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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 virus 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-

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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 efficiency 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′-CCTGGCCTTGGCAGATATAATATATTCATT TCCTGATCCGCC-3′ and (8926 to 8884) 5′-GGCGGATCAGGAAATGAAT ATATTATATCTGCCAAGGCCAGG-3′. The primers were purchased from Sigma-Genosys Biotechnologies, Inc. (The Woodlands, TX). Mutation E767stop did 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). Full-length versions of all the 3′ mutants were generated by insertion of the clone p239SpSp5′ using T4 DNA ligase. For envelope complementation assays, expression-optimized SIV316open and SIV316 E767stop Env expression vectors were used. The SIV316E767stop expression-optimized plasmid was generated by mutation of the expression-optimized SIV316open envelope with the primers (3064 to 3088) 5′-TGGCAG ATCTAATACATCCACTTTC-3′ and (3088 to 3064) 5′-GAAAGTGGATGTA TTAGATCTGCCA-3′. The RNA expression-optimized (codon-optimized) SIV239 Env expression vector (64S) has been recently described (47).

DNA sequencing. 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-CEMx174 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 was 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-CEMx174 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 $\times$ 10^4 LTR-SEAP-CEMx174 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 in columns 3 through 12. 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-CEMx-174) 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 gradient gel. 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 1 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-rhesus 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.

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

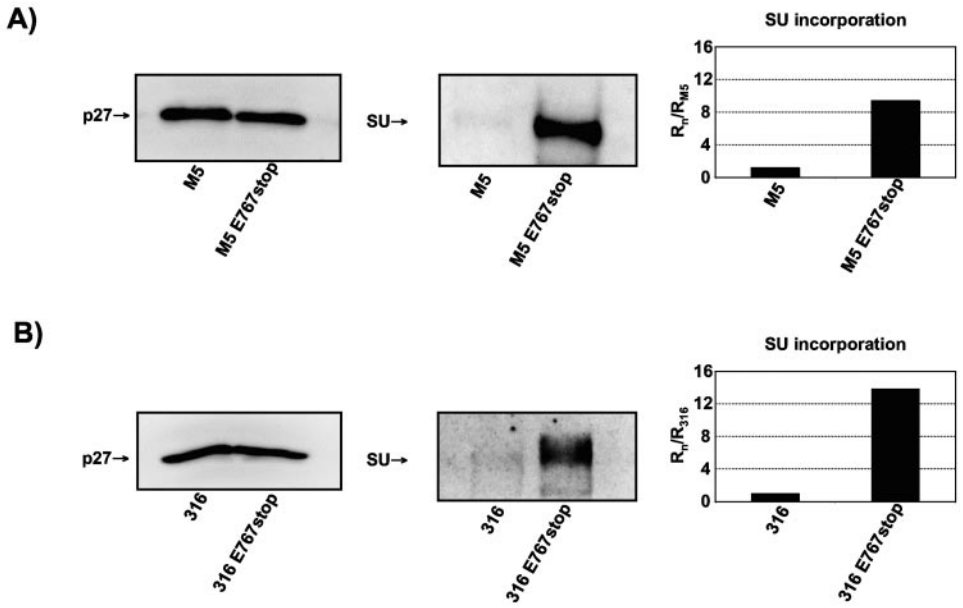

FIG. 1. Effect of mutation E767stop in Env on incorporation into virions in two different viruses: SIV239-M5 (A) and SIV316 (B). Viruses were produced by transfection into 293T cells, and virions were pelleted from the clarified supernatants. gp120 and p27 were detected by Western blotting using 3.11H 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).

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

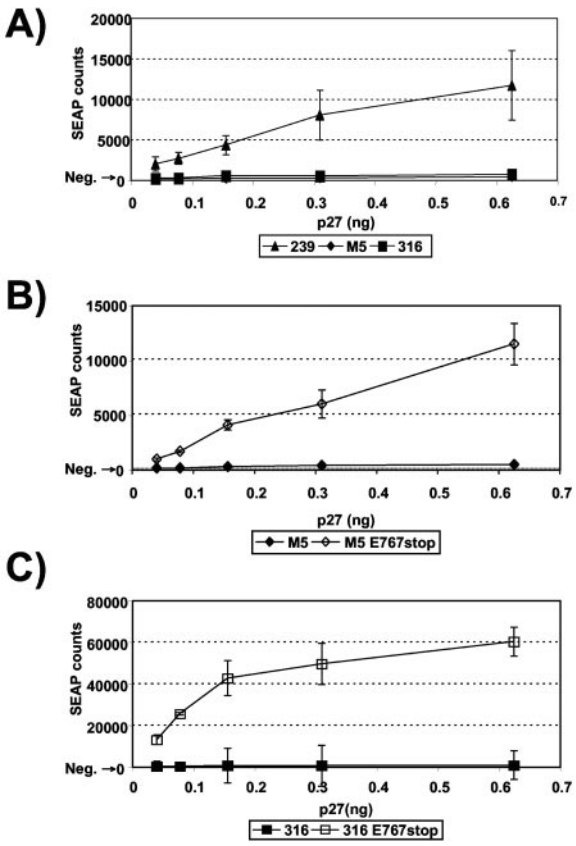

FIG. 2. Comparative infectivity of SIV239, SIV239-M5, SIV316, and the truncating mutants SIV239-M5 E767stop and SIV316 E767stop. SIV239, SIV239-M5, and SIV316 (A), SIV239-M5 versus SIV239-M5 E767stop (B), and SIV316 versus SIV316 E767stop (C) are compared. Virus stocks were obtained from transfection of 293T cells. Stocks were normalized for 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.

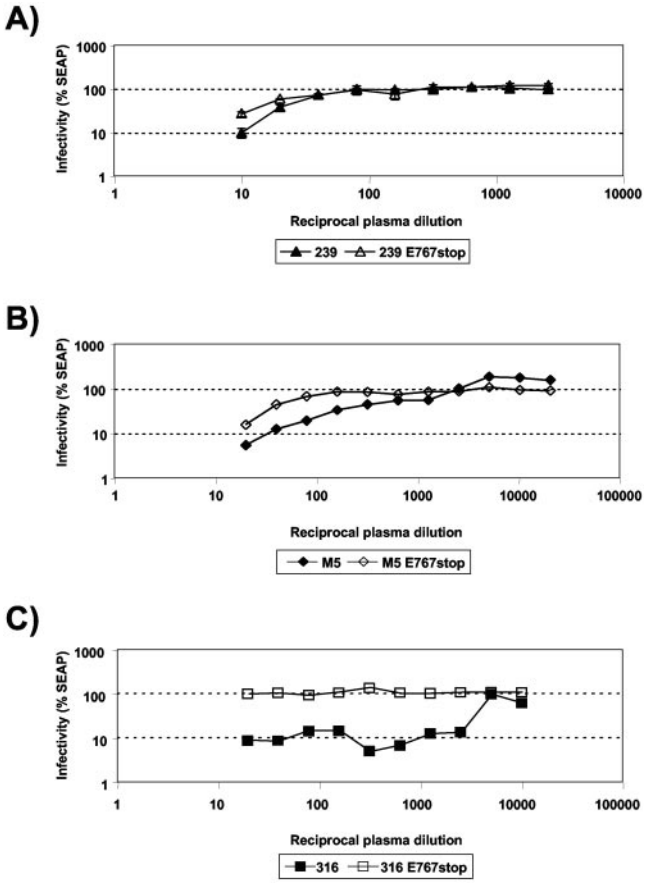

FIG. 3. Comparative neutralization of SIV239, SIV239-M5, SIV316, and their corresponding E767stop mutants by a pool of SIV-positive plasma. (A) SIV239 versus SIV239 E767stop; (B) SIV239-M5 versus SIV239-M5 E767stop; (C) SIV316 versus SIV316 E767stop.

percent neutralization of SIV239-M5 by 3.11E decreased from 2.8 μg/ml to 4.8 μg/ml when mutation E767stop was introduced. Fifty percent neutralization of SIV239-M5 was achieved with a concentration of 12.5 μg/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 μg/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 μg/ml for 3.11E, 0.008 μg/ml for 1.9C, and 0.0009 μg/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).

TABLE 1. Strain comparisons

Comparison strain	Env content in virions[a]	Infectivity[b]
SIV239	1	1
SIV239 E767stop	25	2.5
SIV239-M5	0.2	0.04
SIV239-M5 E767stop	2	1
SIV316	0.5	0.05
SIV316 E767stop	7	24

[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).

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. Virions obtained in this way may have different spike densities, but there will be no difference in sequence. 293T cells were cotransfected with increasing amounts (0.039 μg to 10 μg) 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

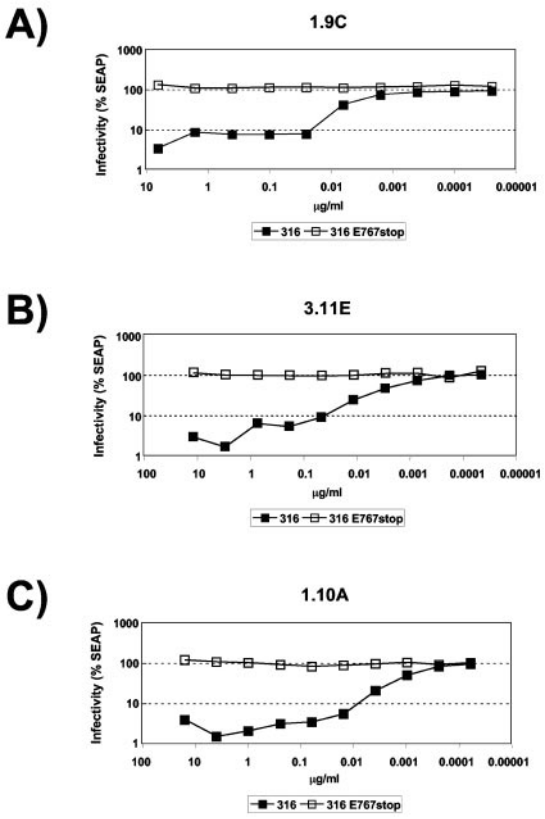

FIG. 4. Comparative neutralization of SIV316 and SIV316 E767stop by rhesus anti-gp120 MAbs from three different competition groups. (A) 1.9C; (B) 3.11E; (C) 1.10A.

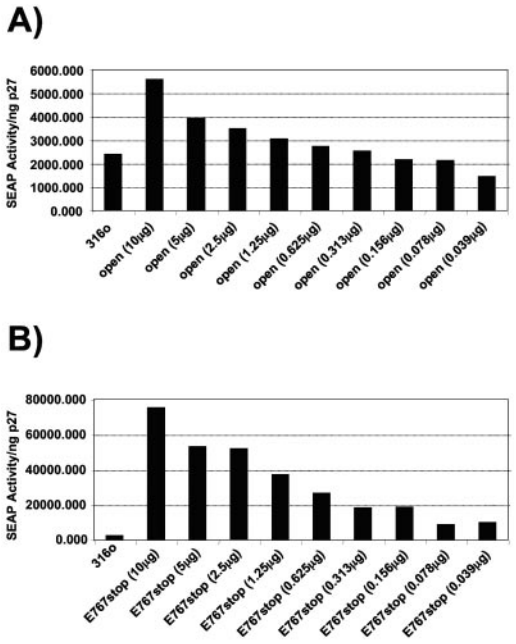

FIG. 5. Effects on infectivity of increasing amounts of envelope incorporated in virions in *trans*. Viruses were produced by cotransfection of SIV316open full-length plasmid and two different envelope expression-optimized plasmids (SIV316 and SIV316 E767stop). (A) SIV316 Env expression-optimized plasmid 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.

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

TABLE 2. Neutralization by anti-gp 120 RhMAbs

MAb	Concn of MAb (µg/ml) reducing infectivity by 50%[a]					
	SIVmac239	SIV239 E767Stop	SIV239-M5	SIV239-M5 E767Stop	SIV316	SIV316 E767Stop
3.11E	—	—	2.8	4.8	0.004	—
1.9C	—	—	—	—	0.008	—
1.10A	—	—	12.5	—	0.0009	—

[a] 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).

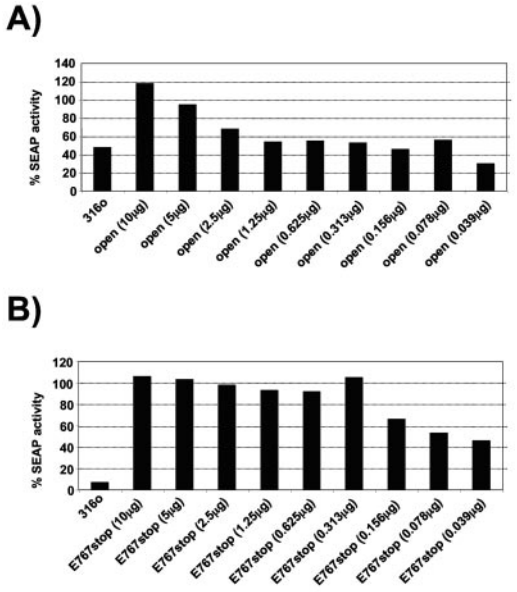

FIG. 6. Comparative neutralization of viruses with increasing amounts of envelope incorporated in virions in *trans* with a pool of SIV-positive plasma. Viruses were produced by cotransfection of SIV316open full-length plasmid and two different envelope expression-optimized plasmids (SIV316 and SIV316 E767stop). (A) SEAP activity after neutralization with a 1/51,200 dilution of a pool of SIV-positive plasma when SIV316 Env expression-optimized plasmid was incorporated in *trans*. (B) SEAP activity after neutralization with a 1/200 dilution of a pool of SIV-positive plasma when SIV316 E767stop Env expression-optimized plasmid was incorporated in *trans*.

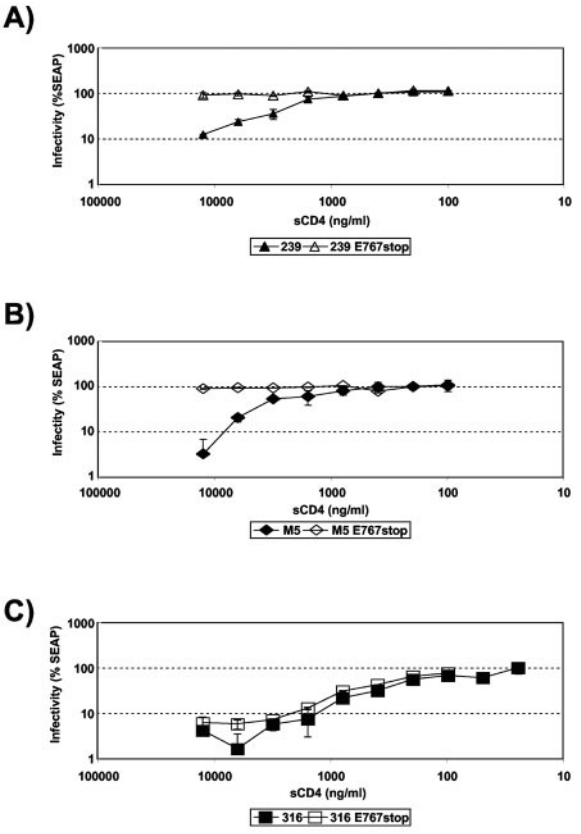

FIG. 7. Comparative inhibition of SIV239, SIV239-M5, SIV316, and their corresponding E767stop mutants by soluble CD4. (A) SIV239 versus SIV239 E767stop; (B) SIV239-M5 versus SIV239-M5 E767stop; (C) SIV316 versus SIV316 E767stop.

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).

Truncation of 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).

DISCUSSION

Our findings demonstrate that truncation at E767 results in increased envelope content in virions in all three SIV genetic

TABLE 3. Inhibition of infectivity by sCD4

Virus	Concn of sCD4 (μg/ml) reducing infectivity by 50%[a]
SIVmac239	2.5
SIVmac239 E767Stop	>12.5[b]
SIVmac239-M5	3.3
SIVmac239-M5 E767Stop	>12.5[b]
SIVmac316	0.25
SIVmac316 E767Stop	0.33

[a] The numbers indicate the concentration of sCD4 required to reduce infectivity of the indicated virus by 50%.

[b] A 50% inhibition was not achieved at the highest concentration tested (12.5 μg/ml).

backgrounds tested: SIV239, SIV239-M5, and SIV316. These results are consistent with a number of earlier publications reporting increased envelope content in virions as a result of truncation (31, 51, 56, 59). The 767 truncation was used because it occurred naturally in the lung compartment of a rhesus monkey during the course of *env* sequence evolution following infection by cloned SIV239 (37). The amount of envelope protein incorporated into virions varies with the location of the truncation (56) and correlates strictly with the level of envelope protein expression on the cell surface (56). This suggests that the rate or extent of endocytosis from the cell surface may be a critical determinant of the level of Env incorporated into virions, as suggested earlier by LaBranche et al. (26). Our previous estimate of 7 to 16 trimer spikes per SIV239 virion (56) agrees well with estimates by Chertova et al. (7), who utilized different biochemical methodologies, and with estimates of Zhu et al. (58) obtained using electron tomography analysis. The range of 7 to 16 reflects uncertainty in the number of Gag molecules per virion on which the calculations are based. Here we show that SIV316 and SIV239-M5 have similar or slightly lower amounts of Env per p27 content than SIV239. Truncation at E767 increased envelope content in virions from 10-fold (SIV239-M5) to 25-fold (SIV239).

Two factors likely contribute to the increased infectivity associated with the E767 truncation: increased virion Env content and an increase in the inherent efficiency of viral entry on a per spike basis. Increased efficiency of productive entry appears to be particularly prominent for SIV316E767stop. In contrast to SIV239 and SIV239-M5, truncation in the context of SIV316 produced an increase in infectivity that was far disproportional to the increase in Env content. Furthermore, when increasing amounts of SIV316 Env-open were titrated into SIV316, only modest increases in infectivity were observed. When increasing amounts of 316 Env-truncated were titrated into SIV316, dramatic increases in infectivity were observed. Truncation at E767 increased infectivity from 2.5-fold (SIV239) (56) to 480-fold (SIV316). Thus, the extent to which these two factors, virion Env content versus inherent efficiency of productive entry, contribute to the increased infectivity of truncated derivatives appears to vary with the SIV genetic context. Similar results have been described for SIV when a truncation of gp41 was introduced in the context of MA mutations (32). In this study, truncations of gp41 increased infectivity 13- to 18-fold. However, in the context of MA mutations that compromised infectivity, the truncation resulted in dramatic increases in infectivity, from 100- to 1,300-fold depending on the MA mutation.

Decreased sensitivity of truncated derivatives to antibody-mediated neutralization similarly appears to have two contributing components: Env content in virions and inherent efficiency of entry. The shift to increased resistance to neutralization of truncated SIV239-M5 was modest with both polyclonal SIV^+ monkey plasma and with assorted monoclonal antibodies. The resistance of truncated SIV316 to antibody-mediated neutralization, in contrast, was dramatic. Although SIV316 with a full-length envelope transmembrane glycoprotein is one of the most neutralization-sensitive strains we have studied (20), we were unable to detect any neutralization of SIV316E767stop with any of the antibodies we tested. The 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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REFERENCES

1. **Akari, H., T. Fukumori, and A. Adachi.** 2000. Cell-dependent requirement of human immunodeficiency virus type 1 gp41 cytoplasmic tail for Env incorporation into virions. J. Virol. **74:**4891–4893.

2. **Berger, E. A., P. M. Murphy, and J. M. Farber.** 1999. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. **17:**657–700.
3. **Berlioz-Torrent, C., B. L. Shacklett, L. Erdtmann, L. Delamarre, I. Bouchaert, P. Sonigo, M. C. Dokhelar, and R. Benarous.** 1999. Interactions of the cytoplasmic domains of human and simian retroviral transmembrane proteins with components of the clathrin adaptor complexes modulate intracellular and cell surface expression of envelope glycoproteins. J. Virol. **73:**1350–1361.
4. **Blot, G., K. Janvier, S. Le Panse, R. Benarous, and C. Berlioz-Torrent.** 2003. Targeting of the human immunodeficiency virus type 1 envelope to the *trans*-Golgi network through binding to TIP47 is required for env incorporation into virions and infectivity. J. Virol. **77:**6931–6945.
5. **Boge, M., S. Wyss, J. S. Bonifacino, and M. Thali.** 1998. A membrane-proximal tyrosine-based signal mediates internalization of the HIV-1 envelope glycoprotein via interaction with the AP-2 clathrin adaptor. J. Biol. Chem. **273:**15773–15778.
6. **Bultmann, A., W. Muranyi, B. Seed, and J. Haas.** 2001. Identification of two sequences in the cytoplasmic tail of the human immunodeficiency virus type 1 envelope glycoprotein that inhibit cell surface expression. J. Virol. **75:** 5263–5276.
7. **Chertova, E., J. W. Bess, Jr., B. J. Crise, I. R. Sowder, T. M. Schaden, J. M. Hilburn, J. A. Hoxie, R. E. Benveniste, J. D. Lifson, L. E. Henderson, and L. O. Arthur.** 2002. Envelope glycoprotein incorporation, not shedding of surface envelope glycoprotein (gp120/SU), is the primary determinant of SU content of purified human immunodeficiency virus type 1 and simian immunodeficiency virus. J. Virol. **76:**5315–5325.
8. **Cole, K. S., M. Alvarez, D. H. Elliott, H. Lam, E. Martin, T. Chau, K. Micken, J. L. Rowles, J. E. Clements, M. Murphey-Corb, R. C. Montelaro, and J. E. Robinson.** 2001. Characterization of neutralization epitopes of simian immunodeficiency virus (SIV) recognized by rhesus monoclonal antibodies derived from monkeys infected with an attenuated SIV strain. Virology **290:**59–73.
9. **Cosson, P.** 1996. Direct interaction between the envelope and matrix proteins of HIV-1. EMBO J. **15:**5783–5788.
10. **Deschambeault, J., J. P. Lalonde, G. Cervantes-Acosta, R. Lodge, E. A. Cohen, and G. Lemay.** 1999. Polarized human immunodeficiency virus budding in lymphocytes involves a tyrosine-based signal and favors cell-to-cell viral transmission. J. Virol. **73:**5010–5017.
11. **Desrosiers, R. C.** 2001. Nonhuman lentiviruses, p. 2095–2122. *In* D. M. Knipe and P. M. Howley. (ed.), Fields virology, vol. 2. Lippincott Williams and Wilkins, Philadelphia, Pa.
12. **Dorfman, T., F. Mammano, W. A. Haseltine, and H. G. Gottlinger.** 1994. Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. J. Virol. **68:**1689–1696.
13. **Dubay, J. W., S. J. Roberts, B. H. Hahn, and E. Hunter.** 1992. Truncation of the human immunodeficiency virus type 1 transmembrane glycoprotein cytoplasmic domain blocks virus infectivity. J. Virol. **66:**6616–6625.
14. **Evans, D. T., K. C. Tillman, and R. C. Desrosiers.** 2002. Envelope glycoprotein cytoplasmic domains from diverse lentiviruses interact with the prenylated Rab acceptor. J. Virol. **76:**327–337.
15. **Facke, M., A. Janetzko, R. L. Shoeman, and H. G. Krausslich.** 1993. A large deletion in the matrix domain of the human immunodeficiency virus *gag* gene redirects virus particle assembly from the plasma membrane to the endoplasmic reticulum. J. Virol. **67:**4972–4980.
16. **Freed, E. O., and M. A. Martin.** 1995. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. J. Virol. **69:**1984–1989.
17. **Gabuzda, D. H., A. Lever, E. Terwilliger, and J. Sodroski.** 1992. Effects of deletions in the cytoplasmic domain on biological functions of human immunodeficiency virus type 1 envelope glycoproteins. J. Virol. **66:**3306–3315.
18. **Haffar, O. K., G. R. Nakamura, and P. W. Berman.** 1990. The carboxy terminus of human immunodeficiency virus type 1 gp160 limits its proteolytic processing and transport in transfected cell lines. J. Virol. **64:**3100–3103.
19. **Higgins, J. R., S. Sutjipto, P. A. Marx, and N. C. Pedersen.** 1992. Shared antigenic epitopes of the major core proteins of human and simian immunodeficiency virus isolates. J. Med. Primatol. **21:**265–269.
20. **Johnson, W. E., H. Sanford, L. Schwall, D. R. Burton, P. W. Parren, J. E. Robinson, and R. C. Desrosiers.** 2003. Assorted mutations in the envelope gene of simian immunodeficiency virus lead to loss of neutralization resistance against antibodies representing a broad spectrum of specificities. J. Virol. **77:**9993–10003.
21. **Kent, K. A., E. Rud, T. Corcoran, C. Powell, C. Thiriart, C. Collignon, and E. J. Stott.** 1992. Identification of two neutralizing and 8 non-neutralizing epitopes on simian immunodeficiency virus envelope using monoclonal antibodies. AIDS Res. Hum. Retrovir. **8:**1147–1151.
22. **Kim, E. M., K. H. Lee, and J. W. Kim.** 1999. The cytoplasmic domain of HIV-1 gp41 interacts with the carboxyl-terminal region of alpha-catenin. Mol. Cell **9:**281–285.
23. **Kodama, T., D. P. Wooley, Y. M. Naidu, H. W. Kestler III, M. D. Daniel, Y. Li, and R. C. Desrosiers.** 1989. Significance of premature stop codons in *env* of simian immunodeficiency virus. J. Virol. **63:**4709–4714.
24. **Kwong, P. D., R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, and W. A. Hendrickson.** 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature **393:**648–659.
25. **LaBranche, C. C., M. M. Sauter, B. S. Haggarty, P. J. Vance, J. Romano, T. K. Hart, P. J. Bugelski, and J. A. Hoxie.** 1994. Biological, molecular, and structural analysis of a cytopathic variant from a molecularly cloned simian immunodeficiency virus. J. Virol. **68:**5509–5522.
26. **LaBranche, C. C., M. M. Sauter, B. S. Haggarty, P. J. Vance, J. Romano, T. K. Hart, P. J. Bugelski, M. Marsh, and J. A. Hoxie.** 1995. A single amino acid change in the cytoplasmic domain of the simian immunodeficiency virus transmembrane molecule increases envelope glycoprotein expression on infected cells. J. Virol. **69:**5217–5227.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
28. **Lodge, R., H. Gottlinger, D. Gabuzda, E. A. Cohen, and G. Lemay.** 1994. The intracytoplasmic domain of gp41 mediates polarized budding of human immunodeficiency virus type 1 in MDCK cells. J. Virol. **68:**4857–4861.
29. **Lodge, R., J. P. Lalonde, G. Lemay, and E. A. Cohen.** 1997. The membrane-proximal intracytoplasmic tyrosine residue of HIV-1 envelope glycoprotein is critical for basolateral targeting of viral budding in MDCK cells. EMBO J. **16:**695–705.
30. **Luciw, P. A., K. E. Shaw, B. L. Shacklett, and M. L. Marthas.** 1998. Importance of the intracytoplasmic domain of the simian immunodeficiency virus (SIV) envelope glycoprotein for pathogenesis. Virology **252:**9–16.
31. **Manrique, J. M., C. C. Celma, J. L. Affranchino, E. Hunter, and S. A. Gonzalez.** 2001. Small variations in the length of the cytoplasmic domain of the simian immunodeficiency virus transmembrane protein drastically affect envelope incorporation and virus entry. AIDS Res. Hum. Retrovir. **17:**1615–1624.
32. **Manrique, J. M., C. C. Celma, E. Hunter, J. L. Affranchino, and S. A. Gonzalez.** 2003. Positive and negative modulation of virus infectivity and envelope glycoprotein incorporation into virions by amino acid substitutions at the N terminus of the simian immunodeficiency virus matrix protein. J. Virol. **77:**10881–10888.
33. **Means, R. E., and R. C. Desrosiers.** 2000. Resistance of native, oligomeric envelope on simian immunodeficiency virus to digestion by glycosidases. J. Virol. **74:**11181–11190.
34. **Means, R. E., T. Greenough, and R. C. Desrosiers.** 1997. Neutralization sensitivity of cell culture-passaged simian immunodeficiency virus. J. Virol. **71:**7895–7902.
35. **Moore, J. P., Q. J. Sattentau, R. Wyatt, and J. Sodroski.** 1994. Probing the structure of the human immunodeficiency virus surface glycoprotein gp120 with a panel of monoclonal antibodies. J. Virol. **68:**469–484.
36. **Moore, J. P., and J. Sodroski.** 1996. Antibody cross-competition analysis of the human immunodeficiency virus type 1 gp120 exterior envelope glycoprotein. J. Virol. **70:**1863–1872.
37. **Mori, K., D. J. Ringler, T. Kodama, and R. C. Desrosiers.** 1992. Complex determinants of macrophage tropism in env of simian immunodeficiency virus. J. Virol. **66:**2067–2075.
38. **Morrison, H. G., F. Kirchhoff, and R. C. Desrosiers.** 1993. Evidence for the cooperation of gp120 amino acids 322 and 448 in SIV_{mac} entry. Virology **195:**167–174.
39. **Murakami, T., and E. O. Freed.** 2000. The long cytoplasmic tail of gp41 is required in a cell type-dependent manner for HIV-1 envelope glycoprotein incorporation into virions. Proc. Natl. Acad. Sci. USA **97:**343–348.
40. **Parren, P. W., J. P. Moore, D. R. Burton, and Q. J. Sattentau.** 1999. The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity. AIDS **13**(Suppl. A)**:**S137–S162.
41. **Pierson, T. C., and R. W. Doms.** 2003. HIV-1 entry and its inhibition. Curr. Top. Microbiol. Immunol. **281:**1–27.
42. **Piller, S. C., J. W. Dubay, C. A. Derdeyn, and E. Hunter.** 2000. Mutational analysis of conserved domains within the cytoplasmic tail of gp41 from human immunodeficiency virus type 1: effects on glycoprotein incorporation and infectivity. J. Virol. **74:**11717–11723.
43. **Reeves, J. D., F. H. Lee, J. L. Miamidian, C. B. Jabara, M. M. Juntilla, and R. W. Doms.** 2005. Enfuvirtide resistance mutations: impact on human immunodeficiency virus envelope function, entry inhibitor sensitivity, and virus neutralization. J. Virol. **79:**4991–4999.
44. **Reitter, J. N., and R. C. Desrosiers.** 1998. Identification of replication-competent strains of simian immunodeficiency virus lacking multiple attachment sites for N-linked carbohydrates in variable regions 1 and 2 of the surface envelope protein. J. Virol. **72:**5399–5407.
45. **Reitter, J. N., R. E. Means, and R. C. Desrosiers.** 1998. A role for carbohydrates in immune evasion in AIDS. Nat. Med. **4:**679–684.
46. **Robinson, J. E., K. S. Cole, D. H. Elliott, H. Lam, A. M. Amedee, R. Means, R. C. Desrosiers, J. Clements, R. C. Montelaro, and M. Murphey-Corb.** 1998. Production and characterization of SIV envelope-specific rhesus monoclonal antibodies from a macaque asymptomatically infected with a live SIV vaccine. AIDS Res. Hum. Retrovir. **14:**1253–1262.

47. **Rosati, M., A. von Gegerfelt, P. Roth, C. Alicea, A. Valentin, M. Robert-Guroff, D. Venzon, D. Montefiori, P. Markham, B. K. Felber, and G. N. Pavlakis.** 2005. DNA vaccines expressing different forms of simian immunodeficiency virus antigens decrease viremia upon SIVmac251 challenge. J. Virol. **79:**8480–8492.
48. **Rousso, I., M. B. Mixon, B. K. Chen, and P. S. Kim.** 2000. Palmitoylation of the HIV-1 envelope glycoprotein is critical for viral infectivity. Proc. Natl. Acad. Sci. USA **97:**13523–13525.
49. **Rowell, J. F., P. E. Stanhope, and R. F. Siliciano.** 1995. Endocytosis of endogenously synthesized HIV-1 envelope protein. Mechanism and role in processing for association with class II MHC. J. Immunol. **155:**473–488.
50. **Schenten, D., L. Marcon, G. B. Karlsson, C. Parolin, T. Kodama, N. Gerard, and J. Sodroski.** 1999. Effects of soluble CD4 on simian immunodeficiency virus infection of CD4-positive and CD4-negative cells. J. Virol. **73:**5373–5380.
51. **Shacklett, B. L., C. Denesvre, B. Boson, and P. Sonigo.** 1998. Features of the SIV_{mac} transmembrane glycoprotein cytoplasmic domain that are important for Env functions. AIDS Res. Hum. Retrovir. **14:**373–383.
52. **Srinivas, S. K., R. V. Srinivas, G. M. Anantharamaiah, R. W. Compans, and J. P. Segrest.** 1993. Cytosolic domain of the human immunodeficiency virus envelope glycoproteins binds to calmodulin and inhibits calmodulin-regulated proteins. J. Biol. Chem. **268:**22895–22899.
53. **Wyss, S., C. Berlioz-Torrent, M. Boge, G. Blot, S. Honing, R. Benarous, and M. Thali.** 2001. The highly conserved C-terminal dileucine motif in the cytosolic domain of the human immunodeficiency virus type 1 envelope glycoprotein is critical for its association with the AP-1 clathrin adaptor [correction of adapter]. J. Virol. **75:**2982–2992.
54. **Yang, C., and R. W. Compans.** 1996. Analysis of the cell fusion activities of chimeric simian immunodeficiency virus-murine leukemia virus envelope proteins: inhibitory effects of the R peptide. J. Virol. **70:**248–254.
55. **Yu, X., X. Yuan, M. F. McLane, T. H. Lee, and M. Essex.** 1993. Mutations in the cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein impair the incorporation of Env proteins into mature virions. J. Virol. **67:**213–221.
56. **Yuste, E., J. D. Reeves, R. W. Doms, and R. C. Desrosiers.** 2004. Modulation of Env content in virions of simian immunodeficiency virus: correlation with cell surface expression and virion infectivity. J. Virol. **78:**6775–6785.
57. **Zhang, H., L. Wang, S. Kao, I. P. Whitehead, M. J. Hart, B. Liu, K. Duus, K. Burridge, C. J. Der, and L. Su.** 1999. Functional interaction between the cytoplasmic leucine-zipper domain of HIV-1 gp41 and p115-RhoGEF. Curr. Biol. **9:**1271–1274.
58. **Zhu, P., E. Chertova, J. Bess, Jr., J. D. Lifson, L. O. Arthur, J. Liu, K. A. Taylor, and K. H. Roux.** 2003. Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. Proc. Natl. Acad. Sci. USA **100:**15812–15817.
59. **Zingler, K., and D. R. Littman.** 1993. Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein increases env incorporation into particles and fusogenicity and infectivity. J. Virol. **67:**2824–2831.