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Analysis of the Interaction of the Human Immunodeficiency Virus Type 1 gp120 Envelope Glycoprotein with the gp41 Transmembrane Glycoprotein

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The human immunodeficiency virus type 1 (HIV-1) gp120 exterior envelope glycoprotein interacts with the viral receptor (CD4) and with the gp41 transmembrane envelope glycoprotein. To study the interaction of the gp120 and gp41 envelope glycoproteins, we compared the abilities of anti-gp120 monoclonal antibodies to bind soluble gp120 and a soluble glycoprotein, sgp140, that contains gp120 and gp41 exterior domains. The occlusion or alteration of a subset of gp120 epitopes on the latter molecule allowed the definition of a gp41 "footprint" on the gp120 antibody competition map. The occlusion of these epitopes on the sgp140 glycoprotein was decreased by the binding of soluble CD4. The gp120 epitopes implicated in the interaction with the gp41 ectodomain were disrupted by deletions of the first (C1) and fifth (C5) conserved gp120 regions. These deletions did not affect the integrity of the discontinuous binding sites for CD4 and neutralizing monoclonal antibodies. Thus, the gp41 interface on the HIV-1 gp120 glycoprotein, which elicits nonneutralizing antibodies, can be removed while retaining immunologically desirable gp120 structures.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS in humans (3, 23, 29). Immunodeficiency in HIV-1-infected humans results from a progressive decline in CD4-positive lymphocytes (21).

The entry of HIV-1 into host cells is mediated by the viral envelope glycoproteins, gp120 and gp41, which are derived by proteolytic cleavage of a gp160 precursor in the Golgi apparatus of the infected cell (2, 20, 62). The gp120 exterior envelope glycoprotein is noncovalently linked to the gp41 transmembrane envelope glycoprotein (20, 41, 46). The HIV-1 envelope glycoproteins are assembled into an oligomeric complex that is transported to the cell surface and is incorporated into budding virions (19, 20, 58, 66). During HIV-1 entry into the target cell, the gp120 exterior envelope glycoprotein binds the CD4 glycoprotein, which serves as a primary receptor for the virus (14, 40, 45). A soluble form of CD4 (sCD4) can inhibit the entry of HIV-1 strains that have been passaged in vitro in transformed T-cell lines (9, 15, 24, 34, 69, 79), and sCD4 can also induce shedding of gp120 from cells or virions possessing HIV-1 oligomeric envelope glycoprotein complexes derived from the laboratory-adapted strains (6, 27, 30, 48, 49, 76). CD4 binding induces conformational changes in the HIV-1 envelope glycoproteins that are postulated to promote subsequent steps in virus entry (64, 65). Members of the family of seven membrane-spanning chemokine receptors have been shown to facilitate the entry of specific HIV-1 variants (1, 13, 16–18, 22). A direct interaction between gp120-CD4 complexes and specific chemokine receptors has been demonstrated (42, 80, 84). Successful HIV-1 entry culminates in the fusion of viral and host cell membranes. By analogy with influenza virus entry (83), the generation of an extended conformation in the gp41 ectodomain is believed to allow the amino terminus of this protein to interact with the target cell membrane (25, 44). Fusion of the viral and host cell membranes allows entry of the viral core and genetic material into the target cell cytoplasm (31, 41, 72).

It has been difficult to obtain crystals of the HIV-1 envelope glycoproteins suitable for detailed structural analysis. Therefore, most of the available information on the structure and function of the HIV-1 envelope glycoproteins has been deduced from biochemical, mutagenic, and antibody binding analyses. Most of the surface-exposed elements of the oligomeric envelope glycoprotein complex are contained on the gp120 exterior envelope glycoprotein (51). When the gp120 glycoproteins derived from different primate immunodeficiency viruses are compared, five conserved regions (C1 to C5) and five variable regions (V1 to V5) can be identified (56, 70). Intramolecular disulfide bonds in the gp120 glycoprotein result in the incorporation of the first four variable regions (V1 to V4) into large loop-like structures (43). Antibody binding studies indicate that the loops are well exposed on the native HIV-1 gp120 glycoprotein, in contrast to the case for most linear epitopes located in conserved regions (51, 52).

Discontinuous structures that are conserved among HIV-1 isolates and that are related to the CD4 binding site (CD4bs) can serve as targets for neutralizing antibodies (33, 38, 39, 53, 60, 71, 74, 75, 77, 78). One group of these conserved epitopes is recognized by antibodies that are abundant in the sera of most individuals infected by HIV-1 for over 1 to 2 years (53, 74). These antibodies compete with CD4 for gp120 binding. The binding of these antibodies is disrupted by changes in conserved gp120 amino acids that have also been implicated in CD4 binding (74, 75). Thus, these epitopes are referred to as CD4bs epitopes.

A second group of neutralizing antibodies recognizes dis-

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continuous gp120 epitopes that are exposed better upon CD4 binding and thus are referred to as CD4-induced (CD4i) epitopes (77, 86). The CD4i epitopes are extremely sensitive to conformational changes in the gp120 glycoprotein, and the integrity of these epitopes is affected by a number of gp120 amino acid changes involving each of the five conserved regions (77).

Antibody mapping studies indicate that of the linear epitopes on the gp120 glycoprotein, those located in the V2 and V3 regions constitute the most exposed elements on the HIV-1 oligomeric envelope glycoprotein complex (51). Both V2 and V3 loops can serve as targets for neutralizing antibodies (28, 37, 47, 50, 61, 63, 68, 73). Changes in V2 and V3 loop amino acids have been shown to affect the membrane fusion process (4, 26, 31, 57, 59, 75, 84) and can determine the tropism of HIV-1 isolates for primary macrophages and T-lymphocyte lines (7, 10–12, 35, 36, 67, 69, 81, 82, 85). The specific structure of the V3 loop dictates which particular chemokine receptor can be utilized as an entry cofactor by HIV-1 (1, 13, 16–18, 22), and binding of the gp120-CD4 complex to the chemokine receptor is dependent, in part, upon the presence of the V3 loop (80, 84).

Consistent with the proposal that the V1/V2 and V3 loops represent surface-exposed elements on the native gp120 glycoprotein, these regions can be deleted from the HIV-1 gp120 glycoprotein without globally disrupting the conformation of the protein (59, 85, 86). Depending upon the specific deletions made, variable loop-deleted gp120 glycoproteins have been shown to retain the ability to bind sCD4 and monoclonal antibodies directed against CD4bs and CD4i epitopes (59, 85, 86). Interestingly, the exposure of CD4bs and CD4i epitopes on such deleted glycoproteins is greater than that on the wild-type gp120 glycoprotein (85, 86), suggesting that the V1/V2 and V3 loops mask these conserved neutralization epitopes. The improved exposure of the CD4i epitopes that occurs upon sCD4 binding appears to involve a demasking of these epitopes mediated by movement of the V2 loop (86).

Recently, a comprehensive competition map of the monomeric HIV-1 gp120 glycoprotein, made by employing an extensive panel of monoclonal antibodies, has been completed (55). This map suggests that CD4 and neutralizing antibodies bind to regions that are clustered on one surface of the gp120 glycoprotein. The ability of these antibodies to neutralize HIV-1 suggests that this surface is exposed on the assembled envelope glycoprotein oligomer. By contrast, antibodies that lack neutralizing activity recognize epitopes that are clustered on a second distinct surface of the gp120 molecule. Some of these epitopes were disrupted by single amino acid changes in the first (C1) and fifth (C5) gp120 regions, which have been implicated in the noncovalent association with the gp41 ectodomain (8, 32). This observation suggested the hypothesis that the interaction of gp120 with the gp41 exterior domain renders the epitopes recognized by nonneutralizing antibodies inaccessible on the assembled envelope glycoprotein complex (54, 55). Here we test this hypothesis by examining the ability of the gp41 ectodomain to compete, in the context of a soluble gp120-gp41 molecule, with monoclonal antibodies recognizing gp120 epitopes. We also determine the effects of gp120 deletions in the C1 and C5 regions on the integrity of gp120 epitopes, in the absence and presence of the major variable loops V1, V2, and V3.

MATERIALS AND METHODS

Plasmid construction and protein production. Soluble HXBc2 gp120 glycoproteins were expressed following calcium phosphate-mediated transient cotransfection of 293T cells with 25 μg of the pSVIIIenvΔ517 expressor plasmid, which encodes a gp120 glycoprotein with seven additional C-terminal residues (41), and 1 μg of a Tat expression plasmid. At approximately 36 h after transfection, radiolabelled glycoproteins were produced by incubation of 293T cells with medium containing 50 μCi of [^{35}S]methionine per ml for 16 h.

Expression of soluble HXBc2 gp140 glycoproteins was accomplished as follows. A stop codon was introduced following the codon for residue 681 in the pSVIIIenv 681Y/P mutant (8) by digestion of the *Msc*I site created by the introduced mutation and blunt-end ligation of an 8-bp *Xba* linker (CTCTAGA G). The construct was then digested with *Xba*I, which released a 5.5-kb fragment containing *env* sequences and a 2.5-kb fragment. The *env*-containing *Xba*I-*Xba*I vector fragment was religated and utilized to transform XL1-Blue *Escherichia coli*. The plasmid DNA was purified and digested with *Bsm*I and *Xba*I to release a 224-bp fragment containing the introduced stop codon. Next, site-directed mutagenesis was used to introduce two mutations into the pBSKS-AB plasmid, which contains the *Asp*718-*Bam*HI HXBc2 *env* sequence subcloned into a pBluescript plasmid, changing the codons for serines 508 and 511 to those encoding arginine. The resulting plasmid, pBSKS-ABcl−, encodes an envelope glycoprotein that is unable to be processed proteolytically at the gp120/gp41 junction. Sequences containing these changes were isolated by *Asp*718 and *Bsm*I digestion of the pBSKS-ABcl− plasmid. The *Asp*718/*Bsm*I fragment and the 224-bp *Bsm*I/*Xba*I fragment (described above) were then ligated into the intermediate vector pMtgp120del0, which had been digested with *Asp*718 (partially) and *Nhe*I. This three-way ligation created a contiguous *env* coding sequence for a cleavage-defective, soluble gp140 (sgp140) glycoprotein. This plasmid was then used as a template to PCR amplify sgp140-coding sequences with 5′ *Asp*718 and 3′ *Bam*HI restriction enzyme sites (primers rw20100 [GACGTCGGTACCAATTCGTTG CAGGACAGG] and rw22453 [AAAAAAGGATCCTCTCTAGAGCCAATTT GTTATGTTAAA]), which were used to directionally clone into the *Asp*718- and *Bam*HI-digested pSVIIIenv plasmid to create pSVIIIexgp140cl−. For the pSVIIIexgp140cl− construct, expression of the Rev protein was provided in *trans* by cotransfection of the pSVIIIenvΔKS plasmid, which expresses a full-length Rev protein but has an extensive deletion in the envelope gene (41). Cotransfection of pSVIIIexgp140cl− with the Tat expressor plasmid and the pSVIIIenvΔKS plasmid and metabolic labelling of envelope glycoproteins were performed as described above.

Intermediate cloning vectors were generated to allow production of plasmids expressing gp120 glycoproteins with conserved region 1 (C1) deletions as follows. The *Sal*I-to-*Bam*HI fragment of pSVIIIexE7, which contains HXBc2 *rev* and *env* coding sequences, was subcloned into the pBSKS+ plasmid digested with *Sal*I and *Bam*HI to create pBSKS+SB. An *Sph*I site was introduced into this plasmid at the HXBc2 proviral positions 5899 to 5904 by PCR amplification of sequences from *Sal*I to *Asp*718 and subcloning of the PCR product back into *Sal*I/*Asp*718-digested pBSKS+S. The *Sph*I site was included in the downstream amplimer which spanned the *Asp*718 site at positions 5925 to 5930, introducing the *Sph*I site, to create pBSKS+SSB. A similar construct, pBSKS+SBB, was generated by site-directed mutagenesis of single-stranded pBSKS+SB DNA, which introduced a *Bgl*II site at the same position (5899 to 5904), and was used in the cloning strategy described below.

The Δ50, Δ74, Δ82, and Δ93 constructs were created by PCR amplification of wild-type or variable loop-deleted HXBc2 sequences by utilizing 5′ amplimers possessing *Sph*I and *Bam*HI sites positioned adjacent to sequences homologous to *env* coding sequences beginning at the codons for residues 51, 75, 83, and 94, respectively. The 3′ amplimer was positioned downstream of the *Nhe*I site at the proviral positions 6838 to 6843, which are 3′ to the coding sequences for the V1, V2, and V3 variable gp120 loops. HXBc2 sequences containing the various C1 deletions were amplified by utilizing the pBSKS-AB plasmid as template DNA. The plasmid pBSKS-ABΔV1/2/3, which contained deletions of coding sequences for residues 121 to 204 and 298 to 329, was used as the template to amplify ΔC1ΔV1/2/3 sequences in a similar manner. The amplified products were digested with *Sph*I and *Nhe*I and cloned into pBSKS+SSB digested with *Sph*I and *Nhe*I. The various pBSKS+SSBΔC1 constructs, with or without loop deletions, were then digested with *Bam*HI, and the *env*-containing sequences were shuttled into the pBSKS+SBB plasmid (described above) digested with *Bgl*II and *Bam*HI, to create pBSKS+SBBΔC1env plasmids. From the various pBSKS+BBΔC1env plasmids, *rev* and *env* sequences were isolated by *Sal*I and *Bam*HI digestion and shuttled into the pSVIIIexenv backbone created by digestion with *Sal*I and *Bam*HI. Cotransfection of the various pSVIIIΔC1env plasmids and the pSVIIIΔC1ΔV1/2/3 plasmid with the Tat expressor plasmid and metabolic labelling of gp120 glycoproteins were performed as described above.

The deletion of HXBc2 gp120 C5 sequences from residue 493 to the natural cleavage site at residue 511 was accomplished by site-directed mutagenesis of single-stranded pBSKS-AB plasmid DNA, which introduced two tandem stop codons after the codon specifying the glutamic acid residue at position 492. The presence of these stop codons was detected in the plasmid DNA by the presence of an *Xba*I site, which had been included in the mutagenic primer as an in-frame substitution at sequences previously specifying Env residues 495 and 496. The *Nhe*I/*Bam*HI fragment containing the premature stop codon was then subcloned into the pBSKS-ABΔV1/2/3 plasmid and into the pBSKS+ clones containing each of the ΔC1 and ΔC1ΔV1/2/3 sequences. The Δ128-194/Δ298-329 and Δ128-194/Δ303-321 deletions were subcloned from pBluescriptAB envelope constructs containing these deletions (85, 86) into the pBSKS+SBBΔC1ΔC5 plasmids by *Nsi*I-*Nhe*I directional cloning. The wild-type and loop-deleted pBSKS-ΔC5 *env*

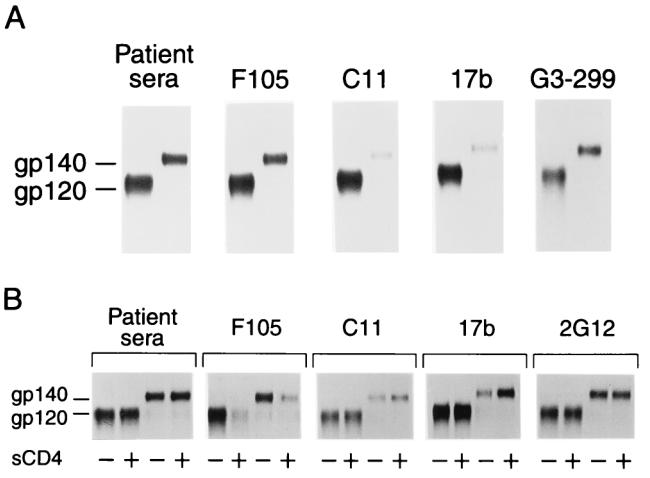

FIG. 1. Immunoprecipitation of soluble gp120 and gp140 glycoproteins. (A) The recognition of gp120 glycoproteins and of soluble, cleavage-defective gp140 glycoproteins by a panel of gp120-directed monoclonal antibodies was compared. Selected representative immunoprecipitations are shown. Pooled AIDS patient antisera were used to assess total envelope glycoprotein protein present in the radiolabelled 293T cell supernatants. Quantitation of recognition by the complete set of antibodies used for immunoprecipitations is presented in Fig. 2. (B) Recognition of envelope glycoproteins in the absence and presence of sCD4. The partial reexposure of the C11 and 17b epitopes on sgp140 glycoproteins in the presence of sCD4 is evident.

sequences were subcloned into pSVIIIexenv by *Asp*718-*Bam*HI directional cloning. Envelope sequences from the pBSKS+SSB clones containing C1 deletions, the C5 premature stop codon, and either wild-type or loop-deleted sequences were subcloned into pSVIIIexenv by *Sal*I-*Bam*HI directional cloning. Cotransfection of the various pSVIIIΔC1ΔC5 constructs and the pSVIIIΔC1ΔV1/2/3ΔC5 construct with the Tat expressor plasmid and metabolic labelling of gp120 glycoproteins were performed as described above.

Immunoprecipitations of radiolabelled envelope glycoproteins. Approximately 0.5 ml of medium containing radiolabelled full-length gp120 or gp140 glycoproteins was immunoprecipitated with 1 μg of antibody and protein A-Sepharose beads for 2 h at room temperature, followed by three washes with phosphate-buffered saline and resuspension in sodium dodecyl sulfate (SDS) gel loading buffer containing β-mercaptoethanol. The samples were then boiled for 3 min, loaded onto SDS–10% polyacrylamide gels, and prepared for autoradiography as previously described (85).

The C-terminally and N/C-terminally deleted gp120 variants, either with or without the major variable loops, were analyzed as described above, with the exception that immunoprecipitations and washes were done at 4°C. The wild-type gp120 glycoprotein was used as a positive control in these experiments.

RESULTS

Exposure of epitopes on soluble forms of HIV-1 gp120 and gp140 glycoproteins. The exposure of epitopes on the HIV-1 gp120 and gp160 glycoproteins was compared by using soluble forms of these proteins. By deleting the transmembrane and intracytoplasmic domains and by altering the amino acid residues near the site of proteolytic cleavage, a soluble glycoprotein, sgp140, that contains covalently linked gp120 and gp41 exterior domains was produced. Radiolabelled gp120 and sgp140 glycoproteins from the laboratory-adapted HXBc2 strain of HIV-1 were produced in transfected 293T cell supernatants and used for immunoprecipitation by a panel of gp120-reactive monoclonal antibodies. The results shown in Fig. 1 indicate that a mixture of sera from HIV-1-infected individuals, which has been shown to recognize a variety of different gp120 and gp41 epitopes (53), precipitated both gp120 and sgp140 glycoproteins. Two patterns were seen when the monoclonal antibodies were used. One group of antibodies recognized both gp120 and sgp140 glycoproteins efficiently. Recognition of the sgp140 glycoprotein by these antibodies was at least 60% of that of the gp120 glycoprotein when the relative amounts of these proteins were normalized by using the patient sera. These antibodies and their epitopes included F105 and 21h (CD4bs), G3-4 and G3-136 (V2), G3-299 (V3/C4 discontinuous), G3-519 and G45-60 (C4 linear), 2G12 (C3/V4 discontinuous) and 0.5β, D47, and 110.I (V3 linear). For a second group of antibodies, recognition of the sgp140 glycoprotein was significantly less efficient than that of the gp120 glycoprotein. These antibodies and their epitopes included M85, 4D4 #85 and 133/290 (C1 linear), 17b and 48d (CD4i), C11 and 212A (C1/C5 discontinuous), 2.3A (C5 linear), M90 and #45 (C1 discontinuous), and A32 and 2/11c (C1/C4 discontinuous) (Fig. 2). These results suggest that the epitopes for some gp120-reactive antibodies are occluded or disrupted in the sgp140 glycoprotein (Fig. 3).

We wished to examine whether the inefficient recognition of some gp120 epitopes on the sgp140 glycoprotein was a consequence of the residue changes introduced near the proteolytic cleavage site. Studies similar to those described above were carried out with a sgp140 glycoprotein containing wild-type sequences near the cleavage site. Since this glycoprotein was only partially processed, both cleaved (gp120) and uncleaved (sgp140) forms were present in the same cell supernatant. A selected subset of anti-gp120 monoclonal antibodies was used to precipitate these supernatants. The relative recognition of the sgp140 and gp120 glycoproteins by the 2G12, C11, and 2.3A antibodies in this experiment was similar to that seen in the experiment described above (data not shown). The results with the C11 and 2.3A antibodies are particularly noteworthy, since gp120 sequences near the gp120-gp41 cleavage site have been implicated in the formation of the epitopes for these antibodies (52, 62a). We conclude that the poor recognition of some epitopes on the sgp140 glycoprotein is not due to the amino acid changes introduced near the proteolytic cleavage site.

The lower efficiency of epitope recognition on sgp140 compared with gp120 could result from effects of the gp41 ectodomain in the context of either a monomeric gp120-gp41 subunit or a soluble oligomer. Soluble HIV-1 envelope glycoproteins equivalent to sgp140 have been reported to exhibit some higher-order structure (19). To examine whether oligomerization might contribute to the observed decreases in exposure of some epitopes, radiolabelled cell supernatants containing sgp140 were precipitated by the T4 monoclonal antibody,

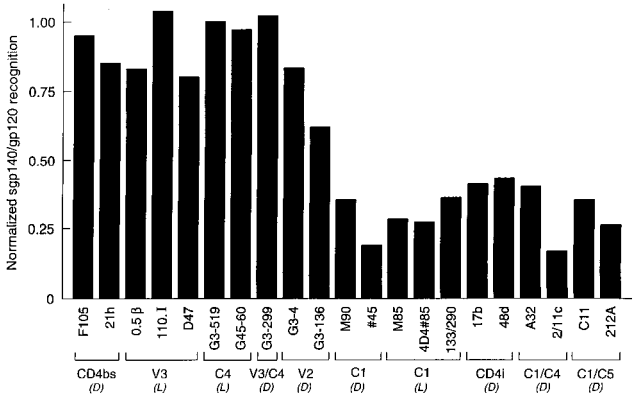

FIG. 2. Relative recognition by gp120-directed antibodies of sgp140 glycoproteins compared to that of gp120 glycoproteins. The data is presented as the ratio of gp140 recognition to gp120 recognition normalized to the gp140/gp120 recognition ratio observed with a mixture of sera from HIV-1-infected individuals. *L*, linear epitope; *D*, discontinuous epitope.

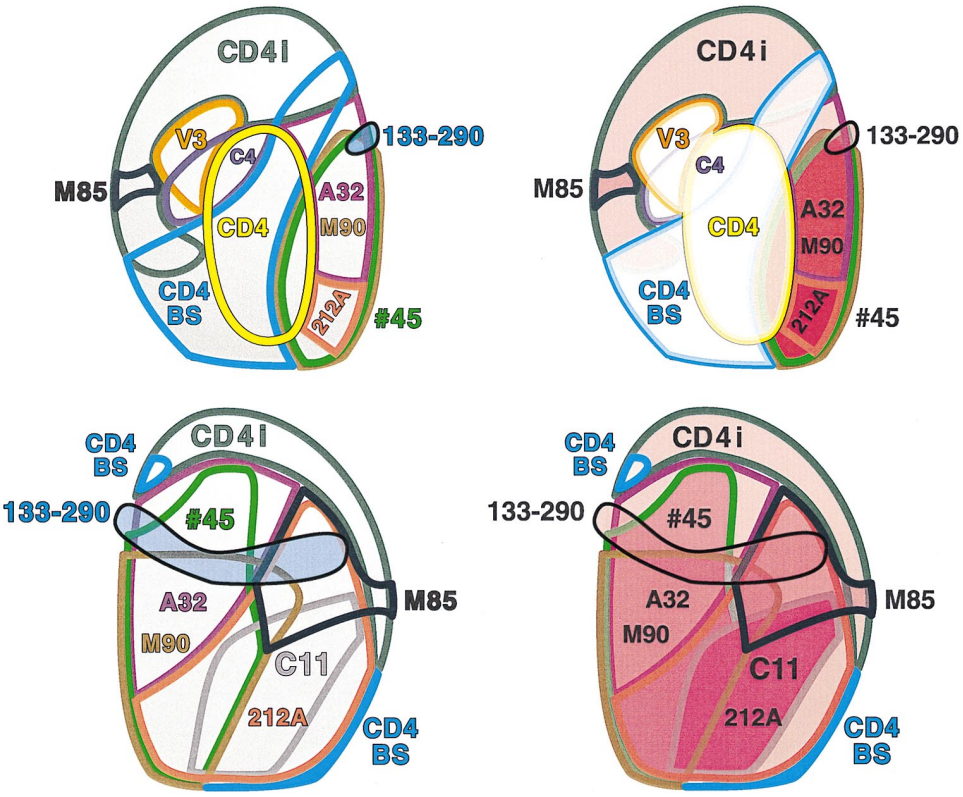

FIG. 3. Schematic representation of the gp41 footprint on gp120. The competition map of the HIV-1 gp120 glycoprotein (55) is shown on the left, with the neutralization face depicted in the upper diagram and the nonneutralizing face depicted in the lower diagram. On the right, the gp120 epitopes whose recognition is decreased by the presence of the gp41 ectodomain are shaded in red. The intensity of the shading of each epitope is proportionate to the observed decrease in antibody recognition. The shading shown is a summation of the shading for each of the overlapping epitopes.

which specifically recognizes HIV-1 envelope glycoprotein oligomers (5). The T4 antibody, even when present at high concentrations, precipitated less than 15% of the sgp140 glycoprotein able to be precipitated by the patient serum mixture (data not shown). This result suggests that only a small fraction of the sgp140 produced under these conditions represents a stable oligomer. The majority of the decreased epitope recognition associated with the sgp140 glycoprotein does not appear to result from interactions specific to the assembled oligomer.

Effect of sCD4 binding on sgp140 epitope recognition. To examine whether CD4 binding would alter the recognition of epitopes on the sgp140 glycoprotein, the radiolabelled HXBc2 gp120 and sgp140 glycoproteins were incubated in the absence and presence of saturating concentrations of sCD4 prior to precipitation by the monoclonal antibodies (Fig. 1B). Precipitation of the gp120 and sgp140 glycoproteins by the mixture of sera from HIV-1-infected individuals was not affected by the presence of sCD4. As expected, sCD4 binding decreased precipitation of both gp120 and gp140 glycoproteins by the F105 antibody, which recognizes a CD4bs epitope (60, 74). For the remaining subset of antibodies that efficiently precipitated the sgp140 glycoprotein, sCD4 binding had little influence on epitope recognition. By contrast, the antibodies that poorly recognized the sgp140 glycoprotein precipitated the protein more efficiently in the presence of sCD4. This observation suggests either that CD4 binding leads to an alteration of gp120-gp41 interaction that decreases the occlusion of epitopes on the glycoprotein subunits or that CD4 binding restores the integrity of gp120 epitopes disrupted by the presence of the gp41 ectodomain. The recognition of the sgp140 glycoprotein by anti-gp120 monoclonal antibodies was also increased when precipitation in the presence of sCD4 was carried out at 4°C or when the V1/V2 and V3 variable loops were completely deleted from the sgp140 glycoprotein (data not shown).

Effect of C1 and C5 deletions on the HIV-1 envelope glycoproteins. Previous studies suggested that the first (C1) and fifth (C5) gp120 regions are important for the interaction of the HIV-1 envelope glycoprotein subunits. To examine the effect of removal of these regions on gp120 epitopes, envelope glycoproteins containing progressive deletions of the C1 region were analyzed. A plasmid encoding the full-length HXBc2 envelope glycoproteins, with an intact proteolytic processing site, was used as the starting construct for these studies. Deletions were introduced into this plasmid so that progressively greater portions of C1 were removed from the encoded envelope glycoproteins, keeping the amino-terminal signal sequence intact in each case (Fig. 4). Precipitation of the wild-type and C1-deleted mutant envelope glycoproteins by a mixture of sera from HIV-1-infected individuals and by the F105 monoclonal antibody is shown in Fig. 5. The proteolytic processing of the precursor envelope glycoprotein was severely impaired for all of the C1-deleted glycoproteins. Decreased gp160 processing has been shown to result from global misfolding or from specific alterations of the gp120-gp41 cleavage site. The latter explanation probably applies to the inefficient proteolytic processing of the Δ50, Δ74, and Δ82 mutants, since these glycoproteins were precipitated by the F105 antibody, which recognizes a conformation-dependent epitope (60, 74).

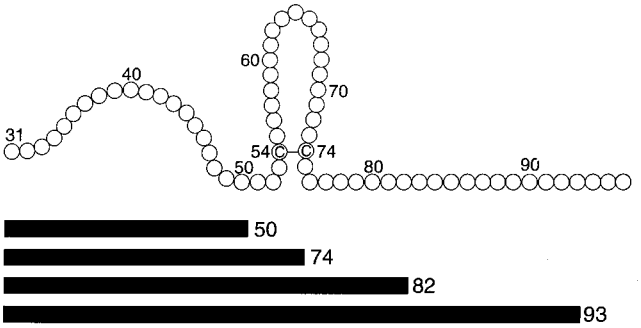

FIG. 4. Schematic representation of the nested C1 deletions in the HIV-1 envelope glycoproteins. Amino acid residues of the mature gp120 glycoprotein N-terminal C1 region are represented by the open circles. The bars indicate residues deleted in the Δ50, Δ74, Δ82, and Δ93 envelope glycoproteins.

Since previous studies have indicated a close proximity of the gp120 C1 and C5 regions (52), it is reasonable that major alterations in C1 could affect sequences near the proteolytic cleavage site. Further support for the correct folding of these glycoproteins was provided by a more comprehensive examination of secreted versions of these proteins (see below). Deletion of additional C1 residues in the Δ93 mutant resulted in a protein that, although recognized by the mixture of sera from HIV-1-infected people, was not precipitated by the F105 antibody. Interestingly, deletion of V1/V2 and V3 variable loops from the Δ93 mutant allowed some F105 recognition of the protein (data not shown). At least in this context, the major gp120 variable loops appear to contribute to masking, disrupting, or impeding the formation of the F105 epitope.

Previous studies suggested that some deletions in the C5 gp120 region could be tolerated without affecting the ability of the mutant glycoprotein to bind CD4 (41, 61). Since these studies indicated that deletion of 39 residues from the gp120 carboxyl terminus abrogated CD4 binding, a more conservative deletion (ΔC5) involving the removal of 19 carboxy-terminal residues was studied. The ΔC5 deletion was introduced into the HXBc2 envelope glycoproteins either alone or in combination with the C1 deletions described above. In addition, a set of HXBc2 glycoproteins containing these C1 and C5 deletions in combination with complete deletion of the V1/V2 stem-loop and V3 variable loop structures was constructed.

The mutant glycoproteins were produced in the supernatants of transfected COS-1 cells and precipitated with a mixture of sera from HIV-1-infected patients or with monoclonal

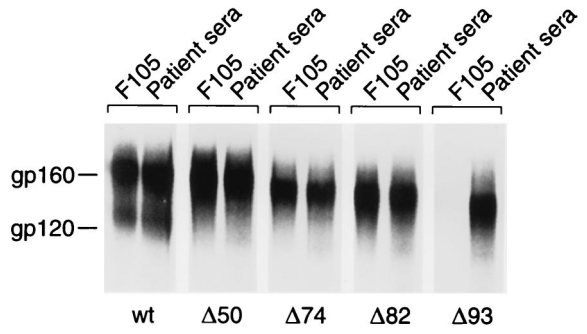

FIG. 5. Immunoprecipitation of wild-type and C1-deleted envelope precursor glycoproteins. Wild-type (wt) and ΔC1 envelope glycoproteins transiently expressed in COS-1 cells, lysed in Nonidet P-40 buffer, and immunoprecipitated with either AIDS patient sera or the F105 antibody are shown.

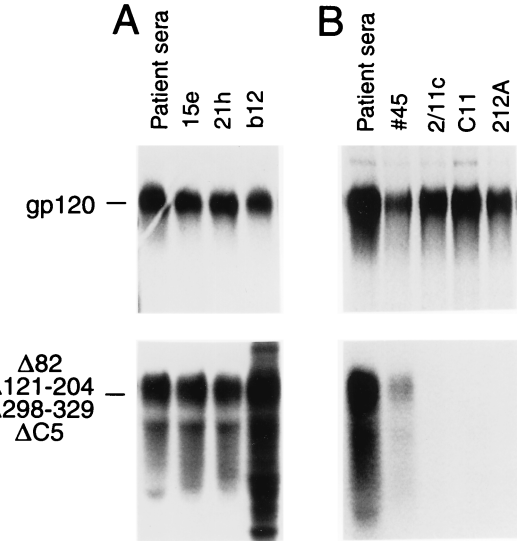

FIG. 6. Immunoprecipitations of soluble wild-type and deleted envelope glycoproteins. (A) The efficient recognition of wild-type and Δ82/Δ128-194/Δ298-329/ΔC5 glycoproteins by several broadly neutralizing, CD4 binding site antibodies is shown. (B) The inefficient recognition of Δ82/Δ128-194/Δ298-329/ΔC5 glycoproteins by several nonneutralizing antibodies that recognize the wild-type gp120 glycoprotein is shown.

antibodies directed against a number of gp120 epitopes. The results are shown in Fig. 6 and Table 1. The ΔC5 mutant glycoprotein was recognized by all of the monoclonal antibodies tested except the C11 and 212A antibodies, whose conformational epitopes have been shown to be sensitive to C1 and C5 changes (52). The Δ50ΔC5 mutant was recognized by all of the antibodies except C11, 212A, and 4D4 #85. The last of these monoclonal antibodies recognizes a linear gp120 epitope within residues 41 to 50, so the loss of recognition of proteins

TABLE 1. Recognition of soluble wild-type and deleted gp120 glycoproteins by a panel of monoclonal antibodies

Envelope glycoprotein	Recognition[a] by:												
	Antibody:												
	Patient sera[b]	4D4 #85	M90	#45	2/11c	C11	212A	F105[b]	15e	21h	b12	2G12	17b
gp120	+	+	+	+	+	+	+	+	+	+	+	+	+
ΔC5	+	+	+	+	+	−	−	+	+	+	+	+	+
Δ50/ΔC5	+	−	+	+	+	−	−	+	+	+	+	+	+
Δ74/ΔC5	+	−	+	+	−	−	−	+	+	+	+	+	+
Δ82/ΔC5	+	−	−	+	−	−	−	+	+	+	+	+	+
Δ93/ΔC5	+	−	−	−	−	−	−	−	−	+	+	+	−[c]
ΔV123/ΔC5	+	+	+	+	+	−	−	+	+	+	+	+	ND[d]
Δ50/ΔV123/ΔC5	+	−	+	+	+	−	−	+	+	+	+	+	ND
Δ74/ΔV123/ΔC5	+	−	−	+	−	−	−	+	+	+	+	+	ND
Δ82/ΔV123/ΔC5	+	−	−	+	−	−	−	+	+	+	+	+	−
Δ93/ΔV123/ΔC5	+/−	−	−	−	−	−	−	−	−	+/−	+/−	+	ND

[a] Symbols: +, efficient envelope glycoprotein recognition; −, envelope glycoprotein not recognized; +/−, Δ93/ΔC5 glycoproteins were not efficiently expressed.

[b] Recognition of the gp120 glycoproteins was also tested in cell lysates with AIDS patient sera and F105. No significant differences in antibody recognition of the various envelope glycoproteins were observed (data not shown).

[c] 17b recognized Δ93E7ΔC5 in the presence of sCD4.

[d] ND, not determined.

bearing a deletion of these residues was expected (51). Recognition by C11 and 212A was also affected by the Δ50 deletion even when the C5 region was intact, in the context of the unprocessed Δ50 gp160 glycoprotein from COS-1 lysates (data not shown). Deletion of an additional 24 amino-terminal residues in the Δ74ΔC5 mutant affected recognition by the 2/11c antibody. The 2/11c and related A32 monoclonal antibodies recognize discontinuous gp120 epitopes thought to involve C1 and C4 residues. Amino acid changes within a conserved, disulfide-linked loop involving C1 residues 54 to 74 decrease 2/11c and A32 recognition, consistent with the observation that the 2/11c antibody does not recognize the Δ74ΔC5 mutant. The Δ82ΔC5 mutant was not precipitated by the M90 antibody, in addition to lack of recognition by the C11, 212A, 4D4 #85, and 2/11c antibodies. The M90 antibody recognizes a poorly characterized discontinuous gp120 epitope affected by some C1 residue changes. The Δ82ΔC5 mutant was recognized by the #45 antibody, which also recognizes a conformational gp120 epitope affected by some C1 changes and which competes with the M90 antibody for gp120 binding (55). Deletion of an additional 11 C1 residues in the Δ93ΔC5 mutant disrupted recognition by most of the monoclonal antibodies tested, although two antibodies, 21h and b12, which recognize CD4bs epitopes, still precipitated this mutant. Two other CD4bs-directed antibodies, F105 and 15e, did not recognize the Δ93ΔC5 protein. Thus, while many gp120 epitopes are disrupted in the Δ93ΔC5 glycoprotein, the preservation of some CD4bs epitopes indicates that the conformation of this protein is not globally disrupted.

The results of the antibody precipitation studies were similar to those described above when mutant gp120 glycoproteins containing complete deletions of the V1/V2 stem-loop (Δ121-204) and V3 loop (Δ298-329) structures were analyzed (Table 1). These results indicate that the effects of C1 and C5 deletions on gp120 epitopes are not dependent upon the major gp120 variable loops.

In some contexts, the deletion of the V1/V2 stem and the base of the V3 loop have been shown to disrupt gp120 recognition by the 17b and 48d antibodies (80, 86), which recognize CD4i epitopes. Therefore, the C1/C5- and variable loop-deleted glycoproteins described above could not be tested for recognition by these antibodies. To determine whether C1 and C5 deletions could be accomplished while preserving the CD4i epitopes, two new constructs containing more conservative deletions affecting V1/V2 and V3 structures in the context of the Δ82/ΔC5 protein were made. The Δ82/Δ128-194/Δ298-329/ΔC5 protein is identical to the Δ82/Δ121-204Δ/298-329/ΔC5 protein except that the conserved V1/V2 stem is preserved in the former. The Δ82/Δ128-194/Δ303-323/ΔC5 protein has, in addition, sequences from the conserved base of the V3 loop preserved. Figure 7 shows that the Δ82/Δ128-194/Δ298-329/ΔC5 protein was efficiently recognized by the 17b antibody only in the presence of sCD4, whereas the 48d antibody did not recognize the mutant protein efficiently. By contrast, both the 17b and 48d antibodies precipitated the Δ82/Δ128-194/Δ303-323/ΔC5 protein in the absence of sCD4, and the efficiency of precipitation was increased in the presence of sCD4. We tested a subset of the ΔC5 and ΔC1/ΔC5 glycoproteins for their ability to bind sCD4. The ΔC5, Δ82/ΔC5, Δ93/ΔC5, Δ82/Δ121-204/Δ298Δ329/ΔC5, and Δ82/Δ128-194/Δ303-323/ΔC5 glycoproteins were analyzed for sCD4 binding as previously described (85). As shown in Fig. 8, the Δ93/ΔC5 glycoprotein did not efficiently recognize sCD4 at the concentrations tested, in agreement with a previous report indicating that deletion of the first 92 residues of gp120 disrupts sCD4 binding (37a). In contrast, the Δ82/ΔC5 glycoprotein bound sCD4 similarly to the wild-type glycoprotein, indicating that the deleted residues are dispensable for sCD4 interaction. Similarly, the other deleted envelope glycoproteins described above showed no significant difference in their ability to bind sCD4 compared to the wild-type envelope glycoprotein. These results indicate that the Δ82 and ΔC5 deletions do not disrupt the CD4i epitopes and demonstrate that HXBc2 gp120 derivatives lacking large portions of C1, V1/V2, V3, and C5 that retain the conserved binding sites for CD4 and neutralizing antibodies can be produced.

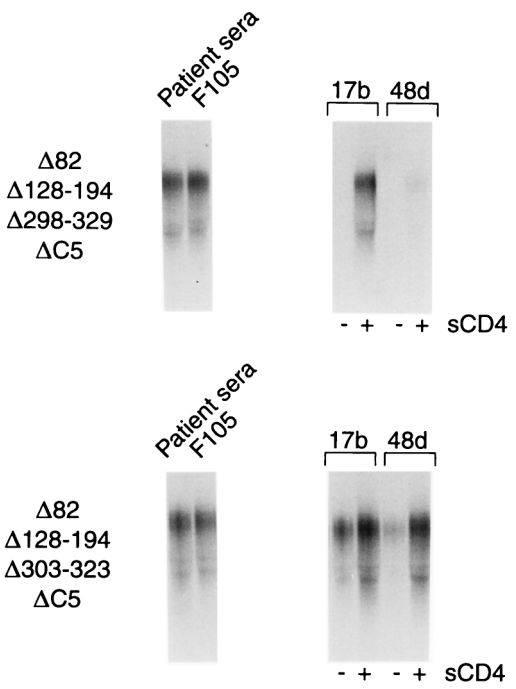

FIG. 7. Immunoprecipitation of the Δ82/Δ128-194/Δ298-329/ΔC5 and Δ82/Δ128-194/Δ303-323/ΔC5 glycoproteins. The precipitation of the Δ82/Δ128-194/Δ298-329/ΔC5 and Δ82/Δ128-194/Δ303-323/Δ82/ΔC5 glycoproteins by the 17b and 48d antibodies in the presence and absence of sCD4 is shown.

DISCUSSION

Previous studies indicated that two surfaces of the HIV-1 gp120 exterior envelope glycoprotein exist. One surface is accessible to sCD4 and neutralizing antibodies and is exposed on the assembled envelope glycoprotein complex. The other surface is recognized by anti-gp120 antibodies that do not neutralize HIV-1. In this study, we demonstrate that the presence of the ectodomain of the gp41 transmembrane envelope glycoprotein interferes with the binding of antibodies to the latter surface of gp120. The gp41 ectodomain may sterically mask the gp120 epitopes recognized by nonneutralizing antibodies, or the interaction of the gp120 and gp41 subunits may involve conformations of these glycoproteins different from those present on the dissociated molecules. By contrast, the presence of the gp41 ectodomain exhibited no effect on the binding of neutralizing antibodies to the CD4bs and to the V3 loop, which are believed to be exposed on the assembled oligomeric complex. The gp41 ectodomain exhibited some inhibitory effect on the binding of a subset of neutralizing antibodies, 17b and 48d, which recognize CD4-induced gp120 epitopes. These results are consistent with a model in which one gp120 surface is exposed on the complex, while the other is inaccessible to

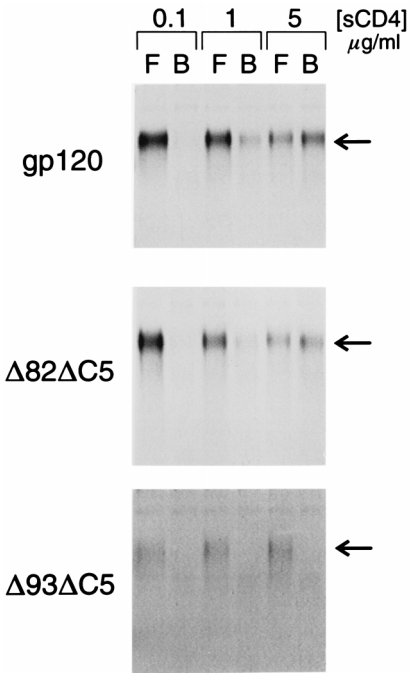

FIG. 8. Evaluation of sCD4 binding of the soluble gp120 glycoproteins. A selected subset of the soluble envelope glycoproteins was compared to wild-type gp120 for sCD4 binding. Supernatants containing radiolabelled envelope glycoproteins were supplemented with 0.1, 1, and 5 μg of sCD4 in a volume of 1 ml. Following overnight incubation at 4°C, the gp120-CD4 complexes were immunoprecipitated with OKT4 and protein A-Sepharose (fraction B). The free fraction (fraction F) of envelope glycoprotein not removed by OKT4 immunoprecipitation was immunoprecipitated from the supernatant with pooled AIDS patient sera and protein A-Sepharose. Shown is a representative autoradiographic exposure of gp120, Δ82/ΔC5, and Δ93/ΔC5 immunoprecipitates analyzed by SDS-polyacrylamide gel electrophoresis. Arrows indicate the positions of the respective radiolabelled envelope glycoproteins.

antibodies as a result of interaction with gp41. The results indicate that the gp41 ectodomain, in the absence of interactions between the subunits of the oligomer, is sufficient to mediate the previously reported inaccessibility of the assembled, native envelope glycoprotein complex to nonneutralizing antibodies (24a, 65a, 73a).

sCD4 binding resulted in a small increase in exposure of the gp120 epitopes occluded on the cleavage-defective gp140 glycoproteins. This effect was also observed on sgp140 glycoproteins that were able to be proteolytically processed, indicating that it is not an artifact resulting from the amino acid substitutions introduced at the natural gp160 cleavage site. While the functional relevance of this phenomenon remains to be established, these results suggest that CD4 binding can alter gp120-gp41 interaction. This effect appears to be distinct from sCD4-induced shedding of the gp120 glycoprotein, since, in contrast to shedding, it occurs efficiently at 4°C, in the absence of the conserved stem of the V1/V2 stem-loop, and with the envelope glycoproteins from a primary HIV-1 isolate. Also, since the decrease in occlusion of gp120 epitopes following sCD4 binding occurs on a molecule lacking the V1/V2 loop, this process appears to occur independently of the exposure of the 17b epitope. Most of the increased exposure of the 17b epitope induced by sCD4 binding has been shown to involve a demasking of the epitope as a result of sCD4-induced movement of the V2 loop. This movement represents a major gp120 conformational change that occurs upon CD4 binding and has been proposed to play a role in chemokine receptor binding and HIV-1 entry (80, 84). Further work will be needed to assess whether CD4-induced realignments of the gp120-gp41 interaction may also play necessary roles in the entry process.

The gp120 epitopes influenced by the gp41 ectodomain were sensitive to deletions affecting the first (C1) and fifth (C5) conserved gp120 regions. The sensitivity of the gp120 epitopes overlapping the gp41-interactive region to C1 and C5 deletions is consistent with the sensitivity of most of these epitopes and of gp120-gp41 association to single amino acid changes in these regions (32). The discontinuous epitope for the #45 monoclonal antibody has been shown to be affected by a single residue change in the C1 region; however, it is clear from our studies that significant portions of C1 are not needed for the integrity of the #45 epitope.

In contrast to gp120 epitopes overlapping the gp41-interactive region, gp120 epitopes recognized by neutralizing antibodies were not affected by N-terminal deletions until C1 residues beyond glutamine 82 were deleted. Even when amino-terminal residues up to residue 93 were removed, many of the neutralization epitopes remained intact. The CD4i epitopes recognized by the 17b and 48d antibodies, which have been reported to be among the most conformationally sensitive gp120 epitopes (77, 86), were disrupted by deletion of the N-terminal 93 residues in the absence of CD4. Some of the CD4bs antibodies also failed to bind to these N-terminally deleted glycoproteins, consistent with the proposed contribution of C1 residues to some of these epitopes (75). Interestingly, in some contexts, the CD4bs antibody F105 recognized the precursor Δ93 envelope glycoprotein only when the major variable loops were deleted. This result suggests that the N-terminal deletion allows interactions of the residual gp120 sequences with portions of the V1, V2, or V3 structures, masking or disrupting some CD4bs epitopes.

The ability to remove substantial portions of the C1 and C5 gp120 regions without globally disrupting the gp120 conformation suggests that the N and C termini of gp120 are somewhat exposed, flexible structures. A similar conclusion applies to the V1/V2 and V3 variable loops, which can be removed from the gp120 glycoprotein while overall conformation and, in some cases, even function can be preserved (59, 85, 86). The exposed, flexible nature of these structures may contribute to their efficient immunogenicity during the course of natural infection or in the generation of antibodies to the HIV-1 envelope glycoproteins in animals. In either of these settings, the generation of nonneutralizing antibodies, many of which are directed against the gp41-interactive region of gp120, and of strain-restricted neutralizing antibodies, directed against the V1/V2 and V3 variable loops, is more efficient than is the generation of more-cross-reactive neutralizing antibodies. Since the latter antibodies recognize discontinuous CD4bs and CD4i epitopes that are composed of amino acid residues derived from conserved gp120 regions, the conformational sensitivity and lower expected flexibility of these structures may contribute to their less efficient immunogenicity. In addition, previous studies indicated that the presence of the V1/V2 and V3 loops masks conserved gp120 epitopes, a process that is particularly efficient in the context of the oligomeric envelope glycoprotein complex (85, 86). The studies reported here indicate that the presumably flexible structures related to the variable loops and gp41-interactive region can be removed from the HIV-1 gp120 glycoprotein, leaving a conserved core that retains the CD4bs and overlapping epitopes for the more broadly neutralizing antibodies. These gp120 core domains may be useful in defining the structural details of these important gp120 epitopes and in directing immune responses towards them.

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REFERENCES

1. **Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. M. Murphy, and E. Berger.** 1996. CC-CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science **272:**1955–1958.
2. **Allan, J., T. H. Lee, M. F. McLane, J. Sodroski, W. Haseltine, and M. Essex.** 1983. Identification of the major envelope glycoprotein product of HTLV-III. Science **228:**1091–1094.
3. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauget, C. Axler-Bin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier.** 1983. Isolation of a T-lymphocyte retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). Science **220:**868–871.
4. **Berger, E., J. Sisler, and P. Earl.** 1992. Human immunodeficiency virus type 1 envelope glycoprotein molecules containing membrane fusion-impairing mutations in the V3 region efficiently undergo soluble CD4-simulated gp120 release. J. Virol. **66:**6208–6212.
5. **Broder, C. C., P. Earl, D. Long, S. Abedon, B. Moss, and R. Doms.** 1994. Antigenic implications of human immunodeficiency virus type 1 envelope quaternary structure: oligomer-specific and -sensitive monoclonal antibodies. Proc. Natl. Acad. Sci. USA **91:**11699–11703.
6. **Bugelski, P., H. Ellens, T. Hart, and R. Kirsh.** 1991. Soluble CD4 and dextran sulfate mediate release of gp120 from HIV-1: implications for clinical trials. J. Acquired Immune Defic. Syndr. **4:**923–924.
7. **Cann, A. J., M. J. Churcher, M. Boyd, W. O'Brien, J.-Q. Zhao, J. Zack, and I. S. Y. Chen.** 1992. The region of the envelope gene of human immunodeficiency virus type 1 responsible for determination of cell tropism. J. Virol. **66:**305–309.
8. **Cao, J., L. Bergeron, E. Helseth, M. Thali, H. Repke, and J. Sodroski.** 1993. Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. J. Virol. **67:**2747–2755.
9. **Capon, D., S. Chamow, J. Mordenti, S. Marsters, T. Gregory, H. Mitsuya, R. Byrn, C. Lucas, F. Wurm, J. Groopman, and D. Smith.** 1989. Designing CD4 immunoadhesins for AIDS therapy. Nature **337:**525–531.
10. **Cheng-Mayer, C., M. Quiroga, J. W. Tung, D. Dina, and J. A. Levy.** 1990. Viral determinants of human immunodeficiency virus type 1 T-cell or macrophage tropism, cytopathogenicity, and CD4 antigen modulation. J. Virol. **64:**4390–4398.
11. **Chesebro, B., J. Nishio, S. Perryman, A. Cann, W. O'Brien, I. S. Y. Chen, and K. Wehrly.** 1991. Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-leukemia cells, and macrophages. J. Virol. **65:**5782–5789.
12. **Chesebro, B., K. Wehrly, J. Nishio, and S. Perryman.** 1992. Macrophage-tropic human immunodeficiency virus isolates from different patients exhibit unusual V3 envelope sequence homogeneity in comparison with T-cell-tropic isolates: definition of critical amino acids involved in cell tropism. J. Virol. **66:**6547–6554.
13. **Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. MacKay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.** 1996. The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell **85:**1–20.
14. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature **312:**763–767.
15. **Deen, K., J. S. McDougal, R. Inacker, G. Folena-Wassterman, J. Arthos, J. Rosenberg, P. Maddon, R. Axel, and R. Sweet.** 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature **331:**82–84.
16. **Deng, H. K., S. Choe, W. Ellmeier, R. Liu, D. Unutmaz, M. Burkhart, P. di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature **381:**661–666.
17. **Doranz, B., J. Rucker, Y. Yi, R. Smyth, M. Samson, S. Peiper, M. Parmentier, R. Collman, and R. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell **85:**1149–1158.
18. **Dragic, T., V. Litwin, G. P. Allaway, S. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton.** 1996. HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. Nature **381:**667–673.
19. **Earl, P. L., R. W. Doms, and B. Moss.** 1990. Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. Proc. Natl. Acad. Sci. USA **87:**648–652.
20. **Earl, P. L., B. Moss, and R. W. Doms.** 1991. Folding, interaction with GRP78-BiP, assembly and transport of the human immunodeficiency virus type 1 envelope protein. J. Virol. **65:**2047–2055.
21. **Fauci, A., A. Macher, D. Longo, H. C. Lane, A. Rook, H. Masur, and E. Gelman.** 1984. Acquired immunodeficiency syndrome: epidemiologic, clinical and therapeutic considerations. Ann. Intern. Med. **100:**92–106.
22. **Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger.** 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science **272:**872–877.
23. **Fisher, R., E. Collati, L. Ratner, R. Gallo, and F. Wong-Staal.** 1985. A molecular clone of HTLV-III with biological activity. Nature **316:**262–265.
24. **Fisher, R., J. Bertonis, W. Meier, V. Johnson, D. Costopoulos, T. Liu, R. Tizard, B. Walker, M. Hirsch, R. Schooley, and R. Flavell.** 1988. HIV infection is blocked *in vitro* by recombinant soluble CD4. Nature **331:**76–78.

24a. **Fouts, T. R., J. M. Binley, A. Trkola, J. E. Robinson, and J. P. Moore.** 1997. Neutralization of the human immunodeficiency virus type 1 primary isolate JR-FL by human monoclonal antibodies correlates with antibody binding to the oligomeric form of the envelope glycoprotein complex. J. Virol. **71:**2779–2785.

25. **Freed, E., D. Myers, and R. Risser.** 1990. Characterization of the fusion domain of the human immunodeficiency virus type 1 envelope glycoprotein gp41. Proc. Natl. Acad. Sci. USA **87:**4650–4654.
26. **Freed, E., D. Myers, and R. Risser.** 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. J. Virol. **65:**190–194.
27. **Fu, Y.-K., T. Hart, Z. Jonak, and P. Bugelski.** 1993. Physicochemical dissociation of CD4-mediated syncytium formation and shedding of HIV-1 gp120. J. Virol. **67:**3818–3825.
28. **Fung, M. S. C., C. R. Y. Sun, W. L. Gordon, R.-S. Liou, T. W. Chang, W. N. C. Sun, E. S. Daar, and D. D. Ho.** 1992. Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. J. Virol. **66:**848–856.
29. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224:**500–503.
30. **Hart, T., R. Kirsch, H. Ellens, R. Sweet, D. Lambert, S. Petteway, J. Leary, and P. Bugelski.** 1991. Binding of soluble CD4 proteins to HIV-1 and infected cells induces release of envelope glycoprotein gp120. Proc. Natl. Acad. Sci. USA **88:**2189–2193.
31. **Helseth, E., M. Kowalski, D. Gabuzda, U. Olshevsky, W. Haseltine, and J. Sodroski.** 1990. Rapid complementation assays measuring replicative potential of human immunodeficiency virus type 1 envelope glycoprotein mutants. J. Virol. **64:**2416–2420.
32. **Helseth, E., U. Olshevsky, C. Furman, and J. Sodroski.** 1991. Human immunodeficiency virus type 1 gp120 envelope glycoprotein regions important for association with the gp41 transmembrane glycoprotein. J. Virol. **65:**2119–2123.
33. **Ho, D., J. McKeating, X. Li, T. Moudgil, E. Daar, N.-C. Sun, and J. Robinson.** 1991. Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by a human monoclonal antibody. J. Virol. **65:**489–493.
34. **Hussey, R., N. Richardson, M. Kowalski, N. Brown, H. Change, R. Siliciano, T. Dorfman, B. Walker, J. Sodroski, and E. Reinherz.** 1988. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. Nature **331:**78–81.
35. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253:**71–74.
36. **Ivanoff, L., D. Looney, C. McDanal, J. Morris, F. Wong-Staal, A. Langlois, S. Petteway, and T. Matthews.** 1991. Alteration of HIV-1 infectivity and neutralization by a single amino acid replacement in the V3 loop domain. AIDS Res. Hum. Retroviruses **7:**595–603.
37. **Javaherian, K., A. J. Langlois, C. McDanal, K. L. Ross, L. I. Eckler, C. L. Jellis, A. T. Profy, J. R. Rusche, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1989. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. Proc. Natl. Acad. Sci. USA **86:**6768–6772.

37a. **Jeffs, S., J. McKeating, S. Lewis, H. Craft, D. Biram, P. Stephens, and R. Brady.** 1996. Antigenicity of truncated forms of the human immunodeficiency virus type 1 envelope glycoprotein. J. Gen. Virol. **77:**1403–1410.

38. **Kang, C.-Y., P. Nara, S. Chamat, V. Caralli, T. Ryskamp, N. Haigwood, R. Newman, and H. Kohler.** 1991. Evidence for non-V3-specific neutralizing antibodies that interfere with gp120/CD4 binding in human immunodefi-

ciency virus-infected humans. Proc. Natl. Acad. Sci. USA **88:**6171–6175.
39. **Kang, C.-Y., K. Hariharan, M. R. Posner, and P. Nara.** 1993. Identification of a new neutralizing epitope conformationally affected by the attachment of CD4 to gp120. J. Immunol. **151:**449–457.
40. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature **312:**767–768.
41. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. G. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the human immunodeficiency virus envelope glycoprotein. Science **237:**1351–1355.
42. **Lapham, C., J. Ouyang, B. Chandrasekhar, N. Nguyen, D. Dimitrov, and H. Golding.** 1996. Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. Science **274:**602–605.
43. **Leonard, C., M. Spellman, L. Riddle, R. Harris, J. Thomas, and T. Gregory.** 1990. Assignment of intrachain disulfide bonds and characterization of potential glycoslation sites of the type 1 human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. **265:**10373–10382.
44. **Lu, M., S. Blackow, and P. Kim.** 1995. A trimeric structural domain of the HIV-1 transmembrane glycoprotein. Nat. Struct. Biol. **2:**1075–1082.
45. **Maddon, P., A. Dalgleish, J. S. McDougal, P. Clapham, R. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS receptor and is expressed in the immune system and the brain. Cell **47:**333–348.
46. **McDougal, J. S., M. Kennedy, J. Sligh, S. Cort, A. Mowie, and J. Nicholson.** 1986. Binding of the HTLV-III/LAV to $T4^+$ T Cells by a complex of the 100 K viral protein and the T4 molecule. Science **231:**382–385.
47. **McKeating, J., C. Shotton, J. Cordell, S. Graham, P. Balfe, N. Sullivan, M. Charles, M. Page, A. Bolmstedt, S. Olofsson, S. Kayman, Z. Wu, A. Pinter, C. Dean, J. Sodroski, and R. Weiss.** 1993. Characterization of neutralizing monoclonal antibodies to linear and conformation-dependent epitopes within the first and second variable domains of human immunodeficiency virus type 1 gp120. J. Virol. **67:**4932–4944.
48. **Moore, J., J. McKeating, R. Weiss, and Q. Sattentau.** 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science **250:**1139–1142.
49. **Moore, J., J. McKeating, Y. Huang, A. Ashkenazi, and D. Ho.** 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J. Virol. **66:**235–243.
50. **Moore, J., Q. Sattentau, H. Yoshiyama, M. Thali, M. Charles, N. Sullivan, S.-W. Poon, M. Fung, F. Traincard, J. Robinson, D. D. Ho, and J. Sodroski.** 1993. Probing the structure of the V2 domain of the human immunodeficiency virus type 1 surface glycoprotein gp120 with a panel of eight monoclonal antibodies: the human immune response to the V1 and V2 domains. J. Virol. **67:**6136–6151.
51. **Moore, J., Q. Sattentau, R. Wyatt, and J. Sodroski.** 1994. Probing the structure of the human immunodeficiency virus surface glycoprotein gp120 with a panel of monoclonal antibodies. J. Virol. **68:**469–484.
52. **Moore, J. P., R. L. Willey, G. K. Lewis, J. Robinson, and J. Sodroski.** 1994. Immunological evidence for interactions between the first, second, and fifth conserved domains of the gp120 surface glycoprotein of human immunodeficiency virus type 1. J. Virol. **68:**6836–6847.
53. **Moore, J. P., and D. D. Ho.** 1993. Antibodies to discontinuous or conformationally sensitive epitopes on the gp120 glycoprotein of human immunodeficiency virus type 1 are highly prevalent in sera of infected humans. J. Virol. **67:**863–875.
54. **Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas III, D. R. Burton, and D. D. Ho.** 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120 and their neutralization is not predicted by studies with monomeric gp120. J. Virol. **69:**101–109.
55. **Moore, J. P., and J. Sodroski.** 1996. Antibody cross-competition analysis of the human immunodeficiency virus type 1 gp120 exterior envelope glycoprotein. J. Virol. **70:**1863–1872.
56. **Myers, G., J. Berzofsky, B. Korber, R. Smith, and G. Pavlakis.** 1992. Human retroviruses and AIDS. A compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Los Alamos, N.Mex.
57. **Page, K., S. Stearns, and D. Littman.** 1992. Analysis of mutations in the V3 domain of gp160 that affect fusion and infectivity. J. Virol. **66:**524–533.
58. **Pinter, A., W. J. Honnen, S. A. Tilley, C. Bona, H. Zaghouani, M. K. Gorny, and S. Zolla-Pazner.** 1989. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. **63:**2674–2679.
59. **Pollard, S., M. D. Rosa, J. Rosa, and D. Wiley.** 1992. Truncated variants of gp120 bind CD4 with high affinity and suggest a minimum CD4 binding region. EMBO J. **11:**585–591.
60. **Posner, M., T. Hideshima, T. Cannon, M. Mukherjee, K. Mayer, and R. Byrn.** 1991. An IgG human monoclonal antibody which reacts with HIV-1 gp120, inhibits virus binding to cells, and neutralizes infection. J. Immunol. **146:**4325–4332.
61. **Putney, S., T. Matthews, W. G. Robey, D. Lynn, M. Robert-Guroff, W. Mueller, A. Langlois, J. Ghrayeb, S. Petteway, K. Weinhold, P. Fischinger, F. Wong-Staal, R. Gallo, and D. Bolognesi.** 1986. HTLV-III/LAV-neutralizing antibodies to an E. Coli-produced fragment of the virus envelope. Science **234:**1392–1395.
62. **Robey, W. G., B. Safai, S. Oroszlan, L. Arthur, M. Gonda, R. Gallo, and P. J. Fischinger.** 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. Science **228:**593–595.
62a. **Robinson, J.** Unpublished observations.
63. **Rusche, J. R., K. Javaherian, C. McDanal, J. Petro, D. L. Lynn, R. Grimaila, A. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino-acid sequence of the viral envelope gp120. Proc. Natl. Acad. Sci. USA **85:**3198–3202.
64. **Sattentau, Q., and J. Moore.** 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J. Exp. Med. **174:**407–415.
65. **Sattentau, Q., J. Moore, F. Vignaux, F. Traincard, and P. Poignard.** 1993. Conformational changes induced in the envelope glycoproteins of human and simian immunodeficiency viruses by soluble receptor binding. J. Virol. **67:**7383–7393.
65a. **Sattentau, Q. J., and J. P. Moore.** 1995. Human immunodeficiency virus type 1 neutralization is determined by epitope exposure on the gp120 oligomer. J. Exp. Med. **182:**185–196.
66. **Schawaller, M., G. E. Smith, J. Skehel, and D. Wiley.** 1989. Studies with cross-linking reagents on the oligomeric structure of the *env* glycoprotein of HIV. Virology **172:**367–369.
67. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature **349:**167–169.
68. **Shotton, C., C. Arnold, Q. Sattentau, J. Sodroski, and J. McKeating.** 1995. Identification and characterization of monoclonal antibodies specific for polymorphic antigenic determinants within the V2 region of the human immunodeficiency virus type 1 envelope glycoprotein. J. Virol. **69:**222–230.
69. **Smith, D., R. Byrn, S. Marsters, T. Gregory, J. Groopman, and D. Capon.** 1987. Blocking of HIV-1 infectivity by a soluble secreted form of the CD4 antigen. Science **238:**1704–1707.
70. **Starcich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions of the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45:**637–648.
71. **Steimer, K. S., C. J. Scandella, P. V. Skiles, and N. L. Haigwood.** 1991. Neutralization of divergent HIV-1 isolates by conformation-dependent human antibodies to gp120. Science **254:**105–108.
72. **Stein, B. S., S. D. Gouda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engelman.** 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell **49:**659–668.
73. **Sullivan, N., M. Thali, C. Furman, D. Ho, and J. Sodroski.** 1993. Effect of amino acid changes in the V2 region of the human immunodeficiency virus type 1 gp120 glycoprotein on subunit association, syncytium formation, and recognition by a neutralizing antibody. J. Virol. **67:**3674–3679.
73a. **Sullivan, N., Y. Sun, J. Li, W. Hoffmann, and J. Sodroski.** 1995. Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T cell line-passaged human immunodeficiency virus type 1 isolates. J. Virol. **69:**4413–4422.
74. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, M. Posner, and J. Sodroski.** 1991. Characterization of a discontinuous human immunodeficiency virus type 1 gp120 epitope recognized by a broadly neutralizing human monoclonal antibody. J. Virol. **65:**6188–6193.
75. **Thali, M., C. Furman, D. D. Ho, J. Robinson, S. Tilley, A. Pinter, and J. Sodroski.** 1992. Discontinuous, conserved neutralization epitopes overlapping the CD4 binding region of the human immunodeficiency virus type 1 gp120 envelope glycoprotein. J. Virol. **66:**5635–5641.
76. **Thali, M., C. Furman, E. Helseth, H. Repke, and J. Sodroski.** 1992. Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. J. Virol. **66:**5516–5524.
77. **Thali, M., J. P. Moore, C. Furman, M. Charles, D. D. Ho, J. Robinson, and J. Sodroski.** 1993. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. J. Virol. **67:**3978–3988.
78. **Tilley, S. A., W. J. Honnen, M. Racho, M. Hilgartner, and A. Pinter.** 1991. A human monoclonal antibody against the CD4 binding site of HIV-1 gp120 exhibits potent, broadly neutralizing activity. Res. Virol. **142:**247–259.
79. **Traunecker, A., W. Luke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature **331:**84–86.
80. **Trkola, A., T. Dragic, J. Arthos, J. Binley, W. Olson, G. Allaway, C. Cheng-Mayer, J. Robinson, P. Maddon, and J. Moore.** 1996. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. Nature **384:**184–187.
81. **Westervelt, P., H. E. Gendelman, and L. Ratner.** 1991. Identification of a determinant within the human immunodeficiency virus type 1 surface envelope glycoprotein critical for productive infection of primary monocytes.

Proc. Natl. Acad. Sci. USA **88:**3097–3101.
82. **Westervelt, P., D. B. Trowbridge, L. G. Epstein, B. M. Blumberg, Y. Li, B. H. Hahn, G. M. Shaw, R. W. Price, and L. Ratner.** 1992. Macrophage tropism determinants of human immunodeficiency virus type 1 in vivo. J. Virol. **66:**2577–2582.
83. **Wiley, D., and J. Skehel.** 1987. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Annu. Rev. Biochem. **56:** 365–394.
84. **Wu, L., N. Gerard, R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Boresetti, A. Cardoso, E. Desjardins, W. Newman, C. Gerard, and J. Sodroski.** 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature **384:**179–183.
85. **Wyatt, R., N. Sullivan, M. Thali, H. Repke, D. Ho, J. Robinson, M. Posner, and J. Sodroski.** 1993. Characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions. J. Virol. **67:**4557–4565.
86. **Wyatt, R., J. Moore, M. Accola, E. Desjardin, J. Robinson, and J. Sodroski.** 1995. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. J. Virol. **69:**5723–5733.