Virion Envelope Content, Infectivity, and Neutralization Sensitivity of Simian Immunodeficiency Virus

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A truncating E767stop mutation was introduced into the envelope glycoprotein of simian immunodeficiency virus (SIV) strain SIV239-M5 (moderately sensitive to antibody-mediated neutralization and lacking five sites for N-linked carbohydrate attachment) and strain SIV316 (very sensitive to neutralization, with eight amino acid changes from the neutralization-resistant parental molecular clone, SIV239). The truncating mutation increased Env content in virions, increased infectivity, and decreased sensitivity to antibody-mediated neutralization in both strains. However, the magnitude of the effect on infectivity and neutralization sensitivity differed considerably between the two strains. In the context of strain SIV239-M5, truncation increased Env content in virions approximately 10-fold and infectivity in a reporter cell assay 24-fold. The truncated SIV239-M5 was only slightly more resistant to neutralization by polyclonal monkey sera and by monoclonal antibodies than SIV239-M5 with a full-length envelope glycoprotein. In the context of strain SIV316, truncation increased infectivity a dramatic 480-fold, while envelope content in virions was increased only about 14-fold. This dramatic increase in infectivity cannot be simply explained by the increase in envelope content and is likely due to an increase in inherent infectivity, i.e., infectivity per spike, that results from truncation. The truncated SIV316 was extremely resistant to antibody-mediated neutralization. In fact, it was not neutralized by any of the antibodies tested. When increasing amounts of SIV316 envelope glycoprotein (full length) were provided in trans to SIV316, infectivity was increased and sensitivity to neutralization was decreased, but to nowhere near the degree that was obtained when truncated SIV316 envelope glycoprotein was used. Truncated forms of SIV239 and SIV239-M5 required higher levels of soluble CD4 for inhibition of infection than their nontruncated forms; truncated SIV316 did not. Our results suggest that envelope content in SIV virions, infectivity, and resistance to antibody-mediated neutralization can be increased not only by truncation of the cytoplasmic domain but also by provision of excess envelope in trans. The striking increase in infectivity that results from truncation in the context of SIV316 appears to be due principally to an increase in inherent infectivity per spike.

Human immunodeficiency virus (HIV) entry into cells is mediated by its envelope glycoprotein (41). The envelope glycoprotein of the human immunodeficiency virus type 1 (HIV-1) is synthesized as a 160-kDa precursor, and it is processed during its passage through the secretory pathway by a host protease to yield the surface subunit (SU) and the transmembrane subunit (TM). The SU (gp120) is responsible for binding to receptors and coreceptors, whereas the TM or gp41 anchors the envelope proteins at the membrane and induces membrane fusion during virus entry. Lentiviruses are unique among retroviruses in having TM glycoproteins with very long cytoplasmic tails (11). Simian immunodeficiency virus (SIV) and HIV, for example, typically express TM glycoproteins with cytoplasmic tails of approximately 170 amino acids. The functions of these gp41 cytoplasmic domains (CDs) are still being elucidated. The contribution of the HIV and SIV TM CD to viral replication appears to be species and cell type dependent. Truncations of the HIV gp41 CD in most cases severely inhibit viral replication in peripheral blood mononuclear cells, macrophages, and most T-cell lines (13, 17), but there are some cell lines, including MT4 and M8166, that are permissive for replication of HIV-1 mutants with a truncated TM (1, 39, 42). In contrast, the gp41 CD of SIV is not absolutely required for viral replication. When SIV strains are propagated in human T-cell lines, premature stop codons that result in a truncated TM CD are often selected (23). Viruses with such changes rapidly revert to restore the full-length gp41 CD during replication in macaque peripheral blood mononuclear cells or infected animals (23, 30). The HIV and SIV Env long cytoplasmic domains have been implicated in modulating Env expression on the cell surface (3, 5, 6, 18, 25, 49, 53, 56), targeting to specific membrane microdomains for assembly (10, 28, 29, 48, 54) and interaction with the viral matrix proteins, as well as interaction with other cellular proteins (9, 12, 15, 16, 55). Interacting cellular proteins include the clathrin-associated adapter complexes AP-1 and AP-2 (3), calmodulin (52), p115-RhoGEF (57), α-catenin (22), the prenylated Rab receptor (14), and Tip-47 (4). These cellular proteins are all known to influence the trafficking of proteins to and from the plasma membrane. Truncations of the CD of SIV that increase cell surface expression to various degrees also increase spike density on virions in a directly proportional manner (56). Increased envelope incorporation into virions has been associ-
ated with increased infectivity of SIV virions with mutations in the matrix (MA) protein (32). The extent to which Env content in virions of SIV and HIV can vary and its influence on different biological properties such as infectivity and sensitivity to neutralization have not been extensively studied.

Neutralizing antibodies are a major component of the immune defense against viral infections (30). These antibodies bind to accessible surface determinants on virions to prevent infection (24, 35, 36, 40). Induction of neutralizing antibodies represents a central protective mechanism of most currently available antiviral vaccines. It will therefore be important to understand the physical basis for neutralization resistance. The structural features of the HIV envelope complex that contribute to its poor immunogenicity include the presence of variable loop sequences on the exposed surface of the complex, the occlusion of protein surfaces by trimer formation, and the presence of extensive N- and O-linked glycosylation (2).

In order to study the influence of spike density on sensitivity to antibody-mediated neutralization, we introduced the truncating mutation E767stop, which has previously been associated with increased levels of envelope incorporation in virions (56), into the gp41 transmembrane protein of different genetic backgrounds. The viruses that we used were chosen to represent a broad range of neutralization sensitivities. The results of our studies indicate that the decreased sensitivity of SIV316 with a truncated CD to neutralization by antibodies results from two contributing factors: increased envelope content in virions and increased resistance of virus entry into the cells.

MATERIALS AND METHODS

Site-specific mutagenesis and subcloning. Mutations in env were created by site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The following mutagenic primers were used: for E767stop, (8884 to 8926) 5'-CCTGCGCCTGGCAGATAATATATATTCCCTGATCCGCC-3' (8926 to 8884) 5'-GGCGGATCAGGAAATGAAT and (8926 to 8884) 5'-GGCGGATCAGGAAATGAATATATATATTCCCTGATCCGCC-3'. The primers were purchased from Sigma-Genosys Biotechnologies, Inc. (The Woodlands, TX). Mutation E767stop did not alter the second exon of rev by changing an AGA arg codon to an AUA ile codon and the second exon of tat by changing a UAG stop codon to a Tyr codon, adding six amino acids at the end of Tat (YNIPIS).

Viral infectivity was measured using LTR-SEAP-CEMx174 infected cells. The amounts of p27 Gag antigen in pelleted virions, we pelleted the virus from the supernatant of transfected 293T cells to investigate the effect of mutation E767stop in different viral backgrounds. To assess Env incorporation into virions, we pelleted the virus from the supernatant of transfected cells. The amounts of p27 Gag antigen in pelleted virions were assessed by an antigen capture assay, and normalized

RESULTS

Truncation of the gp41 cytoplasmic domain results in an increase in envelope incorporation into virions. To investigate the effects of truncation of the cytoplasmic domain of TM, we used site-specific mutagenesis to introduce a stop codon at residue 767, resulting in the truncation of 119 residues from the C-terminal tail of SIV239-M5 and SIV316. This particular mutation was selected because truncation at this residue has been associated with a 25- to 40-fold increase in envelope incorporation in the background of SIV239 (56). SIV239-M5 and SIV316 viruses were selected to represent, together with SIV239, a broad range of neutralization sensitivities: SIV239 (difficult to neutralize), SIV239-M5 (moderately neutralization sensitive, lacking five N-glycans in gp120 sites [44, 45]), and SIV316 (macrophage-tropic and neutralization sensitive [37]). We used virus produced by transfection of cloned DNA into 293T cells to investigate the effect of mutation E767stop in different viral backgrounds. To assess Env incorporation into virions, we pelleted the virus from the supernatant of transfected cells. The amounts of p27 Gag antigen in pelleted virions were assessed by an antigen capture assay, and normalized

DNA cloning. Cloned fragments containing mutated envelope genes were sequenced with an ABI 377 automated DNA sequencer by using the dye terminator cycle-sequencing chemistry as specified by the manufacturer (Perkin-Elmer Inc., Foster City, CA).

Virus stocks and cell culture. The full-length mutants were used to transfect 293T cells using the calcium phosphate method (Promega, Madison, WI). 293T and LTR-SEAP-CMx174 cells were maintained as previously described (37, 38). For virus stocks, 293T cells were transfected as described above. The culture medium was changed on day 2 posttransfection, and supernatants were harvested on day 3. Virus was quantified by determining the concentration of p27 capsid in the supernatant by an antigen capture assay (Coulter Corp., Hialeah, FL).

Envelope complementation assay. Five micrograms of SIV316open full-length plasmids were used to cotransfect 293T cells with different amounts (10 μg to 0.039 μg) of the envelope expression-optimized plasmids (SIV316open or SIV316 E767stop) using the calcium phosphate method (Promega, Madison, WI). For virus stocks, 293T cells were transfected as described above.

Infectivity assay. Viral infectivity was measured using LTR-SEAP-CMx174 indicator cells (34). A 96-well plate was set up with each row containing two uninfected wells and two sets of five twofold dilutions of virus. To these wells, 4 × 10^4 LTR-SEAP-CMx174 cells were added, and the plate was transferred to a humidified CO_2 incubator at 37°C. After 3 days, secreted alkaline phosphatase (SEAP) activity was measured using the Phosphalight kit (Applied Biosystems, Foster City, CA).

Neutralization. The neutralization sensitivity of each virus was tested using the SEAP reporter cell assay previously described (34). Briefly, 96-well plates were set up as follows. To the first three columns, 25 μl of medium (RPMI 1640-10% fetal calf serum) was added. To each of the other columns (no. 4 through 12), 25-μl aliquots of successive twofold dilutions of test antibody or plasma in RPMI 1640-10% fetal calf serum were added. Virus equivalent to 2 ng of p27 in a total volume of 75 μl was then added to each well to a final volume of 3 μl. Virus-free medium was added to columns 1 and 2 (mock). The plate was incubated for 1 h at 37°C. After incubation, 40,000 target cells (LTR-SEAP-CMx174) in a volume of 100 μl were added to each well. The plate was then placed into a humidified chamber within a CO_2 incubator at 37°C for 3 to 7 days. SEAP activity was measured on the earliest days, when levels were sufficiently over background to give reliable measurements. SEAP activity was measured according to the manufacturer's recommendations, with modifications as described previously (34). Neutralization activity for all antibodies and plasma samples was measured in triplicate and reported as the average.

Viral pellets. Virus-containing supernatants were first clarified by two consecutive centrifugations for 10 min at 3,000 rpm. Virus was then pelleted by centrifugation for 2 h at high speed (13,000 rpm) in a refrigerated microcentrifuge. The viral pellet was washed by resuspension in 1 ml of phosphate-buffered saline and pelleted again by centrifugation at high speed. After this second ultracentrifugation, the viral pellets were resuspended in 50 μl of phosphate-buffered saline and the amount of p27 was quantified by antigen capture as described above.

Western blotting. Identical quantities of p27 were mixed with Laemmli buffer (27) and boiled for 4 min. The samples were then electrophoresed through an 8- to 16% polyacrylamide–sodium dodecyl sulfate gel gradient. Following electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membranes were blocked with 5% skim milk in phosphate-buffered saline–0.05% Tween 20 for 2 h. Membranes were then incubated with antibodies recognizing the gp120 (3.11H [8]) and gp41 (KK41 [21]) subunits as well as p27 (2F12 [19]). A horseradish peroxidase-conjugated anti-human immunoglobulin G was used to detect antibody 3.11H, and a horseradish peroxidase-conjugated anti-mouse immunoglobulin G was used to detect monoclonal antibodies 2F12 and KK41. The rhesus monoclonal antibody 3.11H was a gift of J. E. Robinson (Tulane University Medical School). The KK41 and 2F12 murine monoclonal antibodies were obtained through the NIH AIDS Research and Reference Reagent Program. The membranes were treated with a chemiluminescent substrate (Pierce, Rockford, IL). The bands were visualized and analyzed using a Fuji PhosphorImager.
amounts of p27-containing virions were analyzed by Western blotting for SU (gp120) content (Fig. 1). Truncated forms of TM in both viral backgrounds displayed similar elevated levels of Env incorporation into virions after transfection of 293T cells (10- to 15-fold) (Fig. 1). The increase in virion Env content resulting from truncation of SIV316 and SIV239-M5 was slightly less than that observed previously for the same mutation in the SIV239 background (56).

Effects of gp41 cytoplasmic tail truncation on infectivity. LTR-SEAP-CEMx174 cells were used to quantitate the infectivities of the viruses under conditions that approximated a single cycle of infection. LTR-SEAP-CEMx174 cells were infected with normalized amounts of SIV239-M5, SIV316, and the corresponding mutants with the truncation E767stop in the transmembrane protein. LTR-SEAP-CEMx174 cells secrete SEAP into the medium in response to infection by SIV. The amount of SEAP secreted correlates directly with the amount of infecting virus and can be sensitively and rapidly measured by a chemiluminescent assay. The results of a representative experiment are shown in Fig. 2. Mutation E767stop increased infectivity in both genetic backgrounds but to dramatically different degrees. Truncation of CD in SIV239-M5 increased infectivity approximately 24-fold, while truncation of CD in SIV316 increased infectivity approximately 480-fold. A summary of the relative infectivities of SIV239, SIV316, SIV239-M5, and their truncated derivatives is shown in Table 1.

Effects of gp41 cytoplasmic tail truncation on sensitivity to antibody-mediated neutralization. Despite the fact that the sequences of these viruses differ minimally from one another, they displayed a wide range of susceptibility to neutralization by SIV-positive monkey plasma and monoclonal antibodies (20). SIV239 was routinely found to be resistant to antibody-mediated neutralization, with neutralization detectable only at the lowest dilutions (1:20 to 1:40) of pooled plasma from SIV-positive monkeys (Fig. 3). In contrast, SIV316 and SIV239-M5 were found to be sensitive to neutralization by SIV-positive plasma. Fifty-percent neutralization of SIV316 and SIV239-M5 with pooled SIV-positive plasma is typically achieved at dilutions >1,000 or >100, respectively. Mutation E767stop decreased sensitivity to antibody-mediated neutralization by plasma from SIV-positive monkeys in the three viral backgrounds tested (SIV239, SIV239-M5, and SIV316), but the degree of decrease was different for each virus (Fig. 3). Truncation of the transmembrane protein in SIV239 changed the 50% neutralization dilution from 1:40 to 1:20. However, the same mutation (E767stop) in a SIV316 background had a more dramatic effect. Fifty percent neutralization of SIV316 with a full-length TM was achieved with a 1:3,000 dilution of the SIV-positive plasma, but no neutralization was observed at any dilution when the truncating mutation was introduced into SIV316. SIV239-M5 had an intermediate phenotype with a change in 50% neutralization from 1:360 to 1:70 when truncation at position 767 was introduced in the transmembrane protein.

We next measured neutralization of SIV239, SIV239-M5, SIV316, and their corresponding mutants with monoclonal antibodies derived from experimentally infected, SIV-positive rhesus macaques (RhMAbs). Three anti-gp120 RhMAbs from three different competition groups were used for this study (1.9C, 3.11E, and 1.10A). These monoclonal antibodies have been described previously (8, 20, 46). SIV239 and the corresponding truncated mutant were not effectively neutralized by any of the RhMAbs (Table 2). Consistent with the results with positive rhesus monkey sera, sensitivity to neutralization of SIV239-M5 and SIV316 by RhMAbs decreased when the transmembrane protein was truncated at position 767. Fifty

FIG. 1. Effect of mutation E767stop in Env on incorporation into virions in two different viruses: SIV239-M5 (A) and SIV316 (B). Viruses were produced by transfection into 293T cells, and virions were pelleted from the clarified supernatants. gp120 and p27 were detected by Western blotting using 3.11H and 2F12 monoclonal antibodies. Relative SU incorporation into virions was calculated by the ratios of gp120 to p27 using phosphorimaging analysis. Data from the mutants are presented relative to the ratio found for SIV239-M5 (A) or SIV316 (B).
percent neutralization of SIV239-M5 by 3.11E decreased from 2.8 \( /H262 \) g/ml to 4.8 \( /H262 \) g/ml when mutation E767stop was introduced. Fifty percent neutralization of SIV239-M5 was achieved with a concentration of 12.5 \( /H262 \) g/ml of 1.10A, but the corresponding mutant could not be neutralized by this RhMAb at any of the higher concentrations tested. The 1.9C monoclonal antibody could not effectively neutralize SIV239-M5 or SIV239-M5 E767stop at the highest concentration tested (6.38 \( /H262 \) g/ml). The effect of the truncation in the transmembrane protein was more dramatic in SIV316. Fifty percent neutralization of the parental SIV316 was achieved at 0.004 \( /H262 \) g/ml for 3.11E, 0.008 \( /H262 \) g/ml for 1.9C, and 0.0009 \( /H262 \) g/ml for 1.10A but, when mutation E767stop was introduced, the virus could not be neutralized at any of the concentrations tested (Fig. 4 and Table 2).

The differences in levels of envelope incorporated into virions are insufficient to explain the differences in infectivity and sensitivity to antibody-dependent neutralization of SIV316. In order to clarify if envelope incorporation is the only factor responsible for the increase in infectivity and the decrease in sensitivity to antibody-mediated neutralization observed with SIV316, we used envelope transcomplementation to incorporate different levels of Env into virions. 293T cells were cotransfected with increasing amounts (0.039 \( /H262 \) to 10 \( /H262 \)) of the Env expression-optimized plasmids (SIV316open or E767stop) together with full-length SIV316 proviral DNA. To analyze the infectivity of these viruses with various amounts of Env provided in trans, we infected LTR-SEAP-CEMx174 cells under conditions that approximated a single cycle of infection as described above. The

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<th>TABLE 1. Strain comparisons</th>
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<td>Comparison strain</td>
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</tr>
<tr>
<td>SIV239</td>
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<tr>
<td>SIV239 E767stop</td>
</tr>
<tr>
<td>SIV239-M5</td>
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<tr>
<td>SIV239-M5 E767stop</td>
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<tr>
<td>SIV316</td>
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<td>SIV316 E767stop</td>
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\(^a\) Relative to SIV239 (normalized as 1). SIV239 has been previously estimated to contain 7 to 16 trimer spikes per virion (7, 53, 55).

\(^b\) Measured in LTR-SEAP-CEMx174 cells relative to SIV239 (normalized as 1).
infectivities per nanogram of p27 are shown in Fig. 5 (notice the difference in scales in panels A and B). We observed an increase in infectivity when virus was produced by cotransfection with the Env expression plasmid for both SIV316open and SIV316 E767stop envelopes. The increase in infectivity in both cases correlated with the amount of envelope provided in trans. However, the effect was much more dramatic when the Env incorporated in trans had the truncated cytoplasmic domain.

When SIV316open envelope was incorporated in trans, the highest increase in infectivity achieved was 2.3-fold. However, when SIV316 E767stop was incorporated in trans, the amount of alkaline phosphatase secreted into the medium was increased over 30-fold (Fig. 5).

These viruses were also used to investigate effects on sensitivity to antibody-mediated neutralization. In this analysis we measured neutralization of the viruses with different amounts of envelope provided in trans (SIV316open or SIV316 E767stop) with a pooled SIV-positive plasma as described above (Fig. 6). In both cases a decrease in sensitivity to antibody-mediated neutralization was observed when the amount of envelope provided in trans was increased. Percentage of SEAP activity at a 1:51,200 dilution of the SIV-positive plasma for the viruses with SIV316open Env incorporated in trans and a 1:200 dilution for the viruses with SIV316 E767stop Env incorporated in trans are shown in Fig. 6A and B. After neutralization of SIV316 with a 1:51,200 dilution of the SIV-positive plasma, the percentage of SEAP activity was reduced to 48%. However, when the amount of SIV316open Env was increased by envelope expression in trans, the sensitivity to neutralization decreased and the virus could only be neutralized with a 1:51,200 dilution of the SIV-positive plasma when

![Fig. 4. Comparative neutralization of SIV316 and SIV316 E767stop by rhesus anti-gp120 MAbs from three different competition groups. (A) 1.9C; (B) 3.11E; (C) 1.10A.](http://jvi.asm.org/file.php/12459-f4)

![Fig. 5. Effects on infectivity of increasing amounts of envelope incorporated in virions in trans. Viruses were produced by cotransfection of SIV316open full-length plasmid and two different envelope expression-optimized plasmids (SIV316 and SIV316 E767stop). (A) SIV316 Env expression-optimized plasmid incorporated in trans. (B) SIV316 E767stop Env expression-optimized plasmid incorporated in trans. Stocks were normalized to the amount of p27 and used to infect LTR-SEAP-CEMx174 cells. SEAP activity was measured by use of a Phosphalight kit according to the manufacturer's recommendations at 3 days postinfection. SEAP activity was normalized to the amount of p27.](http://jvi.asm.org/file.php/12459-f5)

<table>
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<th>TABLE 2. Neutralization by anti-gp 120 RhMAbs</th>
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<td>MAb</td>
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<td>3.11E</td>
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<td>1.9C</td>
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* The numbers indicate the concentration of MAb required to reduce infectivity of the indicated virus by 50%. —, 50% neutralization was not achieved at the highest concentration tested (11.75 μg/ml for 3.11E, 6.38 μg/ml for 1.9C, and 15 μg/ml for 1.10A).
the lowest amounts of envelope were provided in trans (full-length SIV316 viral DNA cotransfected with 2.5 or less micrograms of the Env expression-optimized plasmid in Fig. 6A). In contrast, sensitivity to neutralization was drastically reduced when SIV316 E767stop envelope was provided in trans. A reduction in the percentage of SEAP activity was only observed with the highest concentration of SIV-positive plasma tested (1:200) and only in the viruses with the lowest amount of envelope provided in trans. After neutralization of SIV316 with a 1:200 dilution of SIV-positive plasma, the percentage of SEAP activity was reduced to 7%. However, when SIV316 E767stop Env was provided in trans, some neutralization was achieved only with the viruses with the lowest amounts of envelope (full-length SIV316 viral DNA cotransfected with 0.156 or less micrograms of the E767stop Env expression-optimized plasmid) (Fig. 6B).

**DISCUSSION**

Our findings demonstrate that truncation at E767 results in increased envelope content in virions in all three SIV genetic backgrounds. Our results suggest that the gp41 cytoplasmic domain in SIV316 results in an increased affinity for soluble CD4. We next studied the effects of soluble CD4 (sCD4) on inhibiting infection by these viruses with and without a truncated CD. Viruses produced by transient transfection in 293T cells were incubated with increasing concentrations of sCD4 for 1 h at 37°C prior to the infection of LTR-SEAP-CEMx174 SEAP cells. sCD4 exhibited a modest inhibitory activity against SIV239. Fifty percent inhibition of infectivity was achieved with 2.5 μg/ml of sCD4. Consistent with previous publications (33, 50), SIV316 was considerably more sensitive to inhibition by sCD4. Only 0.25 μg/ml was required to reduce viral infectivity by 50% (Fig. 7 and Table 3). No significant inhibitory activity was observed against viruses with a truncated envelope protein in a SIV239 or SIV239-M5 background (Fig. 7 and Table 3). In contrast, when the truncating mutation was introduced in a SIV316 background, the inhibitory effect of sCD4 on infection with the resulting virus was similar to the effect observed for SIV316 with a full-length transmembrane protein (Fig. 7 and Table 3).

**TABLE 3. Inhibition of infectivity by sCD4**

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<thead>
<tr>
<th>Virus</th>
<th>Conc of sCD4 (μg/ml) reducing infectivity by 50%&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>SIVmac239</td>
<td>2.5</td>
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<tr>
<td>SIVmac239 E767Stop</td>
<td>&gt;12.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SIVmac239-M5</td>
<td>3.3</td>
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<tr>
<td>SIVmac239-M5 E767Stop</td>
<td>&gt;12.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>SIVmac316</td>
<td>0.25</td>
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<tr>
<td>SIVmac316 E767Stop</td>
<td>0.33</td>
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<sup>a</sup> The numbers indicate the concentration of sCD4 required to reduce infectivity of the indicated virus by 50%.

<sup>b</sup> A 50% inhibition was not achieved at the highest concentration tested (12.5 μg/ml).
polyclonal SIV neutralization of truncated SIV239-M5 was modest with both efficiency of entry. The shift to increased resistance to neutralizing components: Env content in virions and inherent efficiency of virion Env-mediated neutralization similarly appears to have two contributions that compromised infectivity, the truncation resulted in extreme resistance of SIV316E767stop is associated with its extreme efficiency at productive entry into target cells: SIV316E767stop is 10 to 25 times more efficient at productive entry into CEMX174 target cells than SIV239 or SIV239E767 stop (Fig. 2 and Table 1). In support of a role for entry kinetics in neutralization resistance/sensitivity, Reeves et al. recently observed increased neutralization sensitivity in a subset of inhibitor resistance mutants of HIV-1 that also display reduced fusion efficiency and delayed kinetics of entry (43).

The effects of virion Env content on sensitivity to neutralization have relevance for the interpretation of neutralization tests that employ env-deleted provirus and HIV and SIV envelope protein provided in trans, so-called pseudotype assays. Our results (Fig. 6) show that increasing the amount of envelope provided in trans can decrease the sensitivity to antibody-mediated neutralization. It is reasonable to think that provision of lower, limiting amounts of envelope in trans in pseudotype assays will increase the sensitivity to antibody-mediated neutralization. In effect, neutralization titers that are obtained using pseudotype assays will be dependent upon how much envelope protein is provided in trans. Just as increased envelope content requires increased amounts of antibody to achieve the same level of neutralization, increased envelope content would be expected to require increased levels of sCD4 for neutralization. Thus, the higher 50% inhibitory concentration for sCD4 neutralization of truncated derivatives of SIV239 and SIV239-M5 can be explained by the increased envelope content of these strains compared to the parents from which they were derived. However, we cannot rule out a contribution of decreased affinity for sCD4 resulting from the truncation. The equivalent sensitivities of truncated and nontruncated SIV316 to sCD4 suggest that SIV316E767stop may have an even higher affinity for sCD4 than SIV316. If this indeed were the case, the impressive efficiency of productive entry by SIV316E767stop could be explained at least in part by an increased affinity for its initial receptor CD4.

The increased infectivity of SIV316 virions when excess 316 Env (full length) is provided in trans (Fig. 5) is likely to result from increased envelope protein content in virions. Thus, the amount of envelope protein with a full-length cytoplasmic tail that is incorporated into virions can apparently be increased over that which occurs naturally when virions are produced from cells transfected with proviral DNA. However, questions still remain regarding the extent to which the long cytoplasmic domain may limit packing density in virions and the biological advantages that accrue to virions that naturally possess such a low envelope protein content.

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