permissive in the supposed organization of gp120 in the context of the viral spike. This would explain how b12, in contrast to weakly neutralizing CD4bs antibodies, can effectively bind oligomeric gp120 on the viral surface as well as monomeric gp120 (10). Alanine substitutions that significantly diminish b12 binding affinity form a contiguous binding epitope on the CD4-liganded structure (63) (**Figure 5*a*,*b***). However, when the equivalent substitutions are mapped onto the unliganded SIV gp120 structure, a dispersed pattern results (**Figure 5*c*,*d***). Taken together, these observations suggest that b12 likely binds a gp120 conformation that resembles the CD4-bound structure. As discussed in the previous section, the notion that gp120 can adopt conformations resembling the

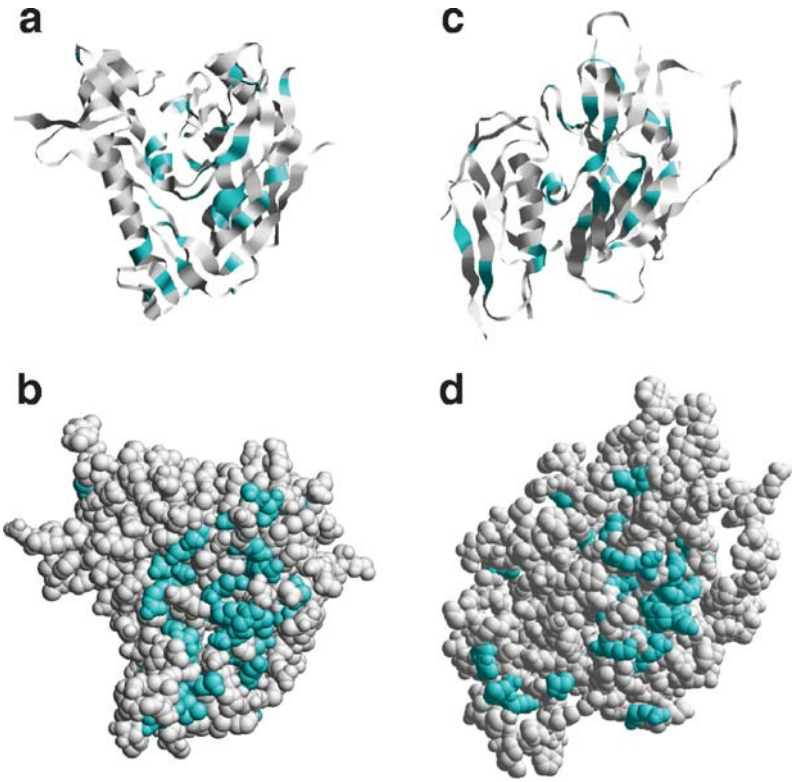

Figure 5

Locations of alanine substitutions that significantly reduce b12 binding. Alanine mutations that caused >50% decrease in apparent b12 binding affinity relative to the apparent antibody binding affinity for wild-type gp120 of HIV-$1_{JR\text{-}CSF}$ were mapped onto the gp120 core structures of HIV-1 and SIV. (*a*) Alanine substitutions in the gp120 core structure of HIV-1, depicted as a ribbon diagram. (*b*) Alanine substitutions in the gp120 core structure of HIV-1, shown as a space-filling model. (*c*) Alanine substitutions in the gp120 core structure of SIV, depicted as a ribbon diagram. (*d*) Alanine substitutions in the gp120 core structure of SIV, shown as a space-filling model. The perspectives are the same as in **Figure 3**.

CD4-bound state is supported by recent molecular dynamics simulations (48). These simulations suggest that the β strands (in particular β2-β3) that comprise the bridging sheet may be highly flexible but that the inner and outer domains of gp120 may largely retain their CD4-bound conformation even when the bridging sheet is unfolded. The seemingly high degree of flexibility of β2-β3 in both the liganded and unliganded structures (48) likely results in movement of V1/V2; this conformational flexibility provides a structural basis for how the V1/V2 regions may generally protect the CD4bs from efficient antibody recognition (2, 44).

Silent face: the 2G12 epitope. A second broadly neutralizing antibody reactive with HIV-1 gp120 is 2G12. This antibody recognizes clustered α1 → 2-linked mannose residues on the distal ends of oligomannose sugars located on the carbohydrate-covered silent face of the gp120 outer domain (76–78). Although the underlying protein surface appears to have an influence on the proper presentation of these sugars to the antibody

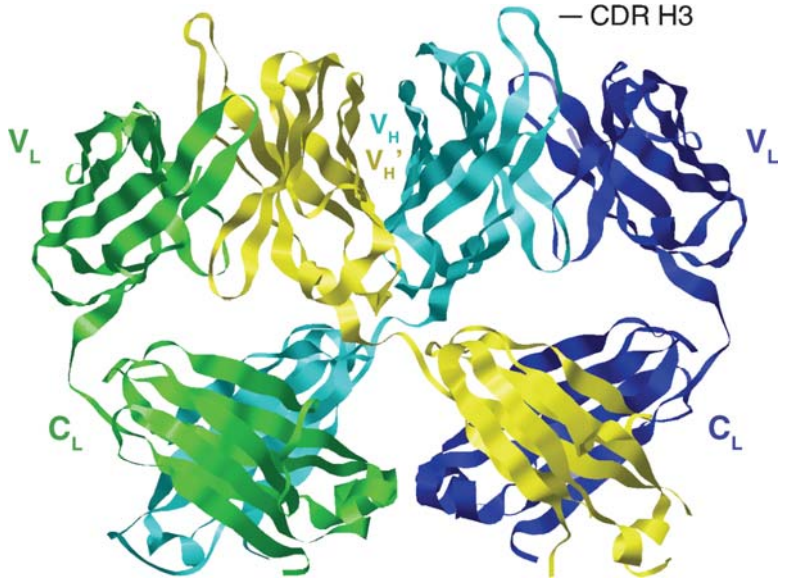

Figure 6

Structure of the domain-swapped Fab molecules of 2G12 as they assemble in the crystal. The Fab light chains (*green* and *blue*) and the corresponding heavy chains (*cyan* and *yellow*) are shown. The V_H/V_H interface and the CDR (complementarity-determining region) H3 are labeled.

(78), there is no evidence for any direct interaction between 2G12 and the side chains of the underlying residues. Like b12, mAb 2G12 has also been studied extensively for its antiviral activity. In vitro, 2G12 achieved an IC_{50} against 41 viruses included in a 90-member virus panel (70), and in passive transfer studies the antibody has been shown to protect against viral infection, particularly when administered in combination with other broadly neutralizing antibodies (79–83). The in vivo efficacy of this antibody has been underscored in a recent study in which it was administered to HIV-positive individuals undergoing interrupted antiretroviral therapy (84). The study showed that acutely infected individuals who were given the antibody had a significant delay in viral rebound compared with controls or chronically infected individuals; the occurrence of 2G12 viral escape mutants shows that the antibody was indeed effective in vivo (84).

The crystal structure of 2G12 (85) reveals that the antibody is able to achieve nanomolar binding affinity to a glycan array owing to the unusual configuration of the antigen-binding fragments (Fabs), which form a domain-swapped Fab dimer (**Figure 6**): Thus, the variable heavy chains (V_H) from each Fab arm have exchanged positions to interact with the light chain (V_L) of the neighboring Fab. The result is a multivalent platform for the binding interaction with carbohydrate (85). The domain swap preserves the conventional antigen binding sites formed by the V_H/V_L interfaces of the two Fab arms, while creating a novel interface for the two V_H domains. Co-crystallization of 2G12 with $Man_9GlcNAc_2$ (85) indicates that this multivalent platform allows 2G12 to interact with up to four oligomannose sugars simultaneously; two of these glycans are located, as expected, within the antigen binding sites formed by V_L and V_H, whereas the other two are bound within the newly formed secondary binding site formed by the V_H/V_H interface. The crystal structure of 2G12 complexed to $Man_9GlcNAc_2$ indicates that the antibody

interacts predominantly with the terminal $\alpha 1 \rightarrow 2$-linked mannose residues that comprise the D1 arm of the oligosaccharide, although more recent studies have shown that the conventional binding sites can also be occupied by the D3 arm (86). These results suggest a lower degree of stringency in the antibody-ligand interaction than previously presumed and are important for the design of synthetic glycoside antigens to elicit 2G12-like anticarbohydrate antibodies.

The 2G12 structure indicates that the humoral arm of the immune system has the capacity to overcome the glycan-masking strategy that HIV employs to protect itself from antibody recognition. However, the uniqueness of the structure does raise the question of whether such domain-swapped antibodies can be elicited by a vaccine. Of note here also is the fact that 2G12, in contrast to mAb b12, is unable to neutralize viruses from clade C (70). Viruses from this clade cause more infections worldwide than viruses from any of the other HIV clades (87). 2G12 also does not frequently neutralize viruses from clades D and AE [circulating recombinant form (CRF) 01]. The general inability of 2G12 to neutralize these viruses is likely due to the absence of one or more glycans that are required for efficient 2G12 binding to gp120 from these viruses (70, 88), although further studies are needed to address this issue fully.

Neutralization-Restricted gp120 Epitopes

V3 region: the 447–52D epitope. The b12 (CD4bs) and 2G12 (glycan) epitopes are presently the most attractive targets for vaccine design owing to their highly conserved nature. Another site on gp120 that has attracted much attention is the epitope in the V3 region recognized by the antibody 447-52D (89). The core sequence of the antibody epitope is Gly-Pro-Gly-Arg (GPGR) (89, 90), which is situated at the center of the V3 region. This sequence, which is predominant in clade B viruses but not in non–clade B viruses (91), causes the V3 region to adopt a marked type-II β-hairpin turn (92, 93); in non–clade B viruses, the Arg residue is generally replaced by Gln, although it appears that the Gln residue preserves the β-hairpin conformation (94). Although the GPGR sequence represents the core epitope for mAb 447-52D, antibody binding is also influenced by certain amino acid substitutions outside of the GPGR sequence, particularly in the N-terminal segment of the V3 region (95, 96).

The X-ray crystal structure of mAb 447-52D in complex with a V3 peptide indicates how this antibody may have the capacity to neutralize more clade B viruses than other anti-V3 antibodies described so far. First, most of the binding interaction between the antibody and the peptide is mediated by main-chain contacts (93), which broadens the ability of the antibody to recognize a variety of V3 sequences. The only side-chain interactions are with the Pro and Arg residues in the GPGR sequence; the side chains from both residues form extensive interactions with residues in the antibody combining site. Second, comparison of the conformation of the 447-52D-complexed V3 peptide to the conformations of V3 peptides solved in complex with other anti-V3 antibodies shows that the side chain of the Arg residue in the GPGR sequence is oriented in the opposite direction in the 447-52D complex relative to its orientation in the other antibody:peptide complexes (93, 96). Thus, the fact that mAb 447-52D neutralizes more viruses than other anti-V3 antibodies that have been described so far may be the result of its unique mode of interaction with V3.

The somewhat broad neutralizing activity of mAb 447-52D (70) makes the antibody epitope a potential vaccine target. However, there are certain caveats associated with targeting the V3 region of HIV-1 (97). For example, some of the main-chain interactions exhibited by 447-52D in binding to the V3 region may be difficult to reproduce upon

CRF: circulating recombinant form (intermixed co-circulating HIV strains)

scFv: single-chain variable fragment (of an antibody)

immunization with antigen. Furthermore, the V3 region on some clade B primary viruses appears to be inaccessible to antibody (70); whether the V3 region in non–clade B viruses is also inaccessible to antibody is uncertain. Occlusion of the V3 region appears to be manifested solely in the context of the gp120 oligomer on the virus (97), and its exposure may be modulated by the presence of glycan moieties (98–104).

Given these caveats, the V3 region may be a target that only yields antibodies with restricted neutralizing ability. It is worth noting here that a 447-52D equivalent has not been identified so far for non–clade B viruses. The V3 region in these viruses contains a GPGQ sequence that is more highly conserved than the GPGR sequence in clade B viruses (91). It is possible, though still largely unproven, that the reduced sequence variability in these viruses may be the result of the V3 region being even less accessible to neutralizing antibodies in non–clade B viruses compared with the V3 region in clade B viruses (97). Experimental support for this notion comes from a recent study that found that although cross-reactive anti-V3 antibodies from individuals infected with a clade A virus were able to neutralize a neutralization-sensitive clade B virus, these antibodies were unable to neutralize the homologous clade A virus or a heterologous clade B virus that is somewhat resistant to neutralization by anti-V3 antibodies, including mAb 447-52D (105). The resistance of these viruses to neutralization was associated with the presence of V1/V2. This observation suggests that the V3 region may be masked by V1/V2 in the context of oligomeric gp120. Although these observations do not unequivocally demonstrate that the V3 regions on non–clade B viruses are inaccessible relative to the V3 region in clade B viruses, they do point to potential difficulties in attempting to elicit cross-clade neutralizing antibodies based on the V3 region.

Coreceptor-binding site. As discussed earlier, the CoRbs is likely not presented until after CD4 has bound gp120. This is reflected in the fact that antibodies to the CoRbs of HIV-1 generally neutralize virus weakly or not at all in vitro, although their ability to neutralize HIV is dramatically enhanced if soluble CD4 is added at sub-neutralizing concentrations (54, 106, 107). The inability of anti-CoRbs antibodies to strongly neutralize HIV in the absence of soluble CD4 is associated with the limited access of these antibodies to the coreceptor site once the virus has engaged membrane-bound CD4 on the target cell (36, 108). Thus, the neutralizing activity of antibodies to the coreceptor site has been shown to be inversely correlated with their size (108, 109); i.e., the neutralizing activity of the single-chain variable fragment (scFv) (~25 kDa in mass) is greater than the neutralizing activity of the Fab fragment (~50 kDa), which is greater than the neutralizing activity of the IgG (~150 kDa). These observations suggest that the CoRbs may not be a good target for vaccine design. However, the conserved nature of the CoRbs does make it an attractive target for small-molecule inhibitors. For example, the chimeric protein sCD4-17b, which is composed of soluble CD4 covalently linked to the scFv of the anti-CoRbs mAb 17b, has been designed as a potential therapeutic agent (110).

V1 and V2 variable regions. Very little is known about the potential of V1 and/or V2 as vaccine targets. However, existing mAbs to epitopes in the V1 or V2 variable regions generally do not exhibit any significant broad neutralizing activity against primary viruses (111–116). Sequence comparison of V1/V2 from different viruses shows that both V1 and V2 exhibit considerable polymorphism in length and sequence (117–120); this polymorphism may explain the inability of antibodies to epitopes in V1/V2 to neutralize broadly. Taken together, these studies indicate that the V1 and V2 regions are likely poor vaccine targets.

Recently, a human mAb (mAb 2909) has been described that is able to bind oligomeric gp120 on the virus but not soluble monomeric

gp120 (57). The epitope of this antibody involves the CD4bs, as well as the V2 and V3 variable regions. Although this antibody only neutralizes the neutralization-sensitive virus SF162, it does so at picomolar antibody concentrations (57). The occurrence of mAb 2909 suggests the possible existence of additional antibodies that are oligomer-specific. Such antibodies, in contrast to mAb 2909, may possess broader neutralizing activity.

ANTIGEN DESIGN STRATEGIES TO ELICIT CROSS-NEUTRALIZING ANTI-GP120 ANTIBODIES

At present, many antigen design strategies are being pursued to evaluate their potential to elicit broadly neutralizing antibodies (121). Two strategies that we focus on here are (*a*) the design of antigens that mimic the functional envelope spike and (*b*) the design of novel, epitope-focused antigens.

Mimicry of the Functional HIV Envelope Spike

Stabilization of recombinant gp120-gp41 complexes. An immense hurdle in the design of antigens that mimic the envelope spike is the stabilization of the gp120-gp41 interaction, which is normally mediated by noncovalent interactions and is relatively labile. An often-used stabilization strategy is mutation of the protease cleavage site between gp120 and gp41 so that the two subunits remain covalently linked upon protein expression (122–130). However, uncleaved envelope proteins appear to be antigenically distinct from their cleaved counterparts (131). This observation is supported by more recent extensive analyses that show that uncleaved gp120-gp41 is antigenically substantially different from its cleaved functional form (24, 132). Thus, whereas both nonneutralizing and neutralizing antibodies bind uncleaved envelope glycoproteins, only neutralizing anti-gp120 antibodies efficiently bind oligomeric glycoprotein when the gp160 precursor protein is effectively cleaved by furin proteases (or equivalent proteases). As an alternative approach to this problem, single cysteine residues have been introduced into gp120 and gp41, which results in the formation of an intermolecular disulfide bridge upon expression of the proteolytically cleaved oligomer (131, 133, 134). To improve cleavage efficiency, optimized cleavage recognition sequences have also been inserted between gp120 and gp41 (135). Oligomeric envelope complexes generated by this approach have better antigenic qualities than uncleaved complexes (134). However, the disulfide bridge-stabilized complexes still display epitopes that are recognized by nonneutralizing antibodies (134), which suggests that mimicry of the native envelope glycoprotein is not fully achieved with these molecules.

Trimerization of recombinant gp120-gp41 complexes. A further challenge in designing envelope spike mimics is the preservation of the trimeric state of the antigen complex. Thus, although mutation of the protease cleavage site or the introduction of a disulfide bridge allows gp120 and gp41 to remain covalently linked, soluble oligomers derived in this manner often either disassociate into single heterodimers of gp120 and gp41, or tend to assemble into varying oligomerization states, such as dimers, trimers and/or tetramers (123, 126–128, 130, 136, 137). One of the strategies that has been employed to circumvent these problems is covalent linkage of heterologous trimerization domains to the C terminus of recombinant oligomeric proteins (138–140). These domains indeed improve trimerization, and the corresponding proteins elicit neutralizing antibodies at modestly improved levels compared with preparations that contain a mixture of oligomeric proteins (141). Notably, immunization studies show that purified trimeric glycoproteins—independent of whether or not they are stabilized by a heterologous trimerization domain—are somewhat superior at eliciting cross-neutralizing

Immunodominant: the epitope on a molecule that provokes the most intense immune response

antibodies as compared to simple monomeric gp120 formulations (142–144). Also of note is that the elicited neutralizing antibodies appear to be directed to epitopes other than the immunodominant V3 region. Further strategies to stabilize the trimeric envelope complex involve the introduction of mutations in the gp41 subunit. For example, cysteine residues have been inserted into the N-terminal heptad repeat of gp41 to introduce intramolecular disulfide bridges (124, 130). This approach has been shown to increase the stability of oligomeric envelope proteins, in particular oligomers that contain appended trimerization domains (124). However, the antigenic profile of such preparations does not seem to improve with the added disulfide bridge (124); immunogenicity studies with these disulfide-stabilized glycoproteins have not been reported. Mutations have also been introduced into gp41 to prevent it from adopting a postfusion conformation and stabilize it in a supposed native-like, prefusogenic conformation (145). Immunogenicity studies in rabbits with oligomeric preparations containing cleaved, disulfide-bridged gp120/gp41, in conjunction with a gp41 prefusion conformation-stabilizing mutation, have recently been reported (146). In a subset of animals, antibodies were elicited that could neutralize the homologous virus that is moderately resistant to neutralizing antibodies. However, direct comparison with neutralizing antibody responses elicited by immunizing with monomeric gp120 was not possible owing to the design of the study (146). Thus, it remains unclear to what extent the introduced modifications are able to enhance the ability of oligomers to elicit cross-neutralizing antibodies.

Presentation of the gp120-gp41 complex on particles. Other antigen design strategies have attempted to present the oligomeric envelope spike in a more native environment, for example, through mild chemical inactivation of virus particles (which appears to preserve the oligomerization state and functionality of the envelope spike) (147, 148) or, alternatively, through the incorporation of uncleaved oligomeric envelope glycoprotein complexes into proteoliposome preparations (149). Though promising, both of these two approaches have so far failed to elicit neutralizing antibodies of the desired breadth and potency (142, 150, 151).

Another attractive approach to presenting the envelope spike in a native-like context is the generation of virus-like particles (VLPs) (152). VLPs represent a unique class of subunit antigens, as they may allow closer mimicry of structures on the surface of virions compared with other types of antigen presentation. VLPs also have the advantage that, in contrast to soluble subunit antigens, they are able to stimulate both cellular and humoral responses, although, unlike live or inactivated virus, they do not contain viral genetic material (152). So far, however, the VLP approach has not achieved the desired success in eliciting high levels of cross-neutralizing antibodies (153–155). A possible reason for the inability of current VLP preparations to elicit more broadly neutralizing antibodies may be due to the expression of both nonfunctional as well as functional forms of the spike on the surface of the virus particle (12, 24). The elicitation of antibodies to cellular proteins that are incorporated into the virus particle may also limit the efficacy of VLP preparations.

Overcoming low spike immunogenicity and genetic diversity. The relatively low accessibility of conserved antibody epitopes on the envelope spike poses another strategic hurdle to the pursuit of envelope spike mimicry as a vaccine approach. To enhance the elicitation of broadly neutralizing antibodies, variant trimeric envelope glycoproteins have been designed with truncated or altered variable regions (156–159). In some cases, alterations have also been made in gp41. Although some of these alterations have resulted in an improvement in the neutralizing capacity of the immune sera (156), additional modifications and other improvements will

be necessary to significantly boost the ability of these envelope spike mimics to elicit antibodies that are capable of neutralizing a wide range of primary viral isolates.

Among the approaches that are currently being evaluated to overcome HIV's genetic diversity are multivalent antigen cocktails (160–163) and the use of antigenic formulations based on consensus or ancestral HIV sequences (164–167). The latter are based on computer-generated "centralized" HIV gene sequences (165, 167). These artificial sequences are genetically equidistant to the sequences of circulating viruses at a given time point, as are the proteins derived from these sequences. It is postulated that immunization with these centralized sequences will yield antibodies to multiple conserved regions across many different viral subtypes (164, 165). So far, however, the multivalent strategies as well as the consensus/ancestral envelope approaches have failed to significantly improve the breadth of the neutralizing antibody response (161, 166). In the multivalent approach, incorporation of additional envelope glycoproteins does result in an increase in the number of viruses that are neutralized, but the observed increase seems to stem from the sum of neutralizing antibodies elicited by the individual components rather than from a significant increase in the overall quality and breadth of the neutralizing antibody response (161). The notion that the observed neutralizing antibody responses are additive and not qualitatively enhanced likely limits the practical application of this approach, as it would probably require the screening and incorporation of numerous envelope glycoproteins to cover all current and future circulating viruses. Similarly, serum antibodies elicited by current consensus envelope glycoproteins do not seem to neutralize a substantially broader range of viral isolates than antibodies elicited by wild-type envelope proteins. These observations suggest that the current synthetic molecules do not display the anticipated conserved regions, or do so in a manner that is not replicated on the functional spike (165, 166). Thus, like wild-type proteins, these synthetic proteins will likely also have to be modified to increase their potential to elicit the desired cross-neutralizing antibodies (166).

N-glycan: a carbohydrate chain covalently linked to an Asn residue in the consensus sequence -Asn-Xaa-Ser/Thr (Xaa: any amino acid except Pro)

Epitope-Focused Antigen Design

Despite incremental progress in the ability of current oligomeric glycoprotein antigens to elicit broadly neutralizing anti-HIV antibodies, type-specific neutralizing antibodies remain the predominant fraction among antibodies that are contained in immune sera. Epitope-focused antigen design may provide a means by which to limit the induction of such antibodies, while preserving the elicitation of antibodies to more conserved epitopes (168). So far, this approach has been aimed mainly at attempts to elicit b12- and 2G12-like antibodies.

Directing antibody responses to the b12 epitope. Antibody responses to gp120 can be influenced by placing glycans at certain positions in the molecule (169). To direct antibody responses to the b12 epitope, monomeric gp120 has been engineered to contain a range of additional N-glycan sites at various locations throughout the molecule to hide gp120 epitopes that could elicit non- or weakly neutralizing antibodies, without altering the accessibility of the b12 epitope (170, 171). This so-called hyperglycosylation strategy has led to the design of a panel of novel glycoproteins that exhibit superior antigenic qualities relative to unmodified gp120, judging from the inability of these modified antigens to be recognized by numerous non- and weakly neutralizing antibodies (170, 171). A recently completed study in which rabbits were immunized with a first-generation of modified antigens did not reveal any improvement in the breadth of serum neutralization compared with sera from animals immunized with wild-type gp120 (172); the induction of antibodies to undesired epitopes was reduced, but no increase in neutralizing antibody responses to the CD4bs was

observed in return. Clearly, further iterative modifications to the design of these antigens will be necessary to boost their ability to elicit neutralizing antibodies, also taking into account factors that may limit the immunogenicity of these antigens (173).

CDR: complementarity-determining region (of an antibody)

Immunity: a natural or acquired resistance to a specific disease; immunity may be partial or complete, long lasting or temporary

Generation of 2G12-like antibodies. Efforts are currently also underway to design an antigen that will elicit antibodies that target the carbohydrate epitope recognized by mAb 2G12. The structure of 2G12 in complex with various carbohydrate ligands shows that the antibody interacts mainly via residues in CDR L3 and CDR H1–H3 with the $\alpha 1 \rightarrow 2$ terminal portion of oligomannose sugars and synthetic glycosides (85, 86); synthetic glycosides and glycans derived from natural sources that contain such $\alpha 1 \rightarrow 2$-linked mannose residues indeed are best at inhibiting 2G12 binding to monomeric gp120 (174–178). However, inhibition of 2G12 binding with monovalent sugars requires concentrations in the millimolar range (174, 176, 178); in contrast, 2G12 binds monomeric gp120 with nanomolar affinity (76), which likely results from its multivalent interaction with gp120. In an attempt to reproduce the high-mannose clustering on gp120, synthetic glycosides and Man9 sugars have been conjugated to protein carrier molecules or to synthetic scaffolds (174–178; H.-K. Lee, C.-Y. Huang, R. Astronomo, C.S. Scanlan, R. Pantophlet, I.A. Wilson, C.-H. Wong & D.R. Burton, unpublished data). Multivalent display of these sugar molecules improves their ability to inhibit 2G12 binding to gp120 ~1000-fold into the micromolar range. Although the improvement in binding affinity by multivalent display is appreciable, these studies clearly show that additional fine-tuning of such multivalently displayed platforms will be required to further improve 2G12 binding affinity, which, by inference, should improve the ability of these antigenic formulations to elicit 2G12-like antibodies. Thus, it is likely that the glycan clusters on current glyco-mimics do not adequately resemble the cluster of glycans on gp120. Improvements to the design of these glyco-antigens will likely require de novo design of synthetic scaffolds that are able to display a high density of oligomannose sugars or synthetic glycosides in spatial orientations that better mimic the 2G12 epitope.

CONCLUDING REMARKS

Advances in HIV therapy have been successful in prolonging the lives of HIV-infected individuals, particularly those who live in the U.S. and Europe. However, in Asian and African nations, the spread of HIV remains a major health concern with dramatic socio-economic consequences. Development of a vaccine offers the best hope of containing the AIDS pandemic. The failure of recent phase III clinical trials that used monomeric gp120 as a candidate vaccine underscores the inability of simple subunit HIV antigens to induce broad humoral immunity, as well as the need for novel scientific approaches to obtain an AIDS vaccine. In this review, we summarize some of the strategies that are currently being pursued to generate an AIDS vaccine component that elicits broadly neutralizing antibodies. These strategies have profited from the elucidation of the crystal structures of various broadly neutralizing mAbs and of the core gp120 structures of HIV and (most recently) of SIV. This structural insight has allowed vaccinologists to understand better the defense mechanisms of the virus, while highlighting chinks in HIV's defensive armor. Efficient exploitation of these chinks will require substantial advances in antigen design, combined with further insight from X-ray crystallography and the identification of additional neutralizing antibody epitopes (see Future Issues To Be Resolved, below). By correlating the antigenic properties of novel antigens to their immunogenicity, it should then be possible to optimize promising antigenic formulations and forward them as potential vaccine candidates into human clinical and efficacy trials.

SUMMARY POINTS

1. The crystal structures of the gp120 cores from HIV-1 and SIV reveal that the viruses are able to minimize effective antibody recognition of conserved regions by restricting access to these regions by bulky carbohydrate chains or variable regions and by only fully assembling functional sites once the interaction between virus and target cell has been initiated.
2. Although oligomeric envelope glycoprotein antigens seem superior at eliciting neutralizing antibodies relative to simple monomeric gp120 formulations, further modifications will be required to optimize the ability of these antigens to elicit broadly neutralizing antibodies.
3. Three epitopes defined by three different mAbs (two broadly neutralizing and one lesser cross-neutralizing) have been identified so far on HIV-1 gp120; specific targeting of these epitopes remains a major challenge in HIV vaccine design.

FUTURE ISSUES TO BE RESOLVED

1. The crystal structures of additional envelope glycoproteins need to be elucidated. The solution of the structures of full-length monomeric gp120 and of the gp120-gp41 trimer complex will provide both (*a*) further insight into how HIV protects itself from antibody and (*b*) possible ways to circumvent these barriers.
2. The broad-neutralizing antibody activity in the sera of long-term HIV-infected individuals needs to be dissected to allow additional neutralizing antibody epitopes on the virus to be identified; these epitopes could then become targets for vaccine design.
3. A comprehensive analysis of antibody responses in sera from immunized animals is required. Delineation of the epitopes recognized by antibodies induced by potential vaccine antigens may reveal correlations between antigenicity and immunogenicity. This information would allow further optimization of the antigens for the elicitation of broadly neutralizing antibodies.
4. An evaluation of the influence of molecular adjuvants on the levels of elicited neutralizing antibodies is also required. The combination of immunostimulatory molecules or equivalent agents with potential vaccine antigens may allow for the improvement of neutralizing antibody titers upon immunization.

NOTE ADDED IN PROOF

Very recently, the crystal structure of a gp120 core of HIV-1 containing the V3 variable region was reported (179). In the structure, which was determined in complex with CD4 and the Fab fragment of an antibody to the CoRbs, the V3 region extends approximately 30 Å away from the core. The extended conformation of the V3 region may explain why anti-V3 antibodies are readily elicited upon immunization with monomeric gp120. Whether V3 is similarly extended in the absence of CD4 and, more importantly, in the context of the viral spike, remains to be determined.

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