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Relationship of the Human Immunodeficiency Virus Type 1 gp120 Third Variable Loop to a Component of the CD4 Binding Site in the Fourth Conserved Region

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Neutralizing antibodies that recognize the human immunodeficiency virus gp120 exterior envelope glycoprotein and are directed against either the third variable (V3) loop or conserved, discontinuous epitopes overlapping the CD4 binding region have been described. Here we report several observations that suggest a structural relationship between the V3 loop and amino acids in the fourth conserved (C4) gp120 region that constitute part of the CD4 binding site and the conserved neutralization epitopes. Treatment of the gp120 glycoprotein with ionic detergents resulted in a V3 loop-dependent masking of both linear C4 epitopes and discontinuous neutralization epitopes overlapping the CD4 binding site. Increased recognition of the native gp120 glycoprotein by an anti-V3 loop monoclonal antibody, 9284, resulted from single amino acid changes either in the base of the V3 loop or in the gp120 C4 region. These amino acid changes also resulted in increased exposure of conserved epitopes overlapping the CD4 binding region. The replication-competent subset of these mutants exhibited increased sensitivity to neutralization by antibody 9284 and anti-CD4 binding site antibodies. The implied relationship of the V3 loop, which mediates post-receptor binding steps in virus entry, and components of the CD4 binding region may be important for the interaction of these functional gp120 domains and for the observed cooperativity of neutralizing antibodies directed against these regions.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (4, 17). HIV-1 establishes a persistent infection in human hosts, eventually resulting in defective cellular immunity secondary to CD4 lymphocyte depletion (33).

The HIV-1 exterior envelope glycoprotein, gp120, and the transmembrane envelope glycoprotein, gp41, are derived by cleavage of the gp160 envelope glycoprotein precursor (2, 53). HIV-1 is tropic for CD4-positive cells by virtue of a high-affinity interaction between the gp120 exterior envelope glycoprotein and the CD4 glycoprotein, which acts as the virus receptor (10, 30, 40). Following gp120-CD4 binding, the fusion of viral and host cell membranes, which involves both gp120 and gp41 envelope glycoproteins, allows virus entry (21, 31, 61). Amino acid changes in the third variable (V3) region of gp120, which forms a disulfide-linked loop (36), the gp41 amino terminus, or the gp41 transmembrane region dramatically reduce the efficiency of the membrane fusion process (15, 16, 18, 18a, 21, 23, 26, 31, 32, 47, 68).

Neutralizing antibodies appear to be an important component of a protective immune response (7, 12, 13, 18). HIV-1 neutralizing antibodies are directed against linear or discontinuous epitopes of the gp120 exterior envelope glycoprotein. Neutralizing antibodies that arise early in infected humans and that are readily generated in animals by immunization are primarily directed against linear determinants in the V3 loop of the gp120 glycoprotein (28, 38, 51, 52, 55). These antibodies generally exhibit the ability to neutralize only a limited number of HIV-1 strains (39, 44, 48), although some subsets of anti-V3 antibodies recognize less variable elements of the region and therefore exhibit broader neutralizing activity (1, 27, 34, 45). Envelope glycoprotein variation within the linear V3 epitope and outside the epitope can allow escape of viruses from neutralization by these antibodies (41, 43, 43a). These antibodies do not block CD4 binding but apparently interfere with post-receptor binding events involved in virus entry and syncytium formation, presumably a component of the membrane fusion process (37, 55, 58).

Later in the course of HIV-1 infection of humans, antibodies capable of neutralizing a wider range of HIV-1 isolates appear (6, 69). These broadly neutralizing antibodies have been difficult to elicit in animals (19) and are not merely the result of additive anti-V3 loop reactivities against diverse HIV-1 isolates that accumulate during active infection (50). A subset of the broadly reactive antibodies, found in most HIV-1-infected individuals, interferes with the binding of gp120 and CD4 (29, 60). At least some of these antibodies recognize discontinuous gp120 epitopes present only on the native glycoprotein (19, 20, 60). Human monoclonal antibodies derived from HIV-1-infected individuals that recognize the gp120 glycoproteins from a diverse range of HIV-1 isolates, that block gp120-CD4 binding, and that neutralize virus infection have been identified (24, 49, 54, 66). Recently, the discontinuous epitopes recognized by five of these human monoclonal antibodies have been characterized (42, 62, 65). Amino acid changes in seven discontinuous gp120 regions, four of which overlap regions defined to be important for CD4 binding, disrupt recognition by these

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antibodies and, in some cases, allow the generation of neutralization escape mutants. The shared components of the antibody epitopes and the discontinuous CD4 binding region include threonine 257, aspartic acid 368 to glutamic acid 370, lysine 421 to tryptophan 427, and aspartic acid 457 (9, 42, 46, 62, 65). The anti-CD4 binding antibodies and anti-V3 loop antibodies exhibit additive or synergistic neutralization of HIV-1 (7a, 63, 65a).

Here we provide evidence suggesting a structural relationship between the V3 loop and the fourth conserved (C4) region of the HIV-1 gp120 glycoprotein, a region that contributes residues to the CD4 binding site and also modulates the affinity of the gp120-gp41 glycoprotein interaction (9, 22, 35, 46).

MATERIALS AND METHODS

Mutant envelope glycoproteins. The HIV-1 (HXBc2 strain) envelope glycoprotein mutants used in this study were previously described (46). The Δ297-329 mutant contains a deletion spanning the V3 loop, with the sequence Gly-Ala-Gly inserted in place of the loop (62, 68). Envelope glycoproteins were expressed by transfection of plasmid pSVIII-env containing either a wild-type or mutated *env* gene into COS-1 cells by the DEAE-dextran technique (46).

Immunoprecipitations. Immunoprecipitations were carried out as previously described (65), using either radioimmunoprecipitation assay (RIPA) buffer (1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 1% sodium deoxycholate, 150 mM NaCl, 10 mM Tris HCl [pH 7.5]) or Nonidet P-40 (NP-40) buffer (0.5% NP-40, 0.5 M NaCl, 10 mM Tris HCl [pH 7.5]) to lyse the transfected COS-1 cells, after labeling with [^{35}S]cysteine. Labeled cell lysates in the buffers described above and cell supernatants in the absence of detergent were precipitated with a mixture of sera from AIDS patients and protein A-Sepharose beads. The precipitates were washed either with RIPA buffer minus sodium deoxycholate or with NP-40 buffer and then analyzed on SDS-polyacrylamide gels.

Iodination of cell surface envelope glycoproteins by lactoperoxidase. For some of the mutant envelope glycoproteins, the envelope glycoproteins on the cell surface were examined by iodination followed by immunoprecipitation with an AIDS patient serum. A 100-mm-diameter dish of transfected COS-1 cells was washed four times with ice-cold phosphate-buffered saline (PBS). Then 900 μl of PBS was added to the dish, after which 100 μl of lactoperoxidase (100 U/ml in PBS; Calbiochem), 0.5 mCi of ^{125}I (New England Nuclear), and 25 μl of a 1:1,000 (vol/vol) solution of 30% hydrogen peroxide (Sigma) in distilled water were added. An additional 25 μl of the hydrogen peroxide solution was added after 5, 10, and 15 min. After 20 min of total incubation time at room temperature, the cells were lysed and used for immunoprecipitation with a mixture of sera derived from AIDS patients.

Binding of radiolabeled anti-gp120 antibody to transfected COS-1 cells. COS-1 cells were transfected with 10 μg of pSVIIIenv DNA expressing wild-type or mutant envelope glycoproteins. Sixty hours after transfection, the COS cells were washed with PBS containing 2% fetal bovine serum and incubated with a ^{35}S-labeled monoclonal antibody against gp120, either F105 (49) or 1.5e (24). Approximately 1.5×10^7 antibody-producing hybridoma cells were labeled overnight in 10 ml of cysteine- and methionine-free RPMI medium containing 100 μCi each of [^{35}S]cysteine and [^{35}S]methionine (New England Nuclear). One milliliter of the antibody-containing medium was added to each 100-mm-diameter dish of transfected COS-1 cells. The envelope-expressing COS-1 cells were incubated with the antibody for 90 min at room temperature, washed three times with ice-cold PBS containing 2% fetal bovine serum, and lysed. The lysates were incubated with protein A-Sepharose beads, which were washed according to the procedure used for immunoprecipitation (65). The labeled antibody was visualized on a reducing SDS-polyacrylamide gel.

Virus neutralization assay. Complementation of a single round of replication of the *env*-deficient chloramphenicol acetyltransferase (CAT)-expressing provirus by the mutant envelope glycoproteins was performed as described previously (21). For inhibition of virus replication, soluble CD4 or monoclonal antibody was incubated with recombinant CAT-expressing virus for 1 h at 37°C before exposure of the virus to target Jurkat lymphocytes. CAT activity in the target cells was measured as described previously (21). A 30-fold range of antibody or soluble CD4 concentrations was used, and the amount needed to inhibit virus entry by 50% was calculated as described elsewhere (64).

RESULTS

Effect of V3 loop deletion on recognition of gp120 by monoclonal antibodies. The effect of a large deletion encompassing the HIV-1 gp120 V3 region on recognition by four human monoclonal antibodies directed against the CD4 binding region was examined, using different detergent conditions. The human monoclonal antibodies chosen for this study all recognize native but not denatured gp120, block the binding of gp120 and CD4, and neutralize a relatively broad range of HIV-1 isolates (24, 24a, 49, 54, 66). As a control in these studies, a mixture of sera from AIDS patients, which recognizes a range of gp120 epitopes, was used. COS-1 cells were transfected with plasmids expressing either the wild-type HIV-1 envelope glycoproteins or the Δ297-329 mutant glycoproteins. The Δ297-329 mutant glycoproteins contain the disulfide linkage at the base of the V3 loop but lack most of the amino acids constituting the loop itself. The transfected COS-1 cells were radiolabeled with [^{35}S]cysteine and then lysed in buffers containing either nonionic detergent (NP-40 buffer) or ionic detergents (RIPA buffer).

Precipitation of the Δ297-329 mutant by the AIDS patient sera and the human monoclonal antibodies was equivalent to that of the wild-type glycoproteins in NP-40 buffer (Fig. 1 and data not shown). Both the gp160 and gp120 forms of the envelope glycoproteins were recognized by all of the antibodies. By contrast, in RIPA buffer containing ionic detergents, while the patient sera precipitated the wild-type and Δ297-329 glycoproteins with equal efficiency, all four human monoclonal antibodies precipitated the wild-type glycoproteins approximately 7- to 25-fold less efficiently than they precipitated the Δ297-329 mutant (Fig. 1). This same phenomenon was observed for the monomeric, soluble gp120 glycoprotein precipitated from transfected COS-1 supernatants in RIPA buffer (data not shown).

The presence of the V3 loop was also found to decrease the precipitation of the wild-type HIV-1 gp120 glycoprotein by a monospecific rabbit antiserum directed against the gp120 C4 region (3). As shown in Fig. 2, the wild-type HIV-1 gp120 glycoprotein was immunoprecipitated by the rabbit anti-C4 peptide serum much less efficiently than was the Δ297-329 mutant, even though in parallel experiments, the two proteins were precipitated to equivalent extents by sera from AIDS patients. By contrast, rabbit antiserum directed against the gp120 C5 region (3) efficiently precipitated both

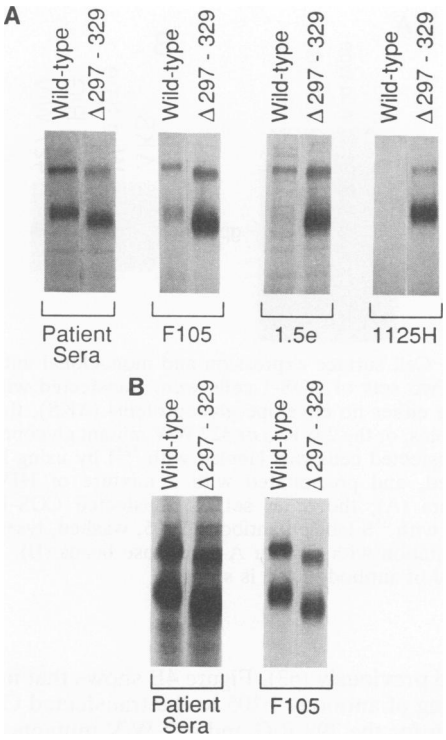

FIG. 1. Precipitation of envelope glycoproteins by serum or monoclonal antibodies. (A) Precipitates from COS-1 cells expressing either wild-type or Δ297-329 envelope glycoproteins. The transfected cells were lysed in RIPA buffer and then precipitated either with a mixture of sera from HIV-1-infected patients or with monoclonal antibody F105, 1.5e, or 1125H. (B) Results of precipitating the wild-type or Δ297-329 envelope glycoproteins from transfected COS-1 cells lysed in NP-40 buffer, using either patient serum or monoclonal antibody F105.

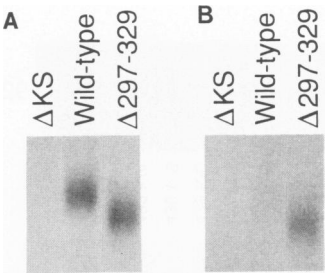

FIG. 2. Precipitation of envelope glycoproteins by an antipeptide serum. Supernatants from COS-1 cells expressing either no envelope glycoproteins (ΔKS), the wild-type HIV-1 gp120 envelope glycoprotein, or the Δ297-329 mutant glycoprotein were precipitated with a mixture of HIV-1-infected patient sera (A) or with a rabbit serum directed against a peptide corresponding in sequence to the gp120 C4 region (B). Precipitations were performed in tissue culture medium without added detergent at 4°C, and precipitates were washed at 4°C with RIPA buffer.

wild-type and Δ297-329 glycoproteins in parallel experiments (data not shown). These results were obtained with use of monomeric gp120 glycoproteins in the supernatants of transfected COS-1 cells (Fig. 2) or multimeric HIV-1 envelope glycoprotein complexes in NP-40 or RIPA cell lysates (data not shown). Apparently, under some buffer conditions, the V3 loop masks both linear C4 epitopes and discontinuous epitopes overlapping the CD4 binding region.

Effects of single amino acid changes on V3 loop conformation. One explanation for the results presented above is that elements of the V3 loop reside in proximity on the native gp120 glycoprotein to components of the epitopes for the anti-CD4 binding antibodies. To test this model, the influence of single amino acid changes in the gp120 glycoprotein on the conformation of the V3 loop was examined by using the murine monoclonal antibody 9284. Antibody 9284 recognizes a sequence on the amino-terminal side of the V3 loop, inefficiently precipitates the native HIV-1 envelope glycoprotein, and weakly neutralizes HIV-1 (57). A series of mutant HIV-1 envelope glycoproteins containing amino acid changes in conserved residues (46) were expressed in COS-1 cells. Of particular interest were gp120 mutants with amino acid alterations affecting recognition by the anti-CD4 binding antibodies (42, 62, 65). Radiolabeled cell supernatants in the absence of detergent were precipitated either with a mixture of sera from AIDS patients or with antibody 9284 (Fig. 3A). As described in the legend to Fig. 3, most of the mutants were recognized by antibody 9284 at a level comparable to that seen for the wild-type gp120 glycoprotein, taking into account differences in the level of mutant gp120 glycoprotein present in the COS-1 supernatants. The latter value was assessed by using the mixture of sera from AIDS patients, which recognizes multiple gp120 epitopes and therefore is not likely to be dramatically affected by alterations in a single epitope. The most significant increases (6- and 19-fold, respectively) in the efficiency with which antibody 9284 precipitated the gp120 glycoprotein were observed for the 427 W/V and 427 W/S mutants (Fig. 3A). Tryptophan 427 is located in the gp120 C4 region and contributes both to the CD4 binding region and to the epitopes for some neutralizing antibodies, such as 1.5e and 1125H (9, 46, 62). Smaller increases in precipitation of the soluble gp120 glycoprotein by antibody 9284 were observed for the 298 R/G (fourfold), 420 I/R (twofold), and 432 K/A (twofold) mutants. Arginine 298 is located on the amino-terminal side and near the base of the V3 region, immediately amino terminal to the 9284 epitope. Isoleucine 420 and lysine 432, like tryptophan 427, reside in the gp120 C4 region. The increased 9284 precipitation observed for the 298 R/G, 427 W/V, 427 W/S, 420 I/R, and 432 K/A mutants is not simply the result of global conformational changes in the gp120 glycoprotein, since these mutants are still recognized by some of the human monoclonal antibodies directed against discontinuous epitopes overlapping the CD4 binding region (42, 46, 62, 65). In addition, the 298 R/G and 420 I/R mutant glycoproteins can mediate virus entry, although the efficiency is less for the 420 I/R mutant than for the wild-type envelope glycoproteins. Thus, local alteration of gp120 conformation by these amino acid changes probably accounts for increased exposure of the 9284 epitope.

In lysates of transfected COS-1 cells in RIPA buffer, antibody 9284 preferentially recognized the wild-type gp160 glycoprotein (Fig. 3B). The wild-type gp120 glycoprotein was not detectably precipitated by antibody 9284 under these conditions. However, as shown in Fig. 3B, the gp120 glycoproteins containing the 298 R/G, 420 I/R, 427 W/V, and 427 W/S changes were precipitated by antibody 9284. These results suggest that the 9284 epitope is more accessible on the gp160 precursor glycoprotein than on the wild-type gp120 glycoprotein and confirm that alterations in either the base of the V3 loop or the gp120 C4 region can alter exposure of this epitope.

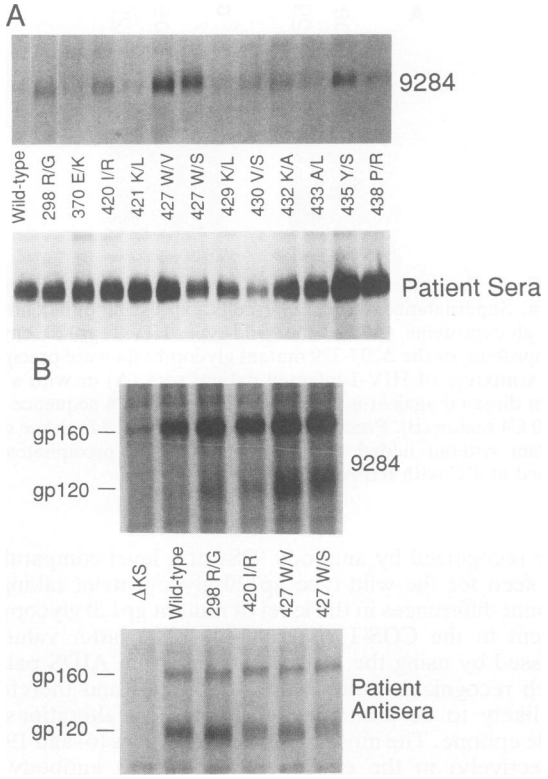

FIG. 3. Precipitation of mutant envelope glycoproteins by sera from HIV-1-infected patients and by monoclonal antibody 9284. (A) Precipitation of the wild-type and mutant HIV-1 gp120 glycoproteins in the supernatant of transfected COS-1 cells by monoclonal antibody 9284 and a mixture HIV-1-infected patient sera. The immunoprecipitations were performed at 4°C in the tissue culture medium without added detergent, and precipitates were washed in RIPA buffer at 4°C. Other HIV-1 envelope glycoproteins that exhibited a level of recognition similar to that of the wild-type gp120 glycoprotein in this assay included the 80 N/R, 102 E/L, 113 D/A, 113 D/R, 120/121 VK/LE, 252 R/W, 257 T/R, 257 T/A, 257 T/G, 266 A/E, 368 D/R, 368 D/T, 368 D/N, 368 D/K, 368 D/E, 370 E/Q, 370 E/R, 370 E/D, 382 F/L, 386 N/Q, 457 D/A, 457 D/R, 457 D/E, 470 P/G, 475 M/S, 477 D/V, and 485 K/V mutants. (B) Precipitation of the wild-type and mutant glycoproteins from transfected COS-1 cells lysed in RIPA buffer by monoclonal antibody 9284 or a mixture of HIV-1-infected patient sera.

Single amino acid changes increase the binding of anti-CD4 binding antibodies. In previous epitope mapping studies, the 298 R/G, 427 W/V, and 427 W/S mutants expressed in COS-1 cell lysates were better able than the wild-type glycoproteins to be precipitated by antibody F105 (65). To determine whether these amino acid changes would alter binding of F105 and related antibodies to the native envelope glycoprotein complex in the absence of detergent, COS-1 cells were transfected with plasmids expressing either wild-type or mutant HIV-1 envelope glycoproteins. Iodination of the transfected cells with lactoperoxidase followed by immunoprecipitation revealed that equivalent amounts of the wild-type glycoprotein and of the 298 R/G and 427 W/V mutants were expressed on the transfected COS-1 cell surface (Fig. 4A). COS-1 cells transfected in parallel were incubated with ^{35}S-labeled monoclonal antibody F105 or 1.5e for 1 h, washed, and lysed. Bound antibody was measured by detection of the labeled heavy and light antibody chains as

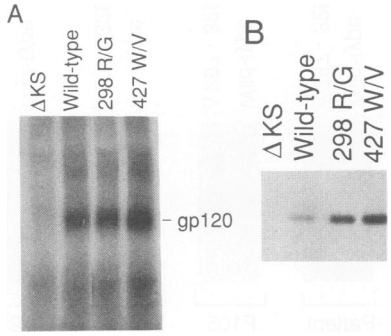

FIG. 4. Cell surface expression and monoclonal antibody F105 binding. Two sets of COS-1 cells were transfected with plasmids expressing either no envelope glycoproteins (ΔKS), the wild-type glycoproteins, or the 298 R/G or 427 W/V mutant glycoproteins. One set of transfected cells was labeled with ^{125}I by using lactoperoxidase, lysed, and precipitated with a mixture of HIV-1-positive patient sera (A); the other set of transfected COS-1 cells was incubated with ^{35}S-labeled antibody F105, washed, lysed, and used for precipitation with protein A-Sepharose beads (B). The heavy-chain band of antibody F105 is shown.

described previously (63). Figure 4B shows that increases in the binding of antibody F105 to the transfected COS-1 cells were seen for the 298 R/G and 427 W/V mutants relative to that observed for the wild-type glycoprotein. In several experiments, 4- and 6-fold increases were observed for the 298 R/G and 427 W/V mutants, respectively. A similar increase in antibody 1.5e binding was observed for the 298 R/G mutant but not for the 427 W/V mutant (data not shown), probably because the 427 W/V change results in decreased recognition of the gp120 glycoprotein by antibody 1.5e (62). These results indicate that a single amino acid change in the V3 loop can increase the recognition of the multimeric envelope glycoprotein complex by antibodies directed against the CD4 binding site. Similarly, changes in tryptophan 427 appear to result in exposure of some of the epitopes recognized by anti-CD4 binding antibodies.

Neutralization of envelope glycoprotein mutants by monoclonal antibodies or soluble CD4. The results presented above suggest that the functional envelope glycoprotein complex might be recognized more efficiently by antibodies recognizing components of the V3 loop or CD4 binding region for the described mutants. The 298 R/G and 420 I/R variants exhibit sufficient ability to support virus entry to allow an assessment of relative sensitivity to neutralization. An assay in which an *env*-defective HIV-1 provirus encoding the CAT gene is complemented by the plasmid expressing wild-type or mutant envelope glycoproteins was used (21). An assessment of the ability of the recombinant virus to be neutralized can be made by measuring CAT activity in the target Jurkat lymphocytes with and without prior incubation of the virus with antiviral agents (64, 65). The abilities of the 298 R/G and 420 I/R mutants to mediate entry into Jurkat lymphocytes in the absence of antibody were 100 and 32%, respectively, that of the wild-type glycoproteins. Table 1 shows that the 298 R/G mutant was more sensitive to neutralization by antibody 9284 than was the wild-type virus, while the 420 I/R mutant exhibited an intermediate degree of sensitivity to neutralization by this antibody. These results are consistent with the immunoprecipitation studies, which indicated that recognition by antibody 9284 exhibited the order 298 R/G > 420 I/R > wild-type glycoproteins. The 298 R/G mutant was also

TABLE 1. Concentrations of soluble CD4 or monoclonal antibodies required for 50% inhibition of entry of viruses with different envelope glycoproteins[a]

Antiviral agent	Concn (μg/ml) required for 50% inhibition of virus with:		
	Wild-type glycoprotein	420 I/R mutant	298 R/G mutant
Soluble CD4	1.3	0.8	0.8
9284	12.0	3.4	0.8
1125H	2.2	0.8	0.8
1.5e	7.2	1.3	1.2
F105	8.0	ND	1.2
2F5	4.5	ND	7.0

[a] The values shown are from a typical experiment. All neutralization assays were performed at least twice, with comparable results. Because of differences in preparations of antibody and virus stocks, the neutralization potency of antibody 2F5 cannot be directly compared with those of the other antibodies. ND, not determined.

more sensitive than the wild-type glycoproteins to neutralization by antibodies directed against the CD4 binding region, F105, 1.5e, and 1125H (Table 1). The 298 R/G mutant was slightly more sensitive than the wild-type glycoproteins to neutralization by soluble CD4 (5, 11, 14, 25, 59, 67) but did not exhibit increased sensitivity to neutralization by 2F5, a human anti-gp41 monoclonal antibody (50a). The 420 I/R mutant, while more sensitive than the wild-type glycoproteins to neutralization by antibodies 1.5e and 1125H, was neutralized comparably to the wild-type glycoproteins by antibodies F105 and 2.1h (62).

DISCUSSION

During HIV-1 entry, binding of the viral envelope glycoproteins to the CD4 receptor is followed by the membrane fusion process, which involves the gp120 V3 loop as well as elements of the gp41 glycoprotein (15, 16, 18, 18a, 21, 23, 26, 31, 34, 47, 68). Both the discontinuous CD4 binding site and continuous epitopes on the V3 loop serve as targets for HIV-1 neutralizing antibodies (19, 20, 28, 29, 38, 51, 52, 55, 60). The results presented herein indicate a structural relationship between the V3 loop and amino acids in the gp120 C4 region that serve as components of both the CD4 binding region and discontinuous epitopes overlapping that site. In buffer containing ionic detergents, the epitopes on the HIV-1 envelope glycoproteins recognized by several human monoclonal antibodies directed against the CD4 binding region appear to be at least partly masked in a V3 loop-dependent manner. This masking was most pronounced for antibodies, such as 1.5e and 1125H, that recognize tryptophan 427 as one component of the epitope (62). Anionic detergents may alter V3 loop conformation as a result of the high concentration of positive charges on the amino-terminal side of the V3 region. Recognition of linear epitopes in the gp120 C4 region by antipeptide antibodies was also increased by deletion of the V3 loop under various buffer conditions.

Changes in the conformation of the V3 loop can also affect recognition and neutralization of HIV-1 by anti-CD4 binding antibodies in the absence of detergents. Changes in an amino acid at the base of the V3 loop (arginine 298) or in the gp120 C4 region (tryptophan 427) resulted in the greatest exposure of epitopes both in the amino-terminal part of the V3 loop and near the CD4 binding region. This conformational alteration must be limited, since both mutants are recognized by antibodies that bind discontinuous gp120 epitopes and since the 298 R/G mutant retains replicative and syncytium-forming ability. These results also indicate that some alteration in the relationship of the V3 loop and the C4 region is tolerated in functional envelope glycoproteins. Proximity of tryptophan 427 to elements of the V3 loop could explain why soluble CD4 binding has been reported to increase the sensitivity of the V3 loop to proteolytic cleavage (8, 56). Whether this change or other changes in the relationship of the gp120 C4 region and V3 loop that occur upon CD4 binding are important for the virus entry process is unknown.

The proposed relationship between tryptophan 427 and elements of the V3 loop is consistent with studies of HIV-1 revertants. A mutation affecting a glycosylation site at asparagine 262 resulted in a revertant virus with an amino acid change at arginine 304, within the amino-terminal part of the V3 loop (70). Some residue changes in the gp120 region, including amino acids 256 to 262, have been previously shown to affect binding of the gp120 glycoprotein either by CD4 or by antibodies recognizing the CD4 binding site (42, 46, 62, 65). These data, taken together, suggest that C2 residues 256 to 262, C4 residues 420 to 427, and the amino-terminal part of the V3 loop may interact on the native glycoprotein.

The gp120 C4 region probably contacts the gp41 glycoprotein, since several amino acid changes in C4 affect the affinity of the noncovalent gp120-gp41 interaction (22). The proposed relationship of this region to the V3 loop provides a structural basis for understanding the potential role of the V3 loop in the membrane fusion process. Changes in V3 conformation, perhaps secondary to interaction with target cell moieties, might alter the relationship of the gp120 and gp41 glycoproteins, allowing exposure of the gp41 amino terminus to the target cell membrane.

The proposed relationship of the V3 loop and the gp120 C4 region also helps to explain some of the cooperative interactions observed between anti-V3 antibodies and anti-CD4 binding antibodies. First, binding of some anti-V3 antibodies results in increased binding of some antibodies directed against the CD4 binding region (63, 65a). Second, various degrees of cooperativity in neutralization have been observed between anti-V3 loop and anti-CD4 binding antibodies. Additive cooperativity has been observed for anti-V3 antibodies used in combination with antibody F105 or 2.1h, whereas synergistic neutralization has been observed for anti-V3 antibodies in combination with antibody 1.5e or 1125H (63, 65a). Recognition of the gp120 glycoprotein by antibodies 1.5e and 1125H is decreased by changes in tryptophan 427, whereas recognition by antibodies F105 and 2.1h is not (62, 65). It is interesting that the same pairing of anti-CD4 binding antibodies was observed with respect to sensitivity of the 420 I/R mutant to neutralization (Table 1) (62). The binding of anti-V3 neutralizing antibodies may induce conformational changes similar to that resulting from the 420 I/R change. These conformational changes would result in increased sensitivity to neutralization by antibodies, such as 1.5e and 1125h, that appear to recognize sequences near tryptophan 427 but not by antibodies, such as F105 and 2.1h, whose epitopes do not include tryptophan 427. Future work should refine our understanding of these structure-function relationships.

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