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Functional Role of the V1/V2 Region of Human Immunodeficiency Virus Type 1 Envelope Glycoprotein gp120 in Infection of Primary Macrophages and Soluble CD4 Neutralization

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We have examined the influence of the V1/V2 region of the human immunodeficiency virus type 1 (HIV-1) gp120 on certain biologic properties of the virus. We observed that on the genomic background of the T-cell-line-tropic strain, HIV-1_{SF2mc}, both the V1 and V2 domains of the macrophage-tropic strain, HIV-$1_{SF162mc}$, in addition to the required V3 domain, are necessary to attain full macrophage tropism. Furthermore, the V2 domain modulates the sensitivity of HIV-1 to soluble CD4 neutralization. Structural studies of recombinant and mutant envelope glycoproteins suggest that the function of the V1/V2 region is to interact with the V3 domain and confer on the envelope gp120 of HIV-1_{SF2mc} a conformation more similar to that of the macrophage-tropic strain HIV-$1_{SF162mc}$. The conformation of the envelope gp120 appears to be strain specific and plays an important role in determining HIV-1 tissue tropism and sensitivity to soluble CD4 neutralization.

Major targets for human immunodeficiency virus (HIV-1) infection in vivo are $CD4^+$ lymphocytes and cells of the mononuclear phagocytic lineage, such as microglial cells in the brain and monocyte-derived macrophages in a variety of tissues (25). HIV-1 isolates display a high degree of sequence divergence as a result of the high error rate of the enzyme reverse transcriptase (RT) (27, 35). Some of this genomic heterogeneity, especially variations in the envelope region, has been shown to control biological properties in vitro, including cellular host range or tropism, kinetics of replication, syncytium induction, and susceptibility to serum or soluble CD4 (sCD4) neutralization (25). Results from studies of recombinant and mutant viruses have shown that the V3 domain of envelope gp120 is a major determinant for these biological properties (5, 7, 11, 13, 19–21, 30, 31, 34, 37, 38, 40, 42, 43).

We previously reported that three amino acid changes within the V3 loop alone (at positions 319, 320, and 323) can confer macrophage tropism on the T-cell-line-tropic isolate HIV-1_{SF2mc}. However, the level of replication of this mutant virus (Mu3) is low compared with that of the parental macrophage-tropic strain HIV-$1_{SF162mc}$ (38). Additional regions outside of the V3 loop, including the V1 and V2 domains of envelope gp120, appear to be necessary for efficient infection of primary peripheral macrophages (37).

In addition to its influence on tissue tropism (2, 37, 43), the V1/V2 domain of envelope gp120 has recently been shown to participate in postbinding events that are involved in membrane fusion (1, 14, 39). Amino acid changes in the V1/V2 domain of the HXBc2 strain of HIV-1 can affect envelope subunit association and syncytium formation (39). Moreover, an interaction of the V2 and V3 domains with other regions in the envelope glycoprotein can modulate some of these properties (14, 37, 43). Furthermore, it is increasingly clear that the V1/V2 region, in addition to the V3 domain, serves as a primary target for neutralizing antibodies (12, 16, 26, 29). However, the mechanism(s) by which the V1/V2 domain mediates these effects is not known.

To examine the functional role of the V1/V2 domain for macrophage tropism, in the presence of the required V3 domain, we generated additional recombinant and mutant viruses by using HIV-1_{SF2mc} and HIV-$1_{SF162mc}$. Their abilities to infect primary macrophages were determined. Furthermore, recombinant and mutant envelopes were transiently expressed in COS-7 cells to assess the synthesis, processing, and structure of each of the envelope glycoproteins. Our results indicate that the V1 and V2 domains modify the overall structure of the envelope gp120 and consequently influence the biological properties of HIV-1.

MATERIALS AND METHODS

Cells. Phytohemagglutinin (3 μg/ml)-stimulated peripheral blood mononuclear cells (PBMC), obtained from normal seronegative blood donors (provided by Irwin Memorial Blood Bank, San Francisco, Calif.), were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum and 10% interleukin-2 (Collaborative Research, Bedford, Mass.). Primary peripheral blood monocytes were obtained from PBMCs by the plastic-adherent technique (6) and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 5% human serum for 10 to 12 days before use. The human rhabdomyosarcoma (RD-4) cells and the simian COS-7 cells were obtained from the American Type Culture Collection and propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Generation of recombinant DNA and viruses. Two principal fragments of the viral genome (5′ and 3′), obtained by *Eco*RI digestion of the biologically active phage clones of HIV-1_{SF2mc} and HIV-$1_{SF162mc}$, were subcloned into plasmid pUC19 as described previously (4). The construction of recombinant plasmid DNA (R19) and mutant plasmid DNA (Mu3) from the 3′ *Eco*RI fragment that contained the *tat*, *rev*, *vpu*, *env*, and *nef* genes and the 3′ long terminal repeat of HIV-1 has been

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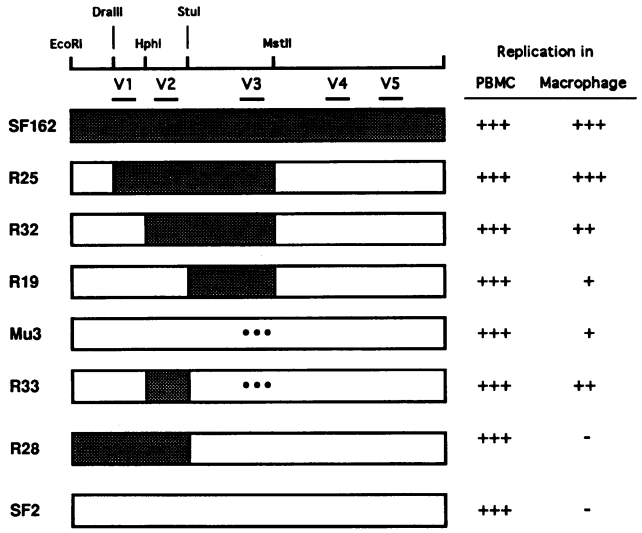

FIG. 1. Genomic structure and biological properties of HIV-$1_{SF162mc}$ and HIV-1_{SF2mc} recombinant viruses. Shaded bars indicate DNA sequences from HIV-$1_{SF162mc}$, and open bars indicate those from HIV-1_{SF2mc}. Solid circles indicate the three HIV-$1_{SF162mc}$-specific amino acids (38) (see Fig. 4). Biological phenotypes of the corresponding molecular clones are indicated. For HIV-1 replication, detected by RT activity in the culture fluid, +++ indicates $>5 \times 10^5$ cpm of RT activity per ml, ++ indicates $>10^5$ cpm/ml, + indicates $>10^4$ cpm/ml, and − indicates $<10^4$ cpm/ml. The background level was $<2 \times 10^3$ cpm of RT activity per ml. Results summarize data from at least three separate experiments.

published (37, 38). Additional recombinant plasmid DNAs (R25, R28, R32, and R33) (Fig. 1) were generated as follows. For R25, a 0.73-kb *Dra*III-*Mst*II fragment of HIV-$1_{SF162mc}$, encompassing the V1, V2, and V3 domains, was substituted into the corresponding region of HIV-1_{SF2mc}. For R28, a 1.1-kb *Eco*RI-*Stu*I fragment of HIV-$1_{SF162mc}$, encompassing the V1 and V2 domains, was substituted into HIV-1_{SF2mc}. For R32 and R33, the common *Hph*I site (nucleotide 6708) that separates the V1 and V2 domains of HIV-1_{SF2mc} and HIV-$1_{SF162mc}$ gp120 was used. Briefly, an *Eco*RI-*Hph*I (0.96-kb) fragment of HIV-1_{SF2mc} and an *Hph*I-*Stu*I (139-bp) fragment of HIV-$1_{SF162mc}$ were subcloned into pSL1180 vectors (Pharmacia, Piscataway, N.J.). These hybrid 1.1-kb *Eco*RI-*Stu*I fragments were then subcloned into pUC19 together with a 3.7-kb *Stu*I-*Eco*RI fragment from R19 or Mu3 DNA. The 3′ *Eco*RI recombinant fragments were then cotransfected with the 5′ *Eco*RI fragment of HIV-1_{SF2mc} into RD-4 cells by the calcium phosphate precipitation method followed by cocultivation with mitogen-activated PBMCs from seronegative donors (4). RT (17)-positive culture supernatants were collected at 7 to 10 days posttransfection and frozen at −70°C as virus stocks.

Construction of N-linked glycosylation site mutants. For mutagenesis, a 2.9-kb *Eco*RI-*Pst*I fragment of HIV-1_{SF2mc} and Mu3 was subcloned into the pTZ19U vector. Single-stranded DNAs were generated and subjected to site-directed mutagenesis with mutant oligodeoxynucleotides as primers according to the procedure recommended by the supplier (Bio-Rad Laboratories, Hercules, Calif.) (23). Mutations generated were confirmed by DNA sequencing, and two clones of each mutated DNA were used for studies.

HIV-1 infection. PBMCs and peripheral blood macrophages were treated with 2 μg of Polybrene (Sigma, St. Louis, Mo.) per ml for 30 min at 37°C and exposed to 1 ml of virus-containing fluid (RT activity, 5×10^5 cpm/ml) for 1.5 h at 37°C as described previously (6). PBMCs were washed once with Hanks balanced salt solution and resuspended in culture medium. In infection of primary macrophages, the cells were treated with 0.05% trypsin for 5 min at 37°C at the end of the adsorption period to remove residual input virus. The infected macrophages were subsequently washed twice with Hanks balanced salt solution and maintained in complete medium. Culture fluids were assayed for RT activity at 3-day intervals.

Expression and immunoblot analysis of envelope protein. *Eco*RI-*Eco*RV (3.5-kb) fragments of wild-type, recombinant, and N-linked glycosylation site-mutated DNAs, which contain full-length proviral *env* sequences, were subcloned into the *Eco*RI and *Sma*I sites in the polylinker of the simian virus 40-based expression vector, pSM (3). A 10-μg portion of each envelope glycoprotein expression vector was cotransfected into COS-7 cells with 5 μg of *rev* expression vector (pRev) (18) by the calcium phosphate precipitation method. Culture supernatants of transfected COS-7 cells were collected 48 h posttransfection and precipitated with a polyclonal goat anti-gp120 antibody (provided by K. Steimer, Chiron Corp., Emeryville, Calif.) and protein G-Sepharose beads (Sigma). The precipitates were washed with washing buffer (100 mM NaCl, 50 mM Tris-HCl, 0.1% sodium dodecyl sulfate [SDS], 1% sodium deoxycholate), resuspended in Laemmli sample buffer containing 2-mercaptoethanol, and eluted by boiling for 10 min. The immunoprecipitated proteins were separated on an SDS–8% polyacrylamide gel and transferred to an Immobilon-P filter (Millipore Corp., Bedford, Mass.). The filters were then reacted with the goat anti-gp120 antibody and incubated with horseradish peroxidase-coupled protein G (Bio-Rad Laboratories). Protein bands were visualized following development with H_2O_2–3,3′-diaminobenzidine (Sigma). Cells were also collected at the same time and washed with phosphate-buffered saline. The cell pellets were then disrupted in buffer (0.05 M Tris hydrochloride [pH 7.8], 0.15 mg of dithiothreitol per ml, 0.1% Triton X-100), and the lysates were denatured and reduced by heating to 90°C for 90 s in sample buffer. Proteins were analyzed on an SDS–8% polyacrylamide gel as described above.

Virus neutralization by sCD4. Virus stocks derived from the provirus clones were incubated with serially diluted recombinant sCD4 (a generous gift of Ray Sweet, Smith Kline Beecham, King of Prussia, Pa.) for 30 min at 37°C. The untreated virus control or mixture of virus and sCD4 was added to 2×10^6 Polybrene-treated mitogen-activated PBMC at 37°C for 2 h. The cells were washed three times with Hanks balanced salt solution and maintained in 2 ml of IL-2-containing medium. On day 4, half the medium of each culture was changed, and the p25 core antigen levels in the culture fluids were determined and compared with those in the untreated control on day 7 by using standard enzyme-linked immunosorbent assay plates (Coulter Immunology, Hialeah, Fla.). Assays were performed in duplicate.

RESULTS

Generation of additional recombinant viruses formed between HIV-1_{SF2mc} and HIV-$1_{SF162mc}$ strains. Our previous studies indicated that efficient infection of primary macrophages required a region of the envelope gp120 that encompassed the V1 and V2 domains (37). To confirm this observation and to investigate whether the V1 and V2 domains are required alone or in combination, we generated additional recombinant viruses between the parental T-cell-line-tropic

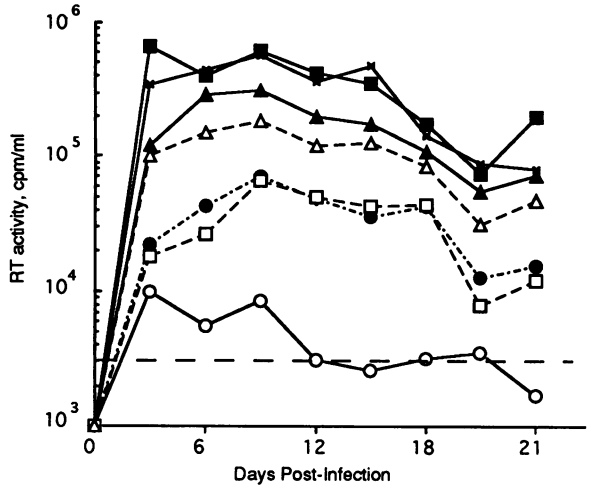

FIG. 2. Kinetics of replication of recombinant and mutant viruses in primary macrophages. Primary macrophages were infected with recombinant viruses as described in Materials and Methods, and supernatants of cultures were assayed for RT activity at 3-day intervals. The horizontal dotted line represents the background RT value. Representative data from at least three separate experiments are shown. Symbols: ○, SF2; ●, R19; □, Mu3; ■, SF162; △, R33; ▲, R32; ×, R25.

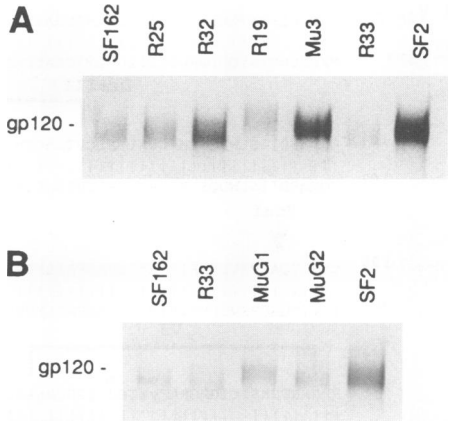

FIG. 3. Expression of envelope protein. Viral envelope glycoproteins (gp120) in the culture medium of transfected COS-7 cells were immunoprecipitated with goat anti-gp120 antibody, and the immunoprecipitated proteins were subjected to SDS-PAGE followed by electroblotting and antibody staining for gp120. (A), gp120s of wild-type and recombinant viruses; (B) gp120s of N-linked glycosylation mutants compared with parental and recombinant virus R33.

HIV-1_{SF2mc} and macrophage-tropic HIV-$1_{SF162mc}$ viruses. Figure 1 summarizes the data obtained with these additional recombinant viruses, R25, R28, R32, and R33. The wild-type, recombinant, and mutant viruses did not differ substantially in their peak virus production in mitogen-activated PBMC as measured by RT activity. However, differences were observed in infection of primary macrophages (Fig. 2). As reported previously, the acquisition of a 0.48-kb *Stu*I-*Mst*II fragment of HIV-$1_{SF162mc}$ by the T-cell-line-tropic HIV-1_{SF2mc} conferred on this virus, R19, the ability to replicate to low levels in primary macrophages (Fig. 2) and, at the same time, abolished its ability to infect T-cell lines (37). Three amino acid changes alone within the *Stu*I-*Mst*II fragment, all located in the V3 domain of envelope gp120, were sufficient to confer the same phenotype in primary macrophages (Mu3; Fig. 1 and 2) (38). A 0.29-kb *Hph*I-*Stu*I fragment of 46 amino acids, containing sequences encoding the complete V2 domain of HIV-$1_{SF162mc}$ gp120, enhanced the efficiency of infection of R19 and Mu3 in primary macrophages about 5- to 10-fold (R32 and R33 versus R19 and Mu3, respectively). However, a 0.73-kb *Dra*III-*Mst*II fragment encompassing the V1, V2, C2, and V3 regions of HIV-$1_{SF162mc}$ was necessary for replication to levels comparable to that of parental HIV-$1_{SF162mc}$ (R25; Fig. 1 and 2). The V1/V2 region of HIV-$1_{SF162mc}$ alone does not alter the tropism of HIV-1_{SF2mc} (R28; Fig. 1). These findings indicate again the importance of the V3 domain in macrophage tropism. Moreover, the results now show that in the context of HIV-1_{SF2mc}, both the V1 and V2 domains of HIV-$1_{SF162mc}$ are necessary to interact with the V3 domain to attain optimal macrophage tropism.

Expression of wild-type and mutant gp160. To examine the possible mechanism(s) by which the V1/V2 domain of envelope gp120 modulates the tropism of HIV-1, envelope glycoproteins of wild-type, recombinant, and mutant viruses were transiently expressed in COS-7 cells. Results show that the envelope gp120s of parental HIV-1_{SF2mc} and HIV-$1_{SF162mc}$ exhibited different electrophoretic patterns in SDS-polyacrylamide gel electrophoresis (PAGE). HIV-$1_{SF162mc}$ gp120 migrated with higher mobility (Fig. 3). The gp120 of recombinant virus R25, which contained the V1 through V3 domains of HIV-$1_{SF162mc}$ on the genomic background of HIV-1_{SF2mc} and which conferred full macrophage tropism on the virus, migrated with a pattern similar to that of HIV-$1_{SF162mc}$. In contrast, the gp120s of recombinant virus R19 and mutant virus Mu3 were similar to HIV-1_{SF2mc} (Fig. 3A). It is noteworthy that the addition of V2 sequences (0.29-kb *Hph*I-*Stu*I) of HIV-$1_{SF162mc}$ to either R19 or Mu3 generated an envelope gp120 that migrated like HIV-$1_{SF162mc}$ (R32, R33; Fig. 3A). In the context of the virus, these sequences also confer enhanced macrophage tropism (Fig. 1 and 2). The addition of the V1/V2 region of HIV-$1_{SF162mc}$ alone, which did not alter the tropism of HIV-1_{SF2mc} (R28; Fig. 1), also did not change the migration pattern of the recombinant gp120; it remains similar to that of HIV-1_{SF2mc} (data not shown).

Effects of N-linked glycosylation. The migration in SDS-PAGE could be the result of differences in the overall conformation of envelope gp120 or the extent of glycosylation (24). Within the *Hph*I-*Stu*I fragment that encompasses the V2 hypervariable region, HIV-1_{SF2mc} envelope sequences contain two additional potential glycosylation sites (amino acids 158 and 184; Fig. 4). The substitutions of this region of HIV-$1_{SF162mc}$ in R25, R32, and R33 therefore result in a decrease in the number of potential N-glycosylation sites compared with that in parental HIV-1_{SF2mc}. To evaluate the effects of these putative glycosylation differences in envelope gp120s on migration in SDS-PAGE, the Asn-Xaa-Ser/Thr sequences at positions 158 and 184 of Mu3 and HIV-1_{SF2mc} envelope gp120s (Fig. 4) were replaced by Asn-Xaa-Ala and Lys-Xaa-Ser to generate MuG1 and MuG2, respectively. Transient-expression assay in COS-7 cells showed that the two mutants with mutations in the N-linked glycosylation sites (MuG1 and MuG2) migrated with patterns more similar to that of HIV-1_{SF2mc} than of HIV-$1_{SF162mc}$ (Fig. 3B). These observations suggest that the difference in potential N-linked glycosylation at these sites is not responsible for the major change in the

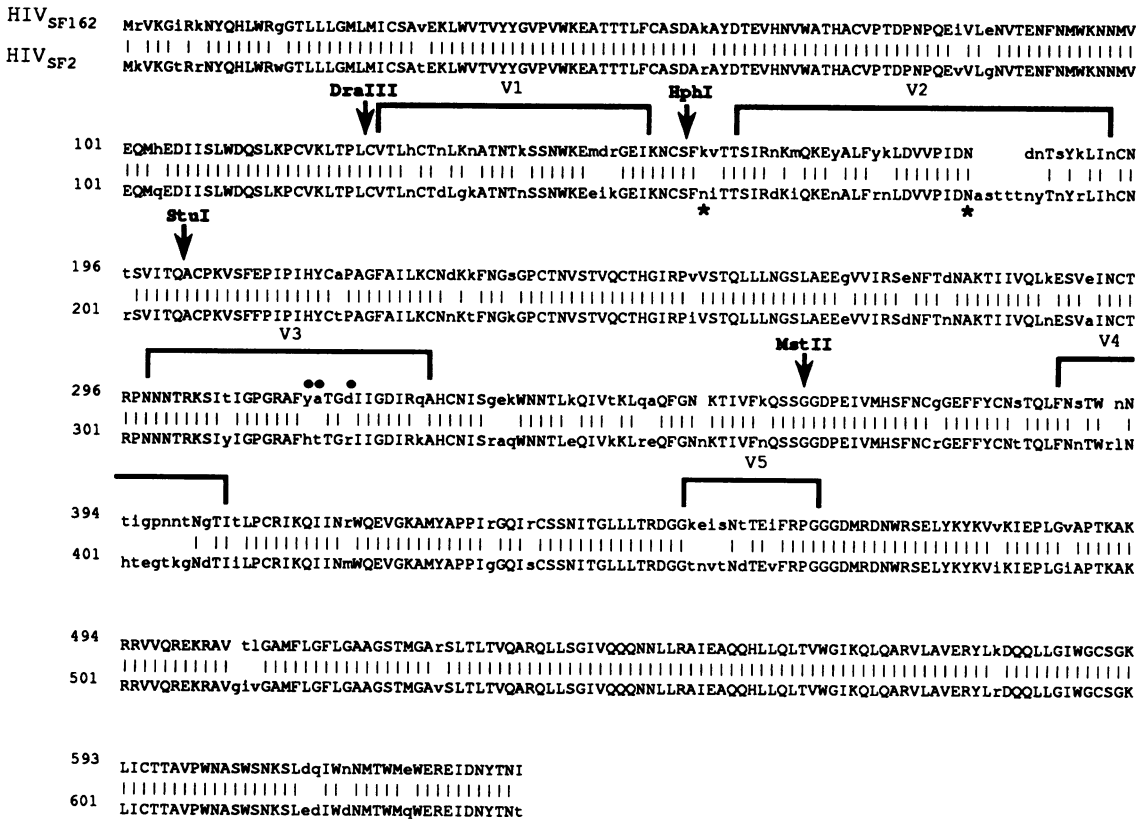

FIG. 4. Comparison of the predicted amino acid sequences of HIV-$1_{SF162mc}$ and HIV-1_{SF2mc} gp120. Five hypervariable regions (V1, V2, V3, V4, and V5) are bracketed. Restriction enzyme cleavage sites used to generate recombinant viruses are indicated by vertical arrows (*Dra*III, *Hph*I, *Stu*I, and *Mst*II). The three HIV-$1_{SF162mc}$-specific amino acid residues, which can confer macrophage tropism on HIV-1_{SF2mc}, are denoted by dots. Nonconserved potential N-linked glycosylation sites in the V2 domain are indicated by asterisks.

migrational mobilities of the envelope glycoproteins of R32 and R33 compared with R19 and Mu3, respectively (Fig. 3A).

Release of recombinant and mutant gp120s. Studies of N-linked glycosylation mutants suggested that the differences in electrophoretic mobility in SDS-PAGE result from differences in the overall conformation of the respective gp120s. Release of gp120 into culture supernatants of transfected cells is indicative of conformational changes in envelope structure that disrupt gp120-gp41 association (15). Therefore, the amounts of gp120s present in supernatants of the various envelope-transfected cultures were examined. Transient expression of wild-type, recombinant, and mutant envelope glycoproteins in COS-7 cells shows comparable levels of synthesis and processing (Fig. 5). However, more gp120 was released into supernatants of HIV-1_{SF2mc} and Mu3-transfected cultures than into those of HIV-$1_{SF162mc}$ and R33 (Fig. 5). These findings suggest that the acquisition of the V2 domain of HIV-$1_{SF162mc}$ by Mu3 alters its conformation such that the association between gp120 and gp41 is now more stable.

Effects of the V2 domain on the sCD4 neutralization sensitivity of HIV-1. Additional support for a conformational change in envelope gp120 as a result of a functional interaction between the V2 and V3 domains was obtained when the sensitivity of the viruses to sCD4 neutralization was examined. Similar to observations reported previously (9, 19, 30, 41), the parental HIV-1_{SF2mc} and HIV-$1_{SF162mc}$ displayed different sensitivities to sCD4 neutralization. Mu3 virus, like HIV-1_{SF2mc}, is easily neutralized. In contrast, R33 virus requires a markedly increased concentration of sCD4 to achieve neutralization (Table 1). These results suggest that the V2 domain interacts with the V3 domain and confers sCD4 resistance to HIV-1 isolates that are initially sensitive to sCD4.

DISCUSSION

We and others have previously demonstrated that the V3 domain of HIV-1 gp120 is required for macrophage tropism (7,

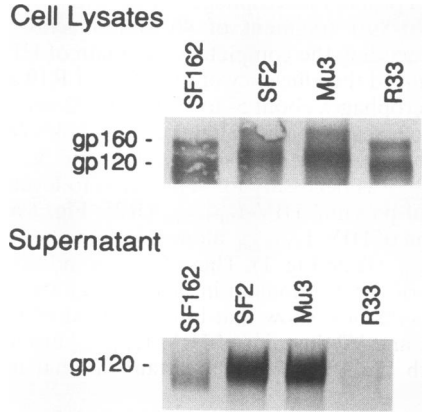

FIG. 5. Effects of the V2 domain on gp120 release from COS-7 cells transfected with wild-type, recombinant, and mutant envelope expression DNA. Immunoprecipitation and detection of viral envelope glycoproteins in the cell lysates and the cell culture medium at 48 h posttransfection were performed as described in Materials and Methods.

19, 31, 37, 38, 43). The present study with additional recombinant viruses shows that, on the genomic background of the T-cell-line-tropic strain HIV-1_{SF2mc}, both sequences in the V1 and V2 domains of the macrophage-tropic strain HIV-$1_{SF162mc}$, in addition to the required V3 domain, are necessary to confer full macrophage tropism. Structural studies indicate that the conformations of the envelope gp120 of the T-cell-line-tropic HIV-1_{SF2mc} and macrophage-tropic HIV-$1_{SF162mc}$ are intrinsically different. Different migration patterns in SDS-PAGE are observed for the gp120s of these isolates (Fig. 3), and the macrophage-tropic strain gp120 is more resistant to spontaneous release from the cell surface (Fig. 5). The latter observation suggests a difference in the association of the macrophage-tropic viral envelope gp120 with its transmembrane gp41.

Most importantly, we observed that the overall structure of gp120 changes concomitantly with the virus cell tropism. This finding is illustrated when R32 and R33 envelope gp120s and infectious viruses are compared with R19 and Mu3. R19 and Mu3 show low levels of macrophage tropism, and their gp120s display a migrational pattern similar to that of the T-cell-line-tropic virus HIV-1_{SF2mc} (Fig. 1 and 3A). With the acquisition by R19 and Mu3 of the V2 domain of the macrophage-tropic strain HIV-$1_{SF162mc}$, the envelope gp120 now confers enhanced macrophage tropism and exhibits an electrophoretic pattern more similar to that of HIV-$1_{SF162mc}$ on SDS-PAGE (R32 and R33; Fig. 2 and 3A). Mutagenesis studies showed that this difference in migration between Mu3 and R33 envelope gp120s was not due to the differences in potential N-linked glycosylation (Fig. 3B). Furthermore, compared with Mu3, R33 virus is resistant to sCD4 neutralization and exhibits a more stable gp120/gp41 association. These properties have been reported to be conformation dependent (15, 29, 33) and are characteristic of the parental macrophage-tropic strain HIV-$1_{SF162mc}$. Thus, these observations suggest that a functional role of the V1/V2 region, in particular the V2 domain, is to interact with the required V3 domain and impart to the envelope gp120 of HIV-1_{SF2mc} a conformation that is more similar to that of the macrophage-tropic strain.

Distinct structures for the V3 loop of T-cell-line-tropic and macrophage-tropic gp120 envelope proteins have been suggested in other studies (10, 22). The V3 loop structure of Mu3 also appears to be different from that of HIV-1_{SF2mc} since it is less susceptible to proteolytic cleavage by human thrombin and binds differently to anti-V3 monoclonal antibodies (38a). The addition of the V1/V2 domain of HIV-$1_{SF162mc}$ to Mu3 could further alter the structure of the V3 loop. Alternatively, the V1/V2 domain has been reported to participate in a postbinding virus-cell fusion process (39), and conformational changes in envelope gp120 that are induced by binding to sCD4 increase the exposure of the V3 domain (8, 36). The V1/V2 domain therefore could influence the presentation of the V3 loop by modulating the types of postbinding conformational changes that the envelope gp120 undergoes. The exact mechanism(s) by which the V1/V2 domain influences these biologic properties requires further investigation.

It has been reported that macrophage tropism and sCD4 resistance are regulated by similar mechanisms via the V3 domain (19, 30). Our observation with Mu3, which shows a macrophage-tropic phenotype but is sensitive to sCD4 neutralization, indicate that the two properties can be segregated. Furthermore, we find that an *Hph*I-*Stu*I (139-bp) region, encompassing the V2 domain, is able to confer resistance to viruses that were previously sensitive to sCD4 neutralization (Mu3 versus R33; Table 1). The V1/V2 domain may fold into the proximity of the viral envelope C4 domain (26, 29). This region is a major component of the CD4-binding epitope in the native gp120 and modulates the association of gp120 and gp41 (15). In addition, amino acid changes in the V1/V2 domain have been reported to affect envelope subunit association (39). The possibility exists, therefore, that the V2 domain confers resistance to sCD4 neutralization by altering the CD4-binding affinity of the envelope gp120 or by affecting its association with gp41. In this regard, the observation that gp120-gp41 association is increased in the R33 envelope glycoprotein (Mu3 versus R33; Fig. 5) would suggest that the increase in resistance of the virus to sCD4 neutralization is the result of increased retention of gp120 on the viral surface (28, 32).

TABLE 1. Relative sensitivity of HIV-1_{SF2mc}/HIV-$1_{SF162mc}$ parental, recombinant, and mutant viruses to sCD4 neutralization[a]

HIV-1	Cell tropism[b]	ID_{50} of sCD4 (μg/ml)[c]
SF2	T	0.3
SF162	mϕ	>10
Mu3	mϕ	0.3
R33	mϕ	>10

[a] Each virus was incubated with sCD4 for 30 min at 37°C. Control and virus-sCD4 mixtures were added to 2×10^6 PBMC for 2 h at 37°C, washed three times with medium, and resuspended in 2 ml of culture medium. On day 7 postinfection, supernatant fluids were assayed for p25 core antigen.

[b] T, T-cell line; mϕ, peripheral blood macrophages.

[c] The sCD4 dose that inhibited p25 antigen produced by 50% (ID_{50}) is indicated. A second experiment yielded comparable results.

It has also been suggested that N-linked glycosylation sites in specific regions of the V2 domain may contribute to the syncytium-inducing capacity of the virus (14). Our mutagenesis studies show that the presence of two additional N-linked glycosylation sites in the V2 domain of HIV-1_{SF2mc} or Mu3 do not have a major effect on the overall structure of envelope gp120s as monitored by migration in SDS-PAGE (Fig. 3B). However, this measurement shows only gross structural changes and may not register any subtle conformational changes in envelope gp120 that are caused by modifications in glycosylation. The effects of these glycosylation differences on the biological function of the virus require further study.

In conclusion, our data strongly suggest a role of the V1/V2 domain, in particular the V2 portion of the envelope gp120, in cell tropism and sensitivity of HIV-1 to sCD4 neutralization. The mechanism appears to involve a functional interaction of these two regions of gp120 with the required V3 domain, leading to a modulation in the overall conformation of gp120. The structure of the gp120 of viruses that display different tissue tropism varies. These differences subsequently could define the types of conformational changes that the external glycoprotein undergoes upon binding to its receptor to mediate viral entry into different cells (8, 36). Conceivably, the differences in conformational changes that T-cell-line-tropic and macrophage-tropic strains undergo expose different regions or epitopes of the envelope gp120 that are required to interact with accessory molecules, other than the CD4 receptor, on the cell surface. These additional interactions may ultimately determine virus entry into cells.

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