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Structural Modulations of the Envelope gp120 Glycoprotein of Human Immunodeficiency Virus Type 1 upon Oligomerization and Differential V3 Loop Epitope Exposure of Isolates Displaying Distinct Tropism upon Virion-Soluble Receptor Binding

LEONIDAS STAMATATOS AND CECILIA CHENG-MAYER*

Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, New York 10016

Received 3 March 1995/Accepted 10 July 1995

We investigated the binding of conformation-dependent anti-V2, anti-V3, and anti-CD4-binding site monoclonal antibodies to monomeric and virion-associated gp120 from human immunodeficiency virus type 1 isolates displaying marked differences in cell tropism. For all viruses examined, we found that the half-maximal binding values of the anti-V2 and anti-CD4-binding site antibodies with virion-associated gp120 were higher than those with monomeric gp120, but the maximum amount of antibodies bound was diminished only for one of the anti-V2 antibodies tested. These observations suggest that upon gp120 oligomerization, the V2 loop and CD4-binding site undergo conformational changes and that particular epitopes within these domains are occluded in the oligomeric gp120. In contrast, although the overall binding patterns and half-maximal binding values of the anti-V3 loop antibodies tested were similar with monomeric and oligomeric gp120, all the V3 loop epitopes examined were less accessible to antibody binding on the virion surface. This masking of the V3 loop is more pronounced for the primary-like macrophage-tropic isolates examined. Lastly, we observe that upon soluble receptor-virion binding, specific V3 loop epitopes that differ for viruses displaying different tropisms are exposed.

Phenotypic and antigenic variations of human immunodeficiency virus type 1 (HIV-1) isolates are believed to play critical roles in viral transmission and pathogenesis (4, 7, 9, 10, 47, 52, 53). An understanding of the molecular mechanisms controlling the different viral biological properties therefore will benefit the development of effective anti-HIV vaccines.

Genetic analyses of the HIV-1 genome have demonstrated that the third hypervariable region (V3 loop) of the viral envelope glycoprotein gp120 contains major determinants for the host range, syncytium induction, and susceptibility to serum neutralization of this virus (8, 13, 14, 19, 20, 29, 33, 36, 41, 42, 46, 48). On the basis of immunochemical analyses, we and others have reported that the structure of the V3 loop of monomeric gp120 may differ among HIV-1 isolates displaying different tropisms (12, 44). To relate these structural differences of the V3 loop of monomeric gp120 to the ability of the virus to enter different cell types, we hypothesized that the extent and/or type of conformational changes that the V3 loop of virion-associated gp120 molecules undergo upon virion-CD4 binding is influenced by the structure of the V3 loop in the context of the oligomeric gp120 (43, 44) and therefore will be different for viruses displaying different cell tropisms. Implicit in this hypothesis is the assumption that the structure of the V3 loop on monomeric and virion-associated gp120 will be similar. Furthermore, we suggested that these differences in postbinding V3 loop conformational changes in turn influence subsequent events that mediate viral entry, among which could be the interaction of the V3 loop with other cellular receptors (29).

* Corresponding author. Mailing address: Aaron Diamond AIDS Research Center, 455 First Ave., 7th Floor, New York, NY 10016. Phone: (212) 725-0018. Fax: (212) 725-1126.

In the present study, we tested this hypothesis by probing the structure of the V3 loop of virion-associated gp120 with human anti-V3 loop monoclonal antibodies (MAbs) in the absence and presence of recombinant soluble CD4 (sCD4), a surrogate for cellular CD4. Furthermore, since the V3 loop has been reported to interact with other regions of gp120 in exerting its function (1, 22, 23, 27, 31, 35, 43, 45, 50, 51), we also examined the structures of the second hypervariable region (V2 loop) and the CD4-binding domain of virion-associated gp120.

Molecularly cloned isolates displaying marked differences in tropism were used in our studies. HIV-1_{SF2} is a T-cell line-adapted virus that does not infect primary macrophages efficiently (41); this virus therefore is T-cell line tropic. HIV-1_{SF162} (41) and HIV-1_{SF128A} (25), in contrast, do not productively infect established $CD4^+$ T-cell lines, but they do replicate efficiently in primary macrophages. Furthermore, both of these viruses, in a manner similar to that of primary isolates (11, 28), are resistant to sCD4 and serum neutralization (4, 44). We also used a mutant virus that contains three amino acid substitutions in the V3 loop of HIV-1_{SF2} (MU3) (41). This virus can productively infect both T-cell lines and primary macrophages, albeit with slower kinetics and to lower titers compared with the parental HIV-1_{SF2} and the macrophage-tropic isolates, respectively.

Our results indicate that although the overall immunochemical structure of the V3 loop on monomeric gp120 is similar to that of the V3 loop on oligomeric, virion-associated gp120, the accessibility of all three epitopes examined on the virion surface is compromised. This masking of the V3 loop is particularly pronounced on the surface of the macrophage-tropic, primary-like viruses. In contrast, both the conformation and the accessibility of epitopes within the V2 and CD4-binding

site domains appear to be different for monomeric and oligomeric gp120.

Upon binding of sCD4 to the virion surface, specific V3 loop epitopes that are different for the T-cell line-tropic (HIV-1_{SF2}) and macrophage-tropic (HIV-1_{SF162} and HIV-1_{SF128A}) HIV-1 strains examined are exposed. Moreover, a simultaneous exposure of those epitopes exposed on the surface of HIV-1_{SF2} and HIV-1_{SF162} (or HIV-1_{SF128A}) virions is observed for the dual-tropic virus MU3. These observations support our hypothesis that the types of conformational changes that the V3 loop of virion-associated gp120 undergoes upon virus-receptor binding are different among viruses displaying different tropisms.

MATERIALS AND METHODS

Viruses. The isolation, molecular cloning, and characterization of HIV-1_{SF2}, HIV-1_{SF162}, and HIV-1_{SF128A} (5, 25, 26), as well as the generation of the HIV-1_{SF2} V3 loop mutant virus MU3, have been reported previously (41). All virus stocks, originally obtained by DNA transfection of RD-4 cells (5), were propagated in phytohemagglutinin-stimulated (3 μg/ml; 72 h) peripheral blood mononuclear cells from HIV-seronegative donors for a maximum of four passages. Viruses were collected at 7 days postinfection and purified from cellular debris and non-virion-associated proteins by sucrose gradient centrifugation as described previously (44).

Antibodies. The production and epitope mapping of the human anti-V3 loop (257D, 268D, and 391-95D) and anti-CD4-binding site (559-64D and 654-30D) MAbs (16, 17, 21) and of the murine anti-V2 loop MAbs (G3.4 and G3.136) (15, 30, 45) have been reported previously. The anti-V3 loop and anti-CD4-binding site antibodies were generously provided by S. Zolla-Pazner (Veterans Hospital, New York University), and the anti-V2 loop antibodies were obtained from Tanox Biosystems Inc. (Houston, Tex.) via D. D. Ho (Aaron Diamond AIDS Research Center, New York University).

Antibody binding to intact and lysed virions. Binding of MAbs to monomeric, soluble gp120 and intact virions was assessed by an enzyme-linked immunosorbent assay (ELISA) (28, 32, 44). To quantitate the amounts of MAbs bound to the virion surface, sucrose gradient-purified viruses were incubated with increasing concentrations of MAbs (0.01 to 10 μg/ml) for 3 h at room temperature. The gp120 content of each sucrose-purified virus stock was determined by the use of human polyclonal anti-HIV-1 sera (32, 44). The stocks were normalized accordingly, so that equal amounts of gp120 for all viruses tested were incubated with the antibodies. Under the experimental conditions used, spontaneous gp120 shedding from the virion surface was minimal and saturation binding of all MAbs to soluble monomeric gp120 was achieved (data not shown). The interaction between virions and MAbs took place in 150 μl of medium (144 mM NaCl, 25 mM Tris [pH 7.5]) containing 10% fetal calf serum. Incubation at 37°C resulted in identical MAb-binding patterns (data not shown). The virion-MAb complexes were then separated from unbound MAbs by pelleting the complexes by centrifugation (15,000 × *g* for 90 min at 15°C) as described by Willey et al. (49). The pellet was then resuspended in 150 μl of the above-described medium supplemented with 4% nonfat dry milk (Carnation) and Nonidet P-40 (1% [vol/vol]). The detergent lysed the virion envelope without disrupting the gp120-MAb interaction, and the gp120-MAb complexes were captured onto ELISA plates (Corning Inc., Corning, N.Y.) precoated with sheep polyclonal antibodies against the carboxy-terminal region of gp120 (D6205) (International Enzymes, Inc., Fallbrook, Calif.) (32, 44). Quantitation of MAbs bound to gp120 was then carried out by determining the A_{490} (optical density at 490 nm [OD_{490}]) with the appropriate secondary antibodies coupled to alkaline phosphatase as previously described (44). The OD_{490} value obtained in the absence of gp120 (negative control wells) had been subtracted from the OD_{490} values presented in the figures. In studies to examine postbinding conformational changes of the V3 loop, increasing concentrations (0 to 200 nM) of recombinant sCD4 (Chiron Corporation, Emeryville, Calif.) were incubated with sucrose gradient-purified virus and 0.1, 1, and 5 μg of anti-V3 loop MAbs per ml. The virion-sCD4-MAb complexes were then separated from unbound MAbs and sCD4 by pelleting, and the amounts of MAbs present in the viral pellet (i.e., bound to the virion-associated gp120 during the preincubation period) were then determined immunochemically as described above.

Binding of MAbs or sCD4 to the virion surface may result in dissociation of gp120-MAb or gp120-MAb-sCD4 complexes. To monitor for gp120 dissociation from the virion surface, supernatants from the virion-MAb or virion-sCD4-MAb pellets were collected and added to ELISA wells coated with D6205. The amounts of MAbs complexed with gp120 were then quantitated as described above. The sum of the OD_{490} values of the viral pellets and supernatants represents the total amount of MAb bound to virion gp120, since soluble gp120 initially present in the culture supernatants had been removed by sucrose gradient purification of the virions.

The binding of MAbs to monomeric soluble gp120 was performed in parallel by prelysing sucrose gradient-purified virions with Nonidet P-40 and incubating the lysates with increasing concentrations of MAbs for 3 h at room temperature. Following this step, the mixtures were added to ELISA wells coated with D6205. Unbound MAbs were removed by washing, and the amounts of MAbs bound to monomeric gp120 were determined as described above.

In the studies in which we compared the extents of MAb binding to monomeric and virion-associated gp120 molecules, we incubated equal amounts of lysed and intact virions with MAbs and we corrected the OD_{490} values for small variations in the amounts of gp120 captured in each well by quantitating in parallel the total amounts of gp120 captured in each well by using pooled human anti-HIV-1 sera (32, 44).

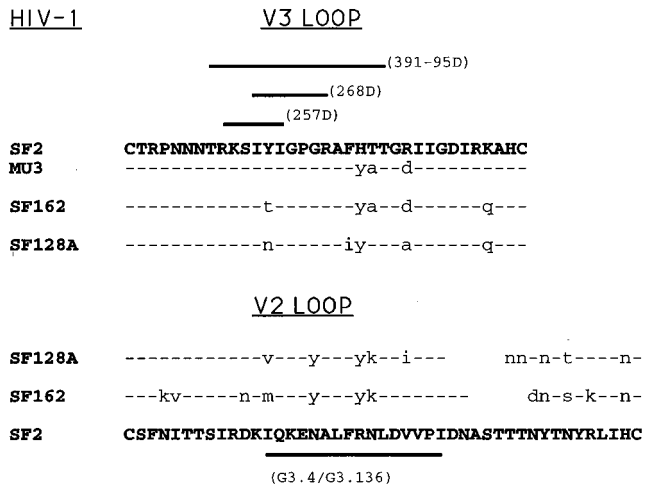

FIG. 1. Primary amino acid sequences of the second and third hypervariable regions of HIV-1_{SF2}, HIV-1_{SF162}, HIV-1_{SF128A}, and MU3. The amino acid sequences of the V2 and V3 loops of the T-cell line-tropic virus HIV-1_{SF2} are shown and are compared with those of the two macrophage-tropic isolates (HIV-1_{SF162} and HIV-1_{SF128A}). Dashes represent amino acid sequence homology with the HIV-1_{SF2} sequence. Amino acids that differ are shown with small letters. MU3 is a mutant virus constructed on the background of HIV-1_{SF2} (41). The entire primary amino acid sequence of the envelope is similar to that of HIV-1_{SF2} except for the three positions indicated within the V3 loop. Compared with those of the two macrophage-tropic isolates, the V2 loop of HIV-1_{SF2} is longer by 5 amino acids. The core epitopes recognized by the anti-V3 loop (257D, 268D, and 391-95D) and anti-V2 (G3.4 and G3.136) MAbs used are also shown.

RESULTS

Binding of anti-V3 loop MAb to monomeric gp120 molecules. We previously reported on the differential binding of three human anti-V3 loop MAbs to monomeric gp120 molecules of the T-cell line-tropic HIV-1_{SF2} and macrophage-tropic HIV-1_{SF162} viruses that were transiently expressed in COS-7 cells (23, 44). Since the glycosylation pattern of gp120 may vary depending on the cell type used to produce it (6) and differences in glycosylation can affect the accessibility of certain gp120 epitopes (50), we first determined the binding pattern of these anti-V3 loop MAbs with gp120 monomers from virions produced in human peripheral blood mononuclear cells. The V3 loop amino acid sequences of the viruses and the core epitopes recognized by the human anti-V3 loop MAbs tested (257D, 268D, and 391-95D) are shown in Fig. 1. For HIV-1_{SF2} monomeric gp120, we observed that MAb 257D bound more extensively than MAb 268D, while the binding of MAb 391-95D was minimal (Fig. 2A, open symbols). The MAb-binding patterns for the two macrophage-tropic isolates, HIV-1_{SF162} and HIV-1_{SF128A}, were similar (Fig. 2B and C, respectively, open symbols), but they differed from that for HIV-1_{SF2}; that is, MAb 268D showed poor reactivity, while MAb 391-95D bound more efficiently than MAb 257D. The binding patterns of the human MAbs with gp120 monomers produced by virion

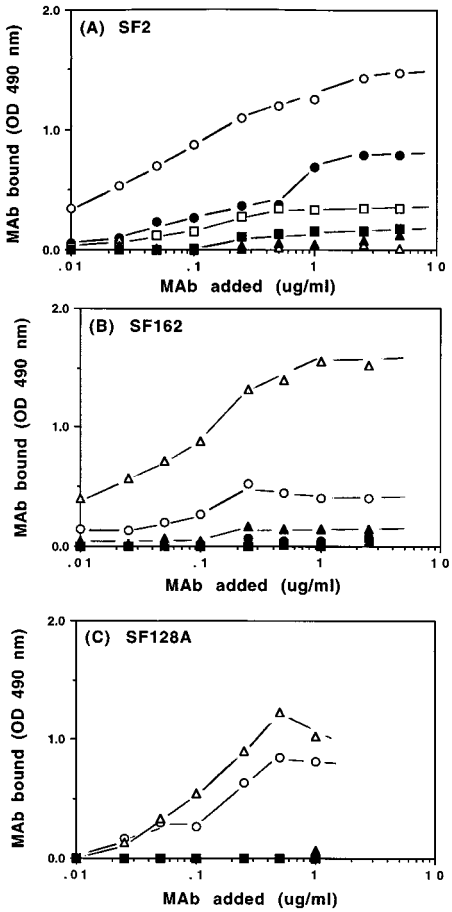

FIG. 2. Comparison of the extents of binding of anti-V3 loop MAbs to monomeric and virion-associated gp120. Increasing amounts of anti-V3 loop MAbs were incubated with the different sucrose gradient-purified viruses. The extents of binding of MAbs 257D (○, ●), 268D (□, ■), and 391-95D (△, ▲) to intact (filled symbols) and detergent-lysed (open symbols) virions of HIV-1_{SF2} (A), HIV-1_{SF162} (B), and HIV-1_{SF128A} (C) were assessed by immunochemical approaches as described in Materials and Methods. For each isolate, equal amounts of lysed and intact virions were used. The OD_{490} values shown for the intact virions are the sums of OD_{490} values obtained for the viral pellets and the supernatants following virus pelleting (see Materials and Methods). The results are representative of at least three independent experiments.

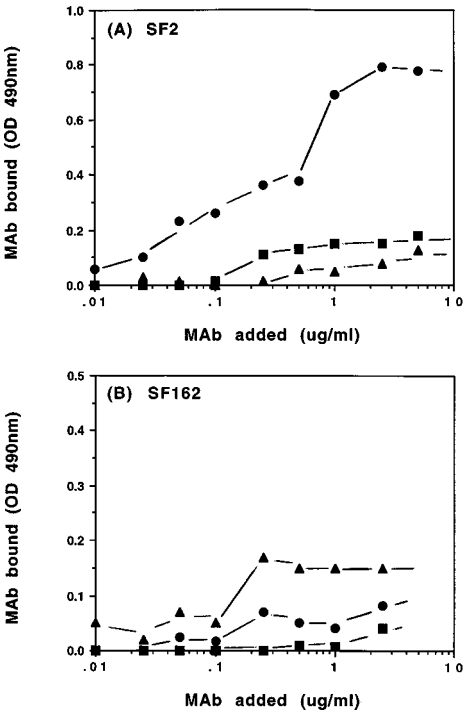

FIG. 3. Binding of anti-V3 loop MAbs to the virion-associated gp120 of isolates displaying different tropisms. The data presented here are similar to those shown in Fig. 2, but the *y* axis is different to emphasize the difference in the extents of binding to the surfaces of intact HIV-1_{SF2} (A) and HIV-1_{SF162} (B) virions. ●, 257D; ■, 268D; ▲, 391-95D.

lysis were therefore similar to those previously reported for soluble gp120 transiently expressed in COS-7 cells (44), and they suggest that the accessibility of the epitopes recognized by these three human MAbs on gp120 monomers is not affected by potential differences in gp120 glycosylation.

Similar anti-V3 loop MAb-binding patterns with monomeric and virion-associated gp120 molecules. To determine if the conformation of the V3 loop of virion-associated gp120 molecules is similar to that of the V3 loop of the corresponding gp120 monomers, we examined the binding of the anti-V3 loop MAbs to virion-associated gp120. For HIV-1_{SF2}, we observed that the virion-associated gp120 molecules bound MAb 257D more extensively than MAb 268D, which in turn bound to the virion surface more extensively than MAb 391-95D (Fig. 3A). This pattern is similar to that for HIV-1_{SF2} gp120 monomers (compare Fig. 2A and 3A; the scale of the *y* axis is different between the two figures for clarity). However, the half-maximal binding values of MAbs 257D and 268D with oligomeric gp120 were increased by three- and twofold, respectively, compared with those observed for monomeric gp120 (Table 1). For HIV-1_{SF162}, MAb 391-95D bound more extensively than MAb 257D, while MAb 268D did not bind significantly to the virion-associated gp120 (Fig. 3B). The binding pattern of HIV-1_{SF128A} was similar to that of HIV-1_{SF162} (data not shown). For these two macrophage-tropic viruses, the half-maximal binding values of the MAbs tested with gp120 monomers and oligomers were similar (Table 1).

With regard to the dual-tropic virus MU3, we previously reported that all three anti-V3 loop MAbs bound to soluble gp120, with MAb 257D and MAb 391-95D binding more extensively than MAb 268D (44). Compared with the values for half-maximal binding of MAb 257D and MAb 268D to soluble gp120, the values for half-maximal binding of these MAbs to the MU3 virion surface were increased by three- and twofold, respectively (as for HIV-1_{SF2}), while the half-maximal binding values of MAb 391-95D with virions and gp120 monomers were similar (data not shown).

Exposure of the V3 loops on monomeric and virion-associated gp120 molecules differs. Although the MAb-binding patterns obtained were qualitatively similar to those seen with gp120 monomers, the third hypervariable region of gp120 appears to be relatively inaccessible on the virion surface. This was indicated by the decrease in the extent of MAb binding to oligomeric, virion-associated gp120 (Fig. 2, filled symbols) compared with that of binding to the gp120 monomers (open symbols) for the viruses and MAbs tested. The masking of the V3 loop appears to be markedly more pronounced on the surface of the primary-like macrophage-tropic HIV-1_{SF162} and HIV-1_{SF128A} viruses (Fig. 2B and C) than on the T-cell line-tropic HIV-1_{SF2} virus (Fig. 2A). For HIV-1_{SF162} and HIV-1_{SF128A}, the extent of binding of MAbs 257D and 391-95D was more than 10-fold reduced compared with that of binding to

TABLE 1. Half-maximal binding values of anti-gp120 MAbs with monomeric and virion-associated gp120

MAb	gp120 region	Half-maximal binding value (μg/ml)[a]					
		SF2		SF162		SF128A	
		M	O	M	O	M	O
257D	V3 loop	0.05	0.16	0.1	0.15	0.1	0.15
268D	V3 loop	0.08	0.15	ND[b]	ND	ND	ND
391-95D	V3 loop	ND	ND	0.08	0.1	0.1	0.15
G3.4	V2 loop	ND	ND	0.1	1	0.03	1.5
G3.136	V2 loop	ND	ND	0.35	1.7	0.5	4.9
654-30D	CD4-binding site	0.025	0.825	0.02	0.36	0.03	0.4
559-64D	CD4-binding site	0.07	0.75	0.023	0.9	0.03	0.8

[a] Data are the average half-maximal binding values (antibody concentrations) from three to four independent experiments. The standard deviation from the mean (not shown) was less than 13% of the mean. M, monomeric gp120; O, virion-associated gp120.
[b] ND, not determined because of poor MAb-gp120 reactivity.

the gp120 monomers. For the T-cell line-tropic virus HIV-1_{SF2} (Fig. 2A), the extent of binding of MAbs 257D and 268D was reduced by only half.

Binding of anti-V2 loop and anti-CD4-binding site MAbs to virion-associated and monomeric gp120. To determine if the structure and exposure of other functional regions of the envelope gp120 are affected by oligomerization of the viral envelope, we examined the binding of two conformation-sensitive murine anti-V2 loop MAbs (G3.4 and G3.136) (15, 30, 45) and two human anti-CD4-binding site MAbs (559-64D and 654-30D) (16, 21) to monomeric and virion-associated gp120 molecules (Fig. 4 and 5). The V2 loop amino acid sequences of the viruses used and the epitopes recognized by these MAbs are shown in Fig. 1. The anti-V2 loop MAbs tested do not bind to gp120 of HIV-1_{SF2} (23, 30), but they reacted with monomeric and oligomeric gp120 molecules of both HIV-1_{SF162} and HIV-1_{SF128A}. MAb G3.4 (Fig. 4A and C) binds to monomeric gp120 with a higher affinity than MAb G3.136 (Fig. 4B and 4D) (half-maximal binding values of 0.1 and 0.35 μg/ml, respectively, for HIV-1_{SF162} and 0.03 and 0.5 μg/ml, respectively, for HIV-1_{SF128A}). The half-maximal binding values of both antibodies with virion-associated gp120 were substantially increased compared with those observed for gp120 monomers (Table 1); the fold increase in the half-maximal binding values was dependent both on the antibody used and on the HIV-1 isolate tested. However, the maximal amount of antibody bound to virion-associated gp120, compared with the maximal amount bound to monomeric gp120, was significantly reduced only for MAb G3.136 (Fig. 4B and D).

The binding of conformation-sensitive human anti-CD4-binding site MAbs (559-64D and 654-30D) was also determined (Fig. 5). We observed that these antibodies bound extensively to both monomeric and virion-associated gp120 molecules of all three isolates examined (the results obtained with HIV-1_{SF128A} were similar to those obtained with HIV-1_{SF162}; data not shown). The maximal amounts of antibodies bound to monomeric and virion-associated gp120 for each virus tested were comparable. However, compared to the half-maximal binding values with monomeric gp120, a 10- to 40-fold increase in the half-maximal binding values with virion-associated gp120 was observed (Fig. 5 and Table 1). Furthermore, although both MAbs bound significantly to all viruses tested, only the binding of these MAbs to the T-cell line-tropic HIV-1_{SF2} virions induced a significant amount of gp120 dissociation from the viral surface (compare Fig. 5A and B with Fig. 5C and D).

Effect of sCD4-virion association on the exposure of the V3 loop. To test the hypothesis that the V3 loops of isolates displaying different cell tropisms undergo different conformational changes during virion-receptor binding as a consequence of their differences in structure, we compared the types and extents of sCD4-induced exposure of V3 loop epitopes on virion-associated gp120 molecules of T-cell line-, macrophage-, and dual-tropic viruses (Fig. 6). The results showed that the degree of postbinding exposure of the epitope recognized by MAb 257D (Fig. 6A) was dependent on the tropism of the virus tested. An increase in binding of MAb 257D to its epitope was observed in all cases, but the degree of increase in exposure of this epitope was more pronounced for the T-cell line- and dual-tropic viruses (HIV-1_{SF2} and MU3, respectively) (Fig. 6A). The epitope recognized by MAb 391-95D became exposed on the surface of both macrophage- and dual-tropic viruses (HIV-1_{SF162} and HIV-1_{SF128A} and MU3, respectively), but such exposure was negligible on the HIV-1_{SF2} surface (Fig. 6B). The exposure of the epitope recognized by MAb 268D was not affected by sCD4-virion binding regardless of the virus used (Fig. 6B). Similar results were obtained when higher antibody concentrations (up to 5 μg/ml) were used (data not shown).

DISCUSSION

To assess whether specific structural features of virion-associated gp120 influence the tropism of HIV-1, we examined and compared the structures of the V2 and V3 loops, together with that of the CD4-binding site region, of monomeric and virion-associated gp120 of three molecularly cloned HIV-1 isolates (HIV-1_{SF2}, HIV-1_{SF162}, and HIV-1_{SF128A}) that display marked differences in tropism.

We find that the pattern of binding of the anti-V3 loop MAbs to the virion surface, i.e., the oligomeric gp120 form, is qualitatively similar to that of their binding to the monomeric gp120 form (Fig. 2 and 3). Although the primary amino acid sequences of the V3 loops of the two macrophage-tropic HIV-1_{SF162} and HIV-1_{SF128A} isolates tested are different (Fig. 1), the binding patterns obtained with these isolates and the anti-V3 loop MAbs are similar to each other (Fig. 2B and C) and differ from that obtained with the T-cell line-tropic HIV-1_{SF2} isolate (Fig. 2A). This is consistent with reports indicating the necessity of preserving a particular V3 loop structure among isolates displaying macrophage tropism (8, 12, 24, 25, 44).

Although the half-maximal binding values of the anti-V3 MAbs tested with monomeric and oligomeric gp120 are comparable (Table 1), the maximal amount of the anti-V3 loop MAbs bound to the virion surface was reduced compared with the maximal amount bound to the gp120 monomers. This re-

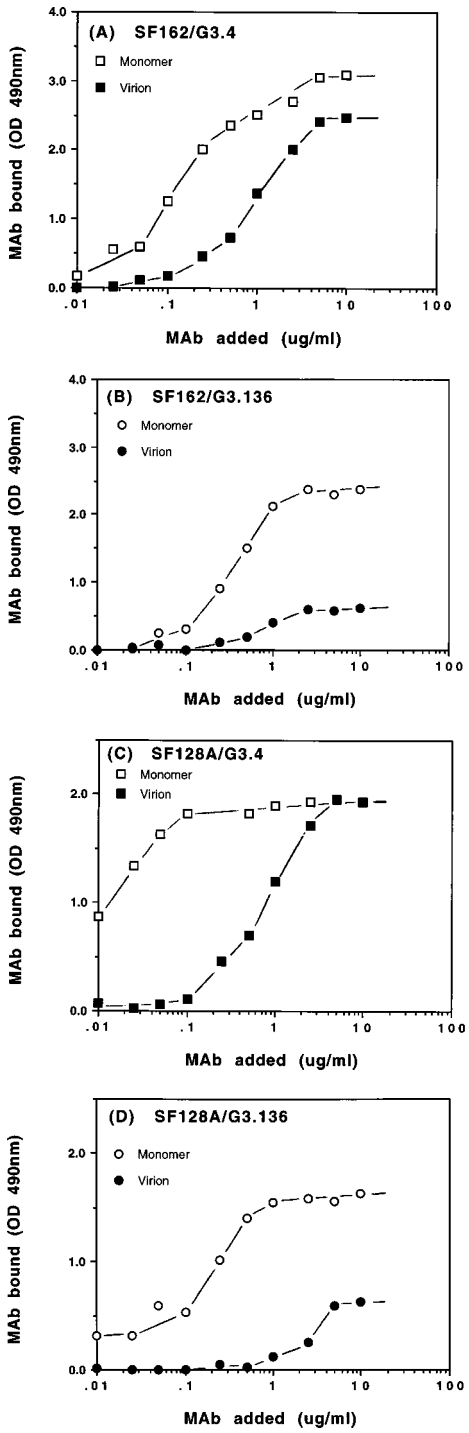

FIG. 4. Extents of binding of anti-V2 loop MAbs to monomeric and virion-associated gp120. The binding of G3.4 and G3.136 to equal amounts of monomeric and virion-associated gp120 of HIV-1_{SF162} and HIV-1_{SF128A} was assessed as described in Materials and Methods. The anti-V2 loop MAbs do not react with the gp120 of HIV-1_{SF2}.

duction in V3 loop epitope binding was more pronounced for the primary-like macrophage-tropic viruses HIV-1_{SF162} and HIV-1_{SF128A} (Fig. 2B and C). These observations made with intact virions are in agreement with those reported recently by Bou-Habib et al., who used a $CD4^+$ T-cell line chronically infected with macrophage-tropic and T-cell line-tropic variants of JR-CSF (3). Several reasons may be responsible for the apparent masking of the V3 loop on the virion and on the surfaces of infected cells: (i) the conformation of the V3 loop itself may change upon oligomerization, resulting in a decrease in the amount of MAbs bound; (ii) the V3 loop may be blocked by other regions of the same molecule upon oligomerization (this possibility implicates structural alterations of these other regions of gp120, but not the V3 loop, during oligomerization); (iii) during juxtaposition of the gp120 subunits, the V3 loop may become buried within the oligomeric structure; and (iv) proteins of the cell plasma membrane, which become incorporated into the virion surface during the process of viral budding (2, 18, 40), may obstruct this region of gp120. However, it is possible that other V3 loop epitopes not examined here are as exposed on the virion surface as on the gp120 monomers. Additional probing of the V3 loop is necessary to address this possibility.

In contrast to the findings made with the anti-V3 loop MAbs, the maximal amounts of both anti-CD4-binding site MAbs and one of the two anti-V2 loop MAbs bound to monomeric and virion-associated gp120 of the macrophage-tropic strains tested appear to be comparable (Fig. 4 and 5). Although we cannot assess the binding of anti-V2 loop MAbs to the V2 region on the virion surface of HIV-1_{SF2}, data for binding to the CD4-binding site indicate that this region of gp120 is also exposed on the T-cell line-tropic HIV-1_{SF2}. However, compared with the values obtained for binding to the gp120 monomers, the half-maximal binding values of the anti-CD4-binding site MAbs tested with the virion surfaces of both macrophage- and T-cell line-tropic isolates were increased (Fig. 5). These findings suggest a change in the conformation and/or extent of exposure of these epitopes upon oligomerization. A similar observation was made with the anti-V2 loop MAb G3.4 (Fig. 4A and C), but for the other anti-V2 loop MAb, G3.136, both the maximal amount of antibody bound and the half-maximal binding values were altered upon envelope oligomerization (Fig. 4B and D). Although these two anti-V2 loop MAbs are reported to compete for binding to gp120, they appear to recognize different but overlapping epitopes (30, 45) whose conformation and/or exposure is differentially modulated upon envelope oligomerization. A similar increase in the half-maximal binding values of MAbs to the V2 loop and the CD4-binding site, but not those of MAbs directed against the V3 loop, for cell-associated envelope gp120 was also observed by Sattentau and Moore (38).

The observation that the maximal extents of binding of the two anti-CD4-binding site MAbs tested are similar for the T-cell line-tropic and macrophage-tropic isolates indicates that certain epitopes of the CD4-binding site are equally well exposed on the surfaces of T-cell line-tropic and macrophage-tropic viruses. The anti-CD4-binding site MAbs tested here have been reported to compete for binding to sCD4 (16, 21). However, macrophage-tropic isolates, like the HIV-1_{SF162} and HIV-1_{SF128A} strains used here, are more resistant to neutralization by sCD4 than are T-cell line-adapted isolates (11, 28, 44). In preliminary studies, we observed that HIV-1_{SF162} and HIV-1_{SF128A} are also more resistant to neutralization by the anti-CD4-binding site MAbs tested here than is HIV-1_{SF2}. In this regard, we observed that the binding of both anti-CD4-binding site MAbs to the T-cell line-tropic HIV-1_{SF2}, but not to the macrophage-tropic viruses, resulted in extensive release of gp120 molecules from the virion surface (up to 60% of the total virion-associated gp120 molecules; Fig. 5), an indication of a weaker gp120-gp41 association for HIV-1_{SF2} than for HIV-1_{SF162} and HIV-1_{SF128A}. These observations are in agree-

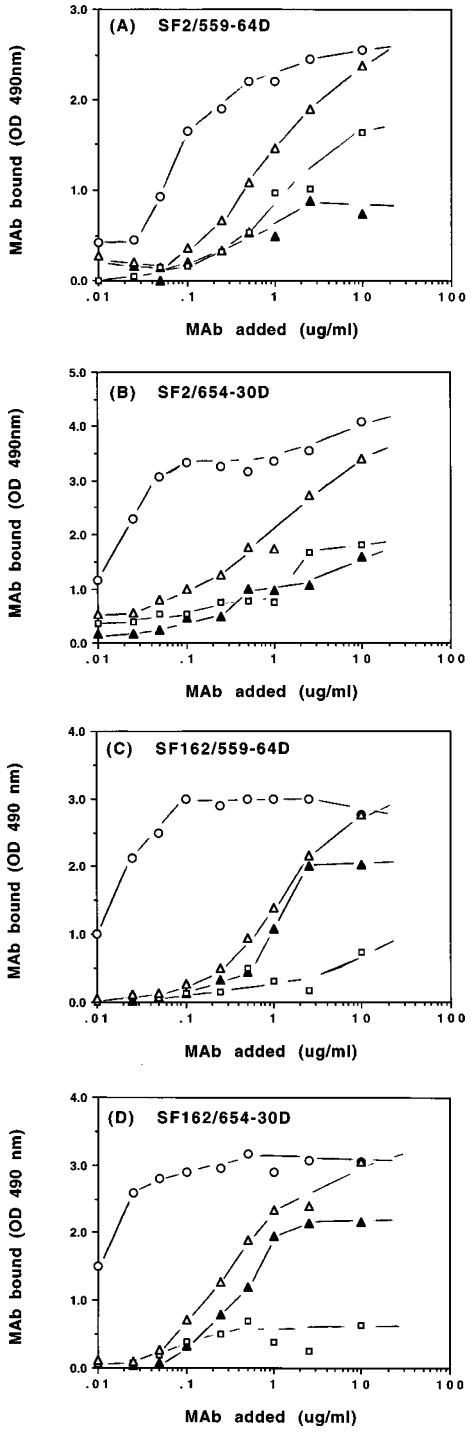

FIG. 5. Extent of binding of MAbs to the anti-CD4-binding site of gp120. The binding of two anti-CD4-binding site MAbs (559-64D and 654-30D) to equal amounts of monomeric and virion-associated gp120 was assessed as described in Materials and Methods. Results for HIV-1_{SF2} and MAb 559-64D, HIV-1_{SF2} and MAb 654-30D, HIV-1_{SF162} and MAb 559-64D, and HIV-1_{SF162} and MAb 654-30D are shown. To control for gp120 dissociation from the virion surface as a result of MAb binding, amounts of MAb bound to gp120 present in the viral pellet and supernatant fractions were determined. ○, MAb bound to soluble monomeric gp120; ▲, MAb bound to intact virions; □, MAb present in the viral supernatant as MAb-gp120 complexes; △, total MAb bound to virions, including the MAb-gp120 complexes released from the viral surface.

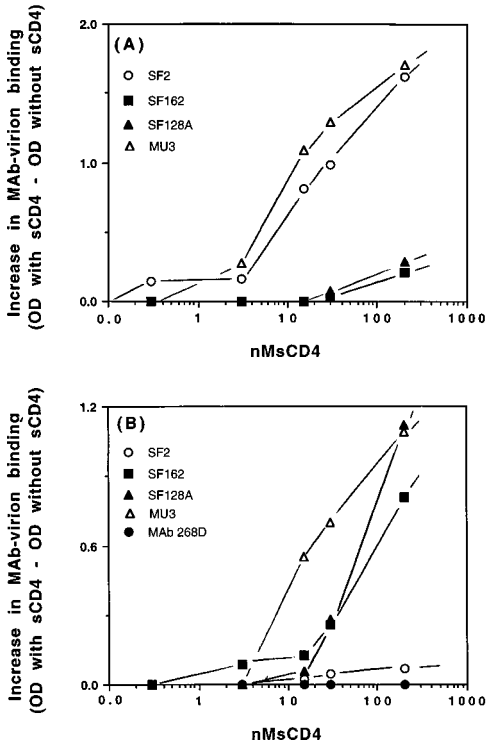

FIG. 6. Increase of exposure of V3 loop epitopes upon sCD4-virion binding. Sucrose gradient-purified viruses were incubated with 0.1 μg of anti-V3 loop MAbs 257D (A) and 391-95D (B) per ml in the presence of increasing sCD4 concentrations (0 to 200 nM). The extent of MAb-virion binding was then determined for each sCD4 concentration. The OD_{490} values obtained in the absence of sCD4 were subtracted from those obtained at the different sCD4 concentrations indicated and are corrected for the sCD4-induced removal of gp120 molecules from the virion surface (i.e., OD_{490} of the viral pellet and OD_{490} of the viral supernatants) as described in the Materials and Methods section. The epitope recognized by the anti-V3 loop MAb 268D remained unexposed on the virion surfaces of all isolates examined (B).

ment with those previously made by examining the sCD4-binding affinity and sCD4 neutralization sensitivity of HIV-1 isolates (28, 34, 44, 49) and suggest that a basis for the increase in susceptibility of T-cell line-adapted viruses, of which HIV-1_{SF2} is an example, to antibody- and sCD4-mediated neutralization is the decrease in stability of the gp120-gp41 association and not the extent of MAb-virion binding.

Regarding the role of the V3 loop in influencing the tropism of HIV-1, of more interest are the observations made in the presence of sCD4. In results similar to those obtained with HIV-1-infected T-cell lines (37, 39) and intact HIV-1 virions (27, 35), we observed an increase in the exposure of the V3 loop upon binding of virion-associated gp120 to sCD4 (Fig. 6). This could be due to conformational changes of the V3 loop itself. Alternatively, since the V3 loop may be masked by other gp120 regions on the virion surface, binding of sCD4 to virions could induce the displacement of these regions and thus increase the V3 loop exposure. In either case, not every V3 loop epitope examined became exposed, suggesting that specific epitopes are exposed during sCD4-induced gp120 conformational changes. The degree of exposure of a particular V3 loop epitope depends on the genomic background and hence the tropism of the virus. For example, although the epitope recognized by MAb 257D is present on the gp120 of all the viruses tested (Fig. 2), it becomes significantly more exposed only on the virion surfaces of the HIV-1_{SF2} T-cell line-tropic virus and

the MU3 dual-tropic virus upon receptor binding, whereas the epitope recognized by MAb 391-95D was preferentially exposed upon binding of macrophage-tropic and dual-tropic virions to sCD4. Thus, upon receptor binding, specific V3 loop epitopes that are different for the T-cell line- and macrophage-tropic viruses are exposed, but both of these V3 loop epitopes are exposed on the surfaces of the dual-tropic viruses. These observations support our hypothesis that the type and extent of CD4-induced gp120 conformational changes differ between isolates displaying different tropisms (44). These differences in conformational changes may form the basis for differences in cell tropism of HIV-1.

In summary, structural differences between monomeric and oligomeric gp120 and between virion-associated gp120 of T-cell line-adapted and primary-like macrophage-tropic isolates are observed upon receptor binding. It will be important to further define these structural differences and determine how these differences influence cell tropism and other antigenic and biological properties of HIV-1.

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