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Role of V1V2 and Other Human Immunodeficiency Virus Type 1 Envelope Domains in Resistance to Autologous Neutralization during Clade C Infection[▽]

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Biologically functional clade C envelope (Env) glycoproteins from the chronically (donor) and newly (recipient) infected partners of four heterosexual transmission pairs in Zambia were cloned and characterized previously. In each case, the donor viral quasispecies contained Envs that were resistant to autologous neutralization by contemporaneous plasma, while the recipient Envs were sensitive to neutralizing antibodies in this donor plasma sample. The donor Envs also varied in length, glycosylation, and amino acid sequence of the V1V2 hypervariable domain of gp120, while the recipient Envs were much more homogeneous. To assess the contribution of V1V2 to the neutralization phenotype of the donor Envs, V1V2 domains from neutralization-sensitive recipient Envs were replaced with donor V1V2 domains, and the autologous neutralization sensitivities of the chimeric Envs were evaluated using a virus-pseudotyping assay. Long donor V1V2 domains regulated sensitivity to autologous neutralization, although the effect was dependent on the Env background. Short donor V1V2 domains did not confer neutralization resistance. Primary sequence differences in V2 were also found to influence neutralization sensitivity in one set of recipient Envs. The results demonstrate that expansion of the V1V2 domain is one pathway to escape from autologous neutralization in subtype C Envs. However, V1V2-independent mechanisms of resistance also exist, suggesting that escape is multifaceted in chronic subtype C infection.

In the course of 25 years, human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, has infected more than 60 million people worldwide (45). Developing countries have been severely impacted by the pandemic, and it is well recognized that viral subtypes that are genetically distinct from those in the United States and Europe circulate in those regions (30, 36). Recently, there has been a movement toward the characterization of viruses representative of globally predominant subtypes, such as A and C, especially with respect to the envelope (Env) glycoproteins (1, 7, 8, 27, 49, 52), which can be as much as 35% divergent between viral clades (11). This extraordinary genetic diversity poses a significant impediment to vaccine development, in which a major goal is induction of broad and potent neutralizing antibodies (NAb) (22). It is therefore important to characterize the mechanisms of escape from autologous neutralization for viruses representative of globally predominant clades, as they could be relevant for the selection and development of immunogens.

The HIV-1 genome encodes two glycoproteins; the surface subunit gp120 facilitates interactions with receptor molecules, while the transmembrane subunit gp41 anchors the Env complex in the viral membrane and mediates fusion with the host cell membrane (16). HIV-1 gp120 contains five "hypervariable" domains that tolerate sequence heterogeneity, and especially in the case of the first two hypervariable domains (V1V2), accommodate dramatic insertions and deletions and various patterns of glycosylation (26). The V1V2 domain facilitates numerous virus entry phenotypes in studies of clade B viruses, including CD4 independence, tropism, receptor utilization, and neutralization sensitivity (2, 3, 6, 14, 18–21, 23, 28, 29, 33–35, 37, 39–43, 46). The V1V2 domain is thought to regulate neutralization sensitivity by masking conserved neutralization targets (23–25, 39, 51) and can also present type-specific neutralization epitopes (9, 10, 12, 15, 17, 31, 38, 47). Because this polymorphic domain is refractory to structural characterization, its position and structure on the native Env trimer have not been defined. A structural model of the unliganded SIVmac239 gp120 molecule places V1V2 in close proximity to the V3 domain on an adjacent protomer (5). This model is supported by the presence of monoclonal antibodies that recognize discontinuous epitopes comprised of sequences in both V1V2 and V3 (9, 13, 53). Upon binding CD4, however, the V1V2 stem is thought to translocate approximately 40 angstroms to mediate formation of the coreceptor binding site (5). It is therefore not difficult to envision how V1V2 influences the exposure of more conserved regions of Env.

We previously investigated the transmission of subtype C HIV-1 in an African setting by characterizing Envs from eight

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chronically infected subjects (donors) who transmitted infection heterosexually to their partners (recipients) in Zambia (8). Inspection of the amino acid sequence of the V1-to-V4 region of gp120, which includes the hypervariable domains V1V2, V3, and V4, revealed that Envs with compact hypervariable domains were frequently transmitted and established infection in the recipients. Furthermore, for the five pairs tested, the newly transmitted recipient Envs were more sensitive to neutralization by plasma from the donor than were the majority of Envs in the donor quasispecies. In addition, a significant association between acquisition of length in V1 to V4 and neutralization resistance against autologous plasma was established for this set of donor and recipient Envs ($P = 0.01$; R. Rong, S. Gnanakaran, J. M. Decker, J. N. Sfakianos, F. Bibollet-Ruche, J. L. Mokili, M. Muldoon, J. Mulenga, S. Allen, B. H. Hahn, G. M. Shaw, J. L. Blackwell, E. Hunter, B. T. Korber, and C. A. Derdeyn, unpublished data).

Because expanded V1V2 domains were common in the quasispecies of the donors and often tracked with neutralization resistance (8), we hypothesized that autologous NAb could be driving changes in this region. To test whether donor V1V2 domains directly modulate sensitivity to autologous neutralization, the recipient Env was utilized as a genetically related, neutralization-sensitive molecular scaffold with which to probe potential neutralization determinants in V1V2. Chimeric Envs in which the V1V2 domain of a recipient Env was replaced with different V1V2 domains derived from the matched donor Envs were created for four transmission pairs, and neutralization sensitivity to contemporaneous donor plasma was evaluated using a pseudovirus reporter assay. Here, we provide strong evidence that expansion of V1V2 results from pressure exerted by autologous NAb in some cases. Moreover, this study describes a direct mechanism of neutralization resistance in chronic subtype C infection and provides indirect evidence that there are additional and perhaps more complex NAb resistance pathways.

MATERIALS AND METHODS

Env clones. Details of the Zambia cohort, sample collection, and processing have been described previously (8, 32, 44). The Envs studied here were derived from four heterosexual transmission pairs from this cohort (8). All Envs are subtype C. All amino acid positions are based on HXB2 gp160 numbering. For this study, Envs were given designations based on the donor and the recipient.

Construction of chimeric Envs. PCR amplification and cloning of the donor and recipient Envs from pairs 53, 55, 106, and 109 have been described previously (8). The *env* genes are in the cytomegalovirus-driven expression vector pCR3.1 (Invitrogen), which is used to generate viral pseudotypes. V1V2 chimeric Envs were constructed using the following domain exchange strategy: the donor V1V2 domains were PCR amplified using primers that anneal to well-conserved sequences that flank V1V2 (Fig. 1A). These primer sequences and their HXB2 locations were as follows: pairs 53 and 109, forward primer 5′-ACCCCACTCT GTGTCACTTTA-3′ (HXB2 nucleotides [nt] 6591 to 6611) and reverse primer 5′-TTGTGTTATGGTTGAGGTATTAC-3′ (HXB2 nt 6833 to 6811); pair 106, forward primer 5′-TGTAAAGTTGACCCCACTCTG-3′ (HXB2 nt 6581 to 6601) and reverse primer 5′-GGCTGAGGTATTACAATTTATTAATC-3′ (HXB2 nt 6826 to 6801); pair 55, forward primer 5′-CCACTCTGTGTCACTT TAAAC-3′ (HXB2 nt 6594 to 6614) and reverse primer 5′-AAAAGAGACCT TTGGACAGGC-3′ (HXB2 nt 6854 to 6834).

The recipient Env backbones (minus the V1V2 domain) with pCR3.1 vector sequences were PCR amplified using primers that anneal to conserved regions adjacent to the V1V2 primers. These primers amplify away from V1V2 (Fig. 1B). A 5′ phosphate group (Phos) was added to these primer sets during synthesis to facilitate ligation to the V1V2 amplicon. The primer sequences and their HXB2 locations were as follows: pair 53, forward primer 5′-Phos-GCCTGTCCAAAG GTCTCTTTTGAT-3′ (HXB2 nt 6834 to 6857) and reverse primer 5′-Phos-CAATTTTACACATGGTTTTAGGCTTTGG-3′ (HXB2 nt 6590 to 6563); pair 106, forward primer 5′-Phos-ATGACACAAGCCTGTCCAAAGGTC-3′ (HXB2 nt 6827 to 6850) and reverse primer 5′-Phos-CATGGCTTTAAGCTTT GATCCCATA-3′ (HXB2 nt 6580 to 6556); pair 109, forward primer 5′-Phos-G CCTGTCCAAAGGTCTCTTTTGAC-3′ (HXB2 nt 6834 to 6857) and reverse primer 5′-Phos-CAATTTTACACATGGCTTTAGGCTTTG-3′ (HXB2 nt 6590 to 6564); pair 55, forward primer 5′-Phos-ATGACACAAGCCTGTCCAAAG GTC-3′ (HXB2 nt 6855 to 6878) and reverse primer 5′-Phos-CATGGCTTTA AGCTTTGATCCCATA-3′ (HXB2 nt 6593 to 6569).

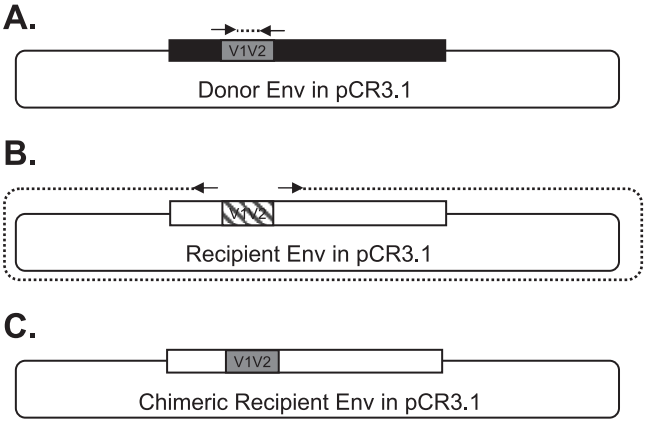

FIG. 1. Construction of V1V2 chimeric Envs. V1V2 chimeric Envs were constructed using a domain exchange strategy. (A) Each donor V1V2 domain (gray box) was PCR amplified from the *env* gene using primers (arrows) that annealed to conserved sequences flanking V1V2 and amplified inward. (B) The recipient Env (open box) plus plasmid vector was PCR amplified using primers (arrows) that annealed to sites adjacent to those of the V1V2 primers and amplified outward. (C) The two fragments were blunt-end ligated together to produce the chimeric recipient Env in pCR3.1 containing a donor V1V2 domain (white and gray boxes).

The PCR amplification conditions for the recipient Env backbones were 1 cycle of 95°C for 3 min; 35 cycles of 95°C for 1 min, 50°C to 60°C for 30 s (the optimal annealing temperature was determined for each primer set), 72°C for 10 min; 1 cycle of 72°C for 15 min; and storage at 4°C. The amplification conditions for the V1V2 domain were the same, except the extension time at 72°C was reduced to 30 s. The 25-μl PCR mixtures contained 50 ng of each primer, 10 ng of the plasmid template, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate, and 1× reaction buffer. *pfuTurbo* DNA polymerase (Stratagene) was used to generate the blunt-ended PCR amplicons, which were digested with DpnI to remove contaminating template DNA and gel purified from an agarose gel using the QIAquick Gel Extraction Kit (QIAGEN) prior to ligation. Each donor V1V2 DNA fragment was then ligated to the purified recipient *env* backbone to produce a V1V2 chimera (Fig. 1C) using T4 DNA ligase (5 U/μl; Roche) at 4°C overnight. The ligation reaction mixture (usually one-third of the volume) was transformed into maximum-efficiency XL2-blue supercompetent cells (1×10^9 CFU/μg DNA; Stratagene) so that the DNA volume did not exceed 5% of the cell volume. The entire transformation was plated onto LB-ampicillin agar plates, generally resulting in 10 to 50 colonies per ligation reaction.

A PCR screen was performed to identify colonies in which the fragments ligated together in the correct orientation using forward primer EnvA (8) and the reverse primer that was used to amplify the V1V2 domain. Colonies that were positive by PCR screen were inoculated into LB-ampicillin broth for overnight cultures, and the plasmid was prepared using the QIAprep Spin Miniprep Kit. Plasmids were then screened for biological function as previously described (8, 27). Briefly, 100 ng of pCR3.1-Env DNA was cotransfected into 293T cells, along with 200 ng of an Env-deficient subtype B proviral plasmid, pSG3ΔEnv, using Fugene-6 according to the manufacturer's instructions (Hoffman-La Roche). Seventy-two hours later, the transfection supernatant was transferred to JC53-BL13 (TZM-bl) indicator cells. At 48 h postinfection, each well was scored positive or negative for blue foci using β-galactosidase staining. For clones that produced functional Env pseudotypes, the plasmids were retransfected into 293T cells on a larger scale to produce a working pseudotype virus stock. The trans-

fection supernatants were collected at 72 h posttransfection, clarified by low-speed centrifugation, aliquoted into 0.5-ml or smaller portions, and stored at −80°C. The titer of each pseudotyped virus stock was determined by infecting JC53-BL cells with fivefold serial dilutions of virus as described previously (8, 27). All V1V2 chimeras were confirmed by nucleotide sequencing.

PCR-based site mutagenesis. To investigate whether amino acid sequence differences in the V2 domain accounted for the difference in neutralization phenotype between Envs 55 recipient 1 (recip1) (F4a) and recip2 (F28a), mutations were created in the V2 domain of recip2. A Thr was changed to Ile, and a Lys was changed to Glu using PCR-based site mutagenesis (see Fig. 6A). Briefly, we designed four primers that either did or did not contain each of the two mutations (substituted nucleotides are underlined). The primer sequences and their HXB2 locations were as follows: reverse primer A with mutation, 5′-TAGTGGTACTATATCAAGTATATC-3′ (HXB2 nt 6776 to 6753); reverse primer B without mutation, 5′-TAGTGGTACTATATCAAGTGTATC-3′ (HXB2 nt 6776 to 6753); forward primer C with mutation, 5′-CTTGATATAG TACCACTAGAACCACC-3′ (HXB2 nt 6759 to 6791); forward primer D without mutation, 5′-CTTGATATAGTACCACTAAAACCACC-3′ (HXB2 nt 6759 to 6791).

recip2 was PCR amplified using primers B and C to mutate Thr to Ile, primers A and D to mutate Lys to Glu, and primers A and C to create the double mutant. The blunt-ended PCR amplicons were generated with *pfuTurbo* DNA polymerase and ligated to the recipient Env backbones using the strategy described above. The strategies for the PCR screen, the biological-function screen, and production of virus stock were the same as those described above. All mutations were confirmed by nucleotide sequencing.

Neutralization assay. All neutralization assays were performed using viral pseudotypes to infect JC53-BL13 (Tzm-bl) indicator cells that produce luciferase, as described previously, and with the modifications described below (8, 27, 48). The neutralizing-antibody activity of plasma collected from donors was evaluated against virions pseudotyped with recipient Envs containing chimeric V1V2 regions in parallel with the parental donor and recipient Envs. In previous studies, a 50% inhibitory concentration (IC_{50}) was calculated for each plasma-Env combination from the virus infectivity curve. However, for the present study, to evaluate the neutralization sensitivities of donor, recipient, and chimeric Envs together, it was necessary to conserve limited amounts of donor plasma. Therefore, the lowest plasma dilution tested in some cases was 1:100 instead of 1:20, which was used previously, and 50% inhibition was not achieved for all Envs.

Nucleotide sequence accession numbers. The Env nucleotide sequences (either V1 to V4 or full length) have been deposited in GenBank under the following accession numbers: pair 53, AY424004, AY424008, AY424010, AY424024, and AY423984; pair 106, AY424151 to -153, AY424155, and AY424138; pair 109, AY424138, AY424141, AY424128 and -129, and AY424133 and -134; pair 55, AY423971, AY423973, AY423938 and -939, AY423944, and AY423946.

RESULTS

To investigate whether exchange of the V1V2 domain from donor Envs influenced the neutralization sensitivity of the recipient Env to donor plasma, we created V1V2 chimeras using Envs from four different clade C transmission pairs (53, female to male [FTM]; 106, male to female (MTF); 109, MTF; and 55, MTF). Each set of V1V2 chimeras was generated from donor and recipient Envs from the same transmission pair; interpair V1V2 exchanges were not performed. The result for each set of donor V1V2 exchanges is presented in the figures (see Fig. 2 through 5) and in the text below, organized by transmission pair.

Pair 53, FTM: long V1V2 domains facilitate resistance to autologous NAb. In FTM transmission pair 53, most of the donor Envs with long V1V2 domains were resistant to autologous NAb (8). The V1V2 domain of a representative neutralization-sensitive recipient Env was therefore replaced with a long domain from a neutralization-resistant donor Env. The first donor Env evaluated (Fig. 2, donor 1) encoded a V1V2 domain that was 19 amino acids longer than that of the recipient Env, with three additional potential N-linked glycosylation (N-gly) sites (Fig. 2A). This donor Env was highly resistant to autologous NAb relative to the recipient Env (Fig. 2B). Its infectivity at the highest plasma concentration tested (1:100) was 83% of the control infection lacking plasma. By contrast, the recipient Env pseudotype was sensitive to neutralization by donor plasma and was almost completely neutralized at 1:100 dilution, with only 5% of its infectivity remaining (Fig. 2B). The chimera carrying the donor 1 V1V2 domain in the recipient Env background had a phenotype similar to that of the parental donor 1 Env (Fig. 2B). A similar result was observed for the donor 3 V1V2 domain (Fig. 2D), which was the same length as that of donor 1 but had a different sequence (Fig. 2A). Thus, for donor 1 and donor 3, the length and perhaps glycosylation of the V1V2 region determined neutralization resistance.

The V1V2 domain of donor 2 was identical to that of donor 1 (Fig. 2A), but donor 2 was almost as sensitive to neutralization by donor plasma as the recipient Env (Fig. 2C). Nevertheless, as might be predicted, when the donor 2 V1V2 domain was placed into the recipient Env background, a neutralization-resistant phenotype was produced (Fig. 2C). Thus, the divergence in neutralization sensitivity between Envs donor 1 and donor 2 must reflect sequence differences outside of V1V2. A comparison between the complete gp160 amino acid sequences of these two Envs revealed 1 amino acid difference in gp120 (at position 269 in C2) but 12 differences scattered throughout gp41 (data not shown). Thus, the effect of the neutralization resistance-conferring V1V2 domain was overridden by one or more sensitive epitopes in donor 2. These results provide a striking illustration of the context-dependent nature of the V1V2 domain. Env donor 4 had a compact V1V2 domain that was identical to that of the recipient (Fig. 2A), but this Env was nevertheless neutralization resistant (Fig. 2E). In this case, the compact V1V2 did not regulate the resistant phenotype of donor 4, since the recipient chimera carrying this V1V2 domain was as sensitive as the wild-type recipient (Fig. 2E). For donor 4, neutralization resistance required determinants located in regions outside of V1V2.

Pair 106, MTF: an unusually long V1V2 domain can confer neutralization sensitivity. The first donor Env (donor 1) evaluated from MTF pair 106 carried a V1V2 domain of 95 residues, the longest in the eight transmission pairs originally studied (8). The V1 loop alone was 54 amino acids in length, resulting from a series of tandem repeats encoding seven N-gly sites (Fig. 3A). Yet this Env was as sensitive to neutralization to donor plasma as the recipient Env, with only 25% infectivity remaining at the 1:100 dilution of plasma (Fig. 3B). When the donor 1 V1V2 domain was placed into the recipient Env, resulting in an insertion of 29 amino acids and five N-gly sites, the chimera became as sensitive to NAb as donor 1 (Fig. 3B). Thus, for this donor Env, the unusually long and heavily glycosylated V1V2 domain was the major determinant of neutralization sensitivity. Unlike donor 1, donor 2 had a V1V2 domain similar in size and sequence to that of the recipient Env (Fig. 3A), and donor 2 was neutralization resistant (Fig. 3C). Substitution of the donor 2 V1V2 domain into the recipient resulted in neutralization sensitivity similar to that of the wild-type recipient (Fig. 3C), arguing that for this donor Env, neutralization resistance also required sequences outside of V1V2. Env donor 3 carried a very short V1V2 domain (Fig. 3A) but

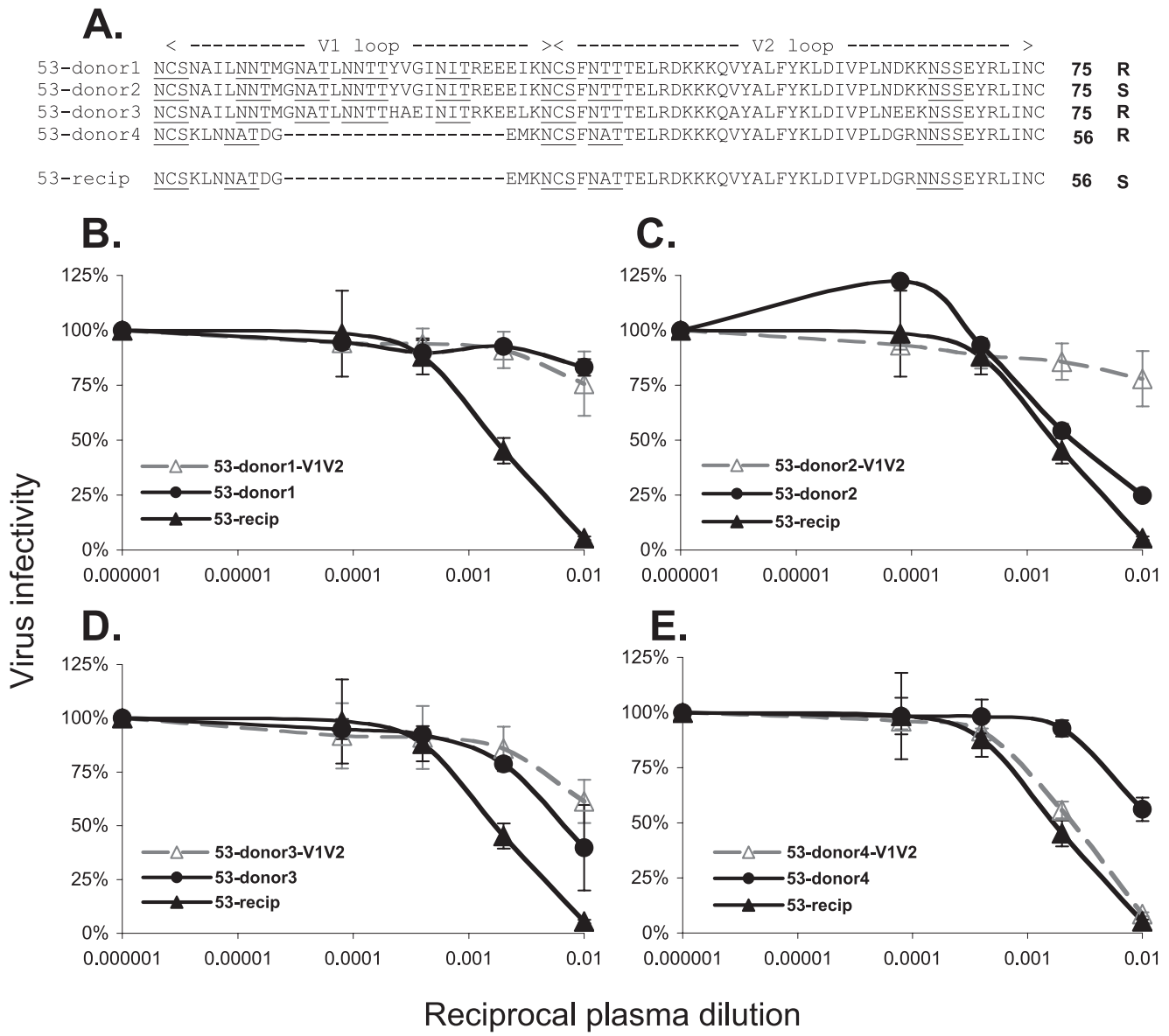

FIG. 2. Effect of donor Env V1V2 exchange on neutralization sensitivity for FTM transmission pair 53. (A) A predicted amino acid alignment of the V1V2 domain (residues HXB2 131 to 196) for donor (top) and recipient (bottom) Envs is shown. Each Env clone is designated by the transmission pair identifier, source (donor or recipient), and a number. All donor and recipient Envs were derived from uncultured patient peripheral blood mononuclear cells. Potential N-linked glycosylation sites (NXS or NXT, where X is any residue except proline) are underlined. Dashes indicate gaps in the sequence relative to the longest donor sequence. The length of each V1V2 domain from cysteine to cysteine is shown. "R" indicates that the Env was resistant to neutralization by donor plasma, while "S" indicates that the Env was sensitive. (B to E) Infectivity curves in the presence of donor plasma are shown for each set of parental donor (filled circles), recipient (filled triangles and diamonds), and V1V2-chimeric (open triangles and diamonds) Env pseudotypes. Virus infectivity (as a percentage of the control lacking plasma) is graphed against the reciprocal dilution factor of the contemporaneous donor plasma on a $\log_{10}$ scale. Each experiment was performed twice independently with duplicate wells. The error bars show the standard deviation for each data point.

was relatively resistant to neutralization (Fig. 3C). When this V1V2 domain was transferred to the recipient Env, resulting in a loss of eight amino acids and four N-gly sites, it caused a sixfold increase in neutralization sensitivity (Fig. 3C). Thus, this donor Env had also acquired resistance in regions outside of V1V2.

Pair 109, MTF: a donor V1V2 domain caused context-dependent changes in phenotypically different recipient Envs. For MTF pair 109, V1V2 chimeras were constructed in two recipient Env backbones that were identical with respect to the V1V2 domain (Fig. 4A) and neutralization sensitivity to donor plasma (Fig. 4B). However, recip1 was more sensitive to inhibition by soluble CD4 than recip2 (Rong et al., unpublished). The V1V2 domain from resistant Env donor 1 (Fig. 4B) was placed into the two phenotypically different recipient Envs, resulting in an increase of 10 amino acid residues and one N-gly site (Fig. 4A). In this case, disparate phenotypes were observed in the two V1V2 chimeras (Fig. 4B). In one recipient

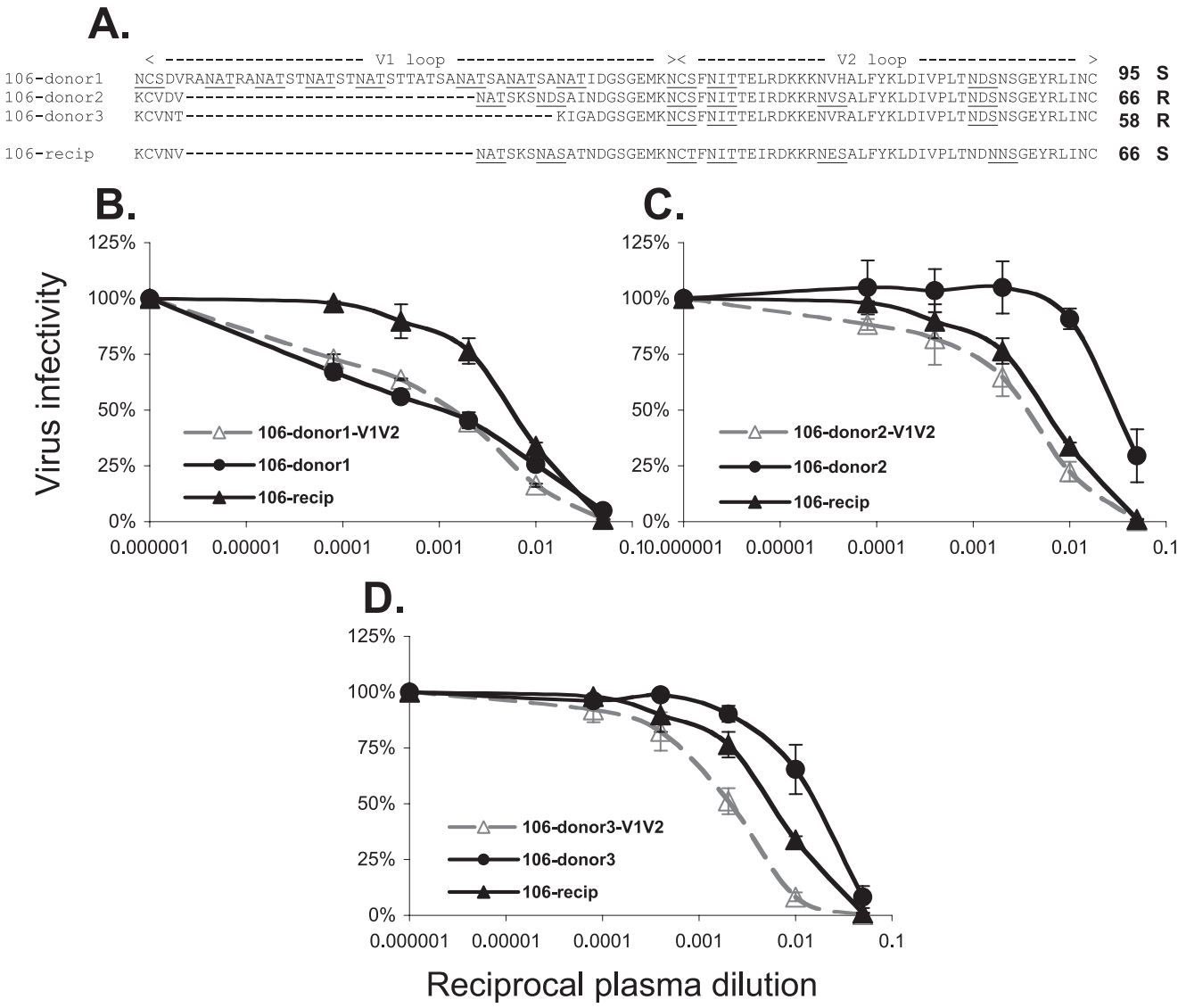

FIG. 3. Effect of donor Env V1V2 exchange on neutralization sensitivity for MTF transmission pair 106. See the legend to Fig. 2 for details.

Env, this V1V2 domain conferred resistance to the level of the donor Env parent, with 60% of infectivity remaining at 1:100 dilution (Fig. 4B). The other recipient-based V1V2 chimera, however, was as neutralization sensitive as the wild-type recipient Envs, with only 30% of infectivity remaining at 1:100 dilution (Fig. 4B). This result illustrates another striking example of the context-dependent nature of V1V2-mediated NAb resistance, where residues outside of V1V2 can modulate its impact.

Env donor 2 carries a long V1V2 domain (Fig. 4A), but this pseudotype is more sensitive to neutralization by donor plasma than the recipient Envs (Fig.4Cs). When this V1V2 domain was exchanged, resulting in the addition of six residues and the shift of two N-gly sites from V2 to V1, a 10-fold increase in sensitivity was produced for both recipient-based chimeras (Fig. 4C). Thus, in this case, the long V1V2 domain contained determinants of neutralization sensitivity that could be translocated to both recipient Envs and was unaffected by sequence differences outside of V1V2.

Pair 55, MTF: differences in primary sequence influence neutralization sensitivity. The results described above for pair 53 and 109 Envs suggested that it was the length and perhaps glycosylation of this region rather than differences in the primary sequence of V1V2 that defined neutralization sensitivity. For MTF pair 55, donor V1V2 domain exchanges were performed using two recipient Envs that differed by 10-fold in neutralization sensitivity to donor plasma (Fig. 5B), despite very homogeneous sequences. These two recipient Envs differed by two residues in V2 (Fig. 5A) and one residue in the gp41 cytoplasmic tail at position 721 (data not shown). When the V1V2 domain from neutralization-resistant Env donor 1 was placed into both recipient Envs, adding one amino acid, the two chimeras had equivalent neutralization sensitivities (Fig. 5B). This result hinted that the amino acid differences in

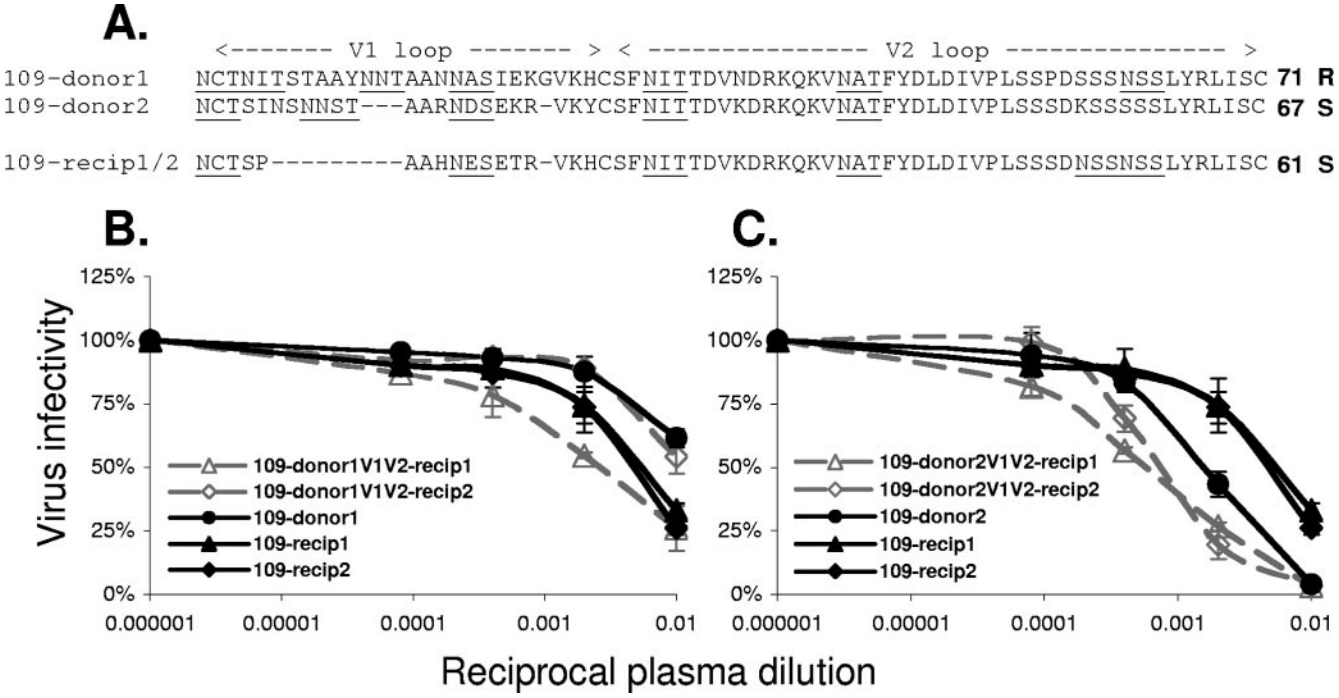

FIG. 4. Effect of donor Env V1V2 exchange on neutralization sensitivity for MTF transmission pair 109. See the legend to Fig. 2 for details.

V2 between the recipient Envs were responsible for the observed differential sensitivities to donor plasma. The chimeras did not, however, reproduce the full resistance of donor 1 (Fig. 5B), arguing again that additional determinants of neutralization resistance exist outside of V1V2 in this donor Env. The V1V2 domain of Env donor 2 was one residue shorter than the recipient's (Fig. 5A). Exchange of the donor 2 V1V2 domain again resulted in equivalent neutralization sensitivities for the two chimeras (Fig. 5C). Thus, unlike the results observed with the previous three pairs, differences in the primary sequence of

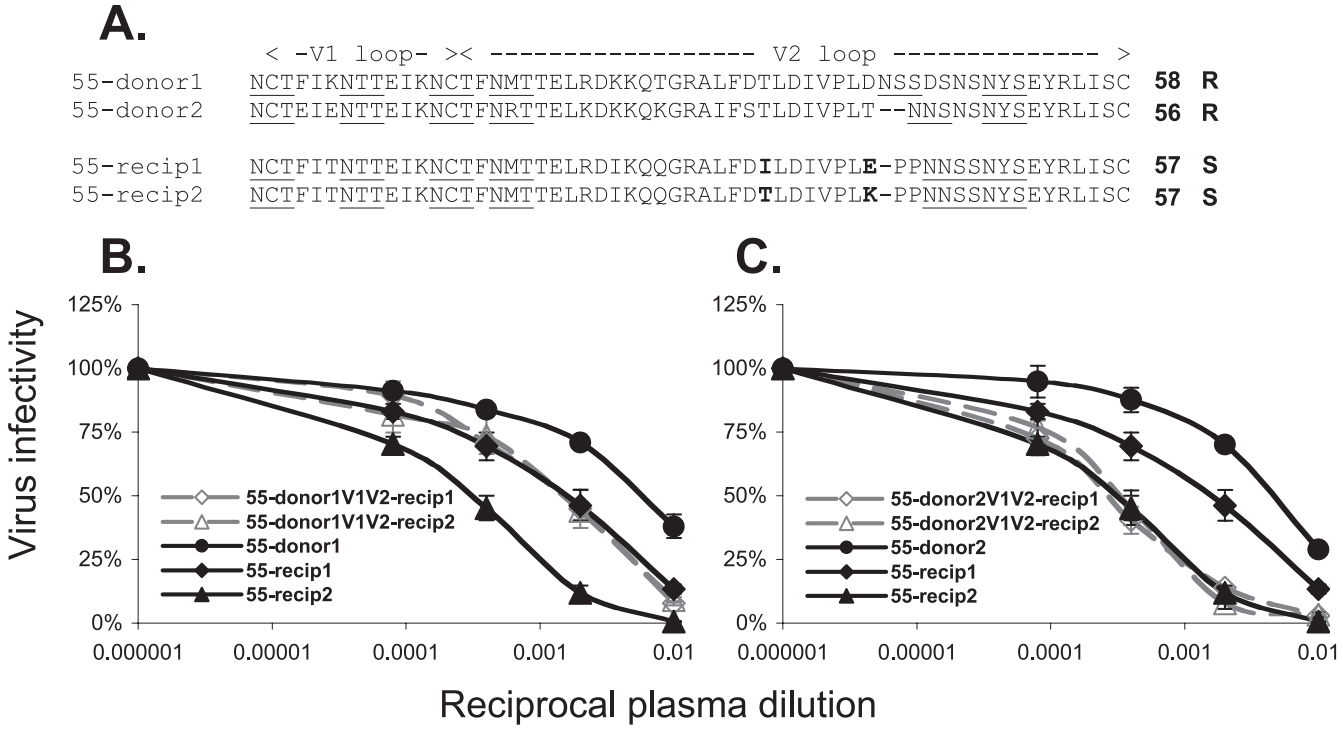

FIG. 5. Effect of donor Env V1V2 exchange on neutralization sensitivity for MTF transmission pair 55. See the legend to Fig. 2 for details.

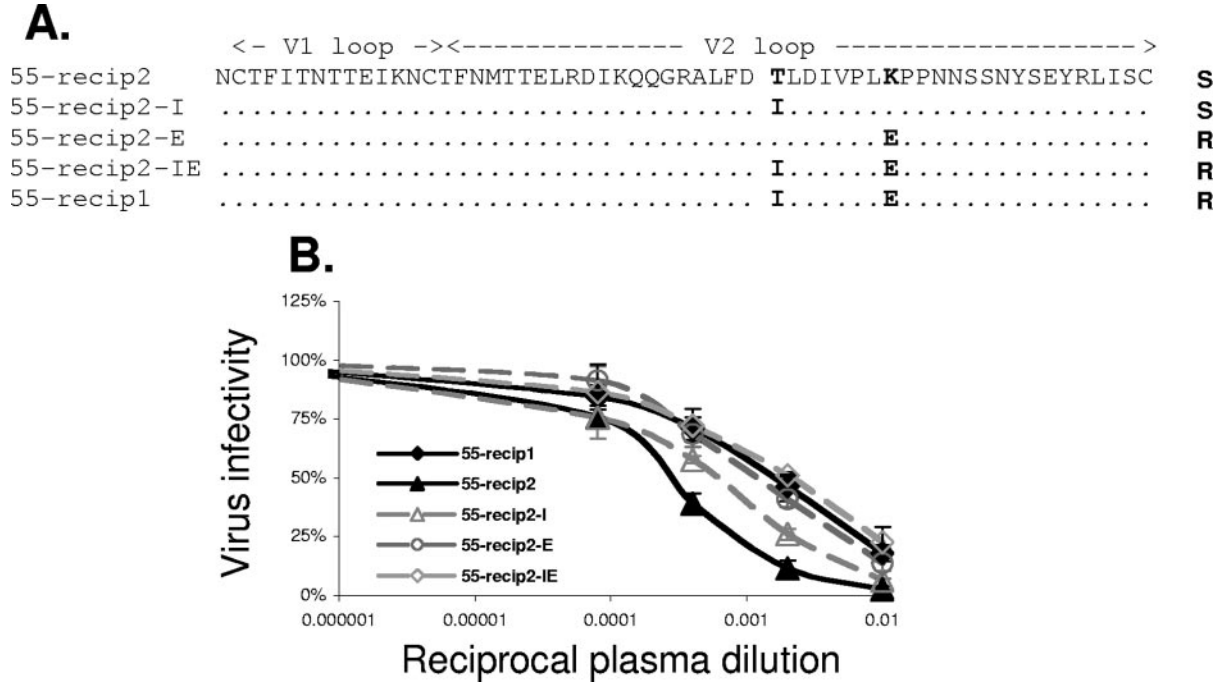

FIG. 6. Effects of V2 mutations on neutralization sensitivities of 55F recipient Envs. (A) A predicted amino acid alignment of the V1V2 domain with the mutations created in F28a (recip2) is shown. Potential N-linked glycosylation sites are underlined. Dots indicate identical sequence relative to recip2, and mutated residues are shown in boldface. (B) Infectivity curves are shown for recip2 (filled triangles), recip1 (filled diamonds), and mutants recip2-I (open triangles), recip2-E (open circles), and recip2-IE (open diamonds) Env pseudotypes against 55M donor plasma dilutions. Virus infectivity (as a percentage of the control lacking plasma) is graphed against the reciprocal plasma dilution on a $\log_{10}$ scale. Each experiment was performed at least twice independently with duplicate wells. The error bars show the standard deviation for each data point.

the recipient Envs appeared to have more dramatic effects on neutralization sensitivity.

Recipient 55F: a single amino acid in the V2 domain modulates neutralization sensitivities of the recipient Envs. Even though recip2 was 10-fold more sensitive to neutralization by donor plasma than recip1, each time a donor V1V2 exchange was performed, the two recipient chimeras had equivalent neutralization sensitivities (Fig. 5B and C). We hypothesized that primary sequence differences in the V2 domain were responsible for the difference in neutralization sensitivity of the recipient Envs and that a single amino acid difference in the cytoplasmic tail had no effect. To test this, two mutations were created in the V2 region of the more neutralization-sensitive Env recip2, singly and in combination, to determine whether they could account for the decreased neutralization sensitivity of recip1. In the first mutant, Thr was changed to Ile, and in the second, Lys was changed to Glu (Fig. 6A). In the third mutant, both changes were introduced (Fig. 6A). Recip2 was the most sensitive to donor NAb (Fig. 6A). The Thr-to-Ile change alone (recip2-I) had a small effect on NAb sensitivity (Fig. 6B), decreasing the sensitivity by approximately twofold. The Lys-to-Glu change (recip2-E) had a more dramatic effect, decreasing the sensitivity of recip2 by sixfold (Fig. 6B). The recip2 pseudovirus carrying both mutations (recip2-IE) had a phenotype similar to that of recip1, which was approximately eightfold less sensitive to NAb (Fig. 6B). Thus, these two nonconservative amino acid differences, which presumably arose during acute/early infection, determined the neutralization phenotypes of the two recipient Envs.

DISCUSSION

In a previous study, we compared Envs from viruses that were transmitted between eight sets of heterosexual partners in Zambia (8). We first analyzed the length and glycosylation of the gp120 V1-to-V4 region and observed that the viral quasispecies in each chronically infected donor partner contained V1V2 and V4 domains that varied in length and glycosylation, while Envs with compact, homogeneous V1V2 and V4 domains were frequently transmitted. We next evaluated the sensitivities of the donor and recipient Envs to neutralization by donor plasma for five of the eight pairs. While the donor quasispecies contained Envs that were resistant to autologous neutralization, the newly transmitted Envs in the recipients were uniformly sensitive to neutralization by plasma from the linked donor partner. In the present study, we created V1V2-chimeric Envs for four of these five transmission pairs to determine whether neutralizing antibodies drive the expansion of V1V2 in chronic infection. A strength of this study is the approach used, where unaltered donor V1V2 domains were transferred into a highly related, neutralization-sensitive "molecular scaffold" to determine their effects on autologous NAb sensitivity. Using this approach, three basic predictions were tested. First, for neutralization-resistant donor Envs that had a long V1V2 domain, we predicted that this region would confer neutralization resistance. Second, for neutralization-resistant donor Envs that had a short V1V2 domain, we predicted that the V1V2 domain would not contain determinants of resistance (i.e., that resistance determinants would be located else-

where). Finally, some donor Envs had a long V1V2 domain but were nevertheless neutralization sensitive. For these, we predicted that the V1V2 domain contained a neutralization epitope and would transfer increased sensitivity to the recipient Env.

In the panel of biologically functional donor Envs studied here, the V1V2 domains ranged in length from 56 to 95 amino acids and contained between 3 and 11 N-gly sites. Of 11 donor V1V2 domains that were evaluated, 7 were longer (and usually more glycosylated) than the matched recipient Env. Of these seven long V1V2 domains, five conferred resistance to NAb. The other two increased the neutralization sensitivity of the recipient Env, possibly because they contained a neutralization epitope. Two V1V2 domains were the same length as the matched recipient Env, and exchange of these domains did not change the neutralization sensitivity of the recipient Env. Two donor V1V2 domains were shorter than the matched recipient Env, and both of these increased neutralization sensitivity. Thus, the main mechanism by which V1V2 appears to confer protection from NAb is by masking, although this effect was dependent on the Env background. The data also suggest that masking is dependent on the size and glycosylation of V1V2.

The donor Env quasispecies has the capacity to stand a high degree of polymorphism in V1V2 without loss of function. Furthermore, the recipient Envs can tolerate insertion of a wide range of V1V2 domains without loss of function and in the absence of any compensatory changes. Many studies have shown that alteration of glycosylation sites or insertion/deletion of amino acid residues in V1V2 can influence a number of molecular interactions critical to virus entry. Although informative in their own context, mutagenesis studies of a single variant may not fully reflect the natural variation of V1V2 within a quasispecies. A further consideration is that previous studies have been based solely on subtype B viral isolates, some of which were laboratory-adapted strains. In addition, few studies have assessed the role of V1V2 in escape from autologous NAb, using instead monoclonal antibodies or heterologous patient plasma or serum samples. In the current study, we probed the determinants of NAb sensitivity contained within naturally occurring V1V2 domains as they pertain to four different subtype C viral quasispecies from chronic infection. Overall, the V1V2 domain can exert a powerful influence on sensitivity to NAb in this setting.

A notable finding that was observed in Envs from two different transmission pairs was the highly context-dependent nature of the effects of the V1V2 domain. Other studies have also reported that V1V2 acts in a context-dependent manner. For instance, SF162 can tolerate deletion of V2 without affecting replication, but in two other Envs, V2 deletion resulted in a loss of function (41). This study suggested that the functional contributions of V1V2 can differ based on the Env background. For FTM pair 53, long V1V2 domains clearly confer resistance when transferred to the recipient Env, and the effect of this V1V2 domain is global, in that V1V2 appears to protect against the entire NAb pool. However, resistance-conferring determinants in V1V2 can be overridden by the presence of neutralization-sensitive sequences in other Env domains. These results suggest that some neutralization epitopes cannot be masked by the V1V2 domain.

Another example of the context-dependent nature of V1V2 was observed when a long V1V2 domain from MTF pair 109 was transferred into two different recipient Envs. This long V1V2 domain conferred full phenotypic resistance to one recipient Env. In the other recipient Env background, however, this V1V2 domain had the opposite effect. The two recipient Envs differed by only two residues in gp120 (one in V3 and one in C3) that did not appear to influence their NAb sensitivity to donor plasma in the context of the native recipient V1V2 domain. In contrast, the long donor V1V2 domain yielded a chimeric Env that was sensitive to these differences. The only residue that was uniquely associated with the neutralization-sensitive Env background of recip1 was a Leu at position 309, located within the V3 crown adjacent to the GPGQ motif. All other pair 109 Envs showed an Ile at this position, and this Ile was highly conserved within subtype C sequences in the database (http://www.hiv.lanl.gov). The recipient Env with Leu substituted is also phenotypically unique in that it is highly sensitive to inhibition by soluble CD4 and relatively resistant to inhibition by anti-CD4 antibody (Rong et al., unpublished). These results argue for an interaction between V1V2 and V3 that is necessary for the masking effects of the long V1V2 domain, which could be disrupted by the uncommon Leu in the V3 of recip1. The significance of this unusual amino acid residue for early infection is under investigation.

V1V2 masking of neutralization epitopes has been described for subtype B Envs, and this domain accounts for the overall neutralization-resistant phenotype of JRFL (39). V1V2 masking is also operative in the context of anti-V3 NAb responses in subtype A patient plasma samples (23, 24), suggesting that this could be a widespread and effective mechanism for blocking NAb epitopes in V3 and other conserved targets. Substitution of the JRFL V1V2 domain into the neutralization-sensitive strain SF162 rendered this Env resistant to neutralization by patient sera and monoclonal antibodies targeted to multiple gp120 domains. Interestingly, the JRFL and SF162 V1V2 domains are the same length, but JRFL contains three additional N-gly sites. In the present study, increases in length were accompanied by increases in the number of N-gly sites, so it is possible that increased glycans are as important as increased length. In contrast, large V1V2 domains from two donor Envs transferred increased sensitivity to the recipient Env. It is possible that these large V1V2 domains contained a potently neutralized epitope. Alternatively, the large, heavily glycosylated structures could result in a conformation that exposes distant neutralization epitopes. These exchanges also produced dramatic shifts in the positions of glycans relative to the native recipient V1V2 domain, which could also influence neutralization sensitivity, as has been reported by others (4, 6, 14, 28, 29, 50).

Clearly, there are also examples of V1V2-independent mechanisms of NAb escape in these subtype C Envs from chronic infection. Exchange of short V1V2 domains was not associated with resistance in any case, even when transferred from donor Envs that were themselves resistant to NAb. This in itself is strong evidence for V1V2-independent mechanisms of escape. Four donor Envs were NAb resistant despite having compact V1V2 domains, and these V1V2 domains did not confer NAb resistance on the matching recipient Env (Fig. 2E, 3C and D, and 5C). Even in donors like 53F where NAb resistance could be effectively achieved by lengthening

V1V2 (Fig. 2B, C, and D), one donor Env had acquired resistance independently of V1V2 (Fig. 2E). These observations are consistent with a previously established association between maximum-likelihood branch length in the V1-to-V4 region (a measure of sequence divergence outside of the hypervariable domains) and sensitivity to donor NAb ($P = 0.0002$; Rong et al., unpublished). The results argue that NAb can drive expansion of V1V2 in chronic infection, but there are other pathways to neutralization resistance, probably because not all neutralization epitopes are effectively masked by V1V2 (Fig. 2C and 4A).

Env sequences in newly clade C-infected recipients are usually homogeneous, differing at most by a few residues in the entire gp160 sequence (8, 27). In recipient 55F, there were two residues located in V2 that influenced sensitivity to donor NAb. The most dramatic effect was observed by changing a positively charged Lys residue present in the V2 domain of the more neutralization-sensitive recipient Env. When the charge was reversed by mutating Lys to a negatively charged Glu, this recipient Env became sixfold less sensitive to NAb. The negatively charged Glu may facilitate some level of epitope masking or may directly disrupt an epitope in the V2 domain. Further support for the importance of this charged residue comes from the observation that one of the 55M donor V1V2 domains has a negatively charged Asp residue at this position (Fig. 5A, donor 1), and exchange of this domain tracks with the less sensitive recip1 (Fig. 5B). On the other hand, the other donor Env V1V2 domain lacks the negative charge, and its V1V2 domain tracks with the more sensitive recip2 (Fig. 5C). The charge differences in V2 notwithstanding, both donor Envs also contain NAb resistance determinants outside of V1V2, illustrating the complexity of resistance in chronic infection.

In summary, the data demonstrate that V1V2 can have dramatic yet context-dependent effects on the autologous neutralization sensitivities of patient-derived subtype C Envs. Exchange of long V1V2 domains conferred neutralization resistance in five cases, but in two cases it increased neutralization sensitivity, suggesting that this domain could also be a target for NAb in subtype C infection. Short V1V2 domains did not confer neutralization resistance, suggesting that a size-dependent conformational masking of neutralization epitopes can occur. Importantly, we have observed four clear cases where V1V2 did not contain determinants of NAb resistance. Taken together, the data suggest that there are intricate pathways to resistance against autologous NAb in subtype C infection that involve V1V2, as well as other Env domains. Identification of the neutralization epitopes targeted in subtype C infection will be necessary to understand how to effectively neutralize viruses of this subtype.

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REFERENCES

1. **Binley, J. M., T. Wrin, B. Korber, M. B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C. J. Petropoulos, and D. R. Burton.** 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. J. Virol. **78:**13232–13252.
2. **Bouma, P., M. Leavitt, P. F. Zhang, I. A. Sidorov, D. S. Dimitrov, and G. V. Quinnan, Jr.** 2003. Multiple interactions across the surface of the gp120 core structure determine the global neutralization resistance phenotype of human immunodeficiency virus type 1. J. Virol. **77:**8061–8071.
3. **Carrillo, A., and L. Ratner.** 1996. Cooperative effects of the human immunodeficiency virus type 1 envelope variable loops V1 and V3 in mediating infectivity for T cells. J. Virol. **70:**1310–1316.
4. **Chackerian, B., L. M. Rudensey, and J. Overbaugh.** 1997. Specific N-linked and O-linked glycosylation modifications in the envelope V1 domain of simian immunodeficiency virus variants that evolve in the host alter recognition by neutralizing antibodies. J. Virol. **71:**7719–7727.
5. **Chen, B., E. M. Vogan, H. Gong, J. J. Skehel, D. C. Wiley, and S. C. Harrison.** 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. Nature **433:**834–841.
6. **Cheng-Mayer, C., A. Brown, J. Harouse, P. A. Luciw, and A. J. Mayer.** 1999. Selection for neutralization resistance of the simian/human immunodeficiency virus SHIVSF33A variant in vivo by virtue of sequence changes in the extracellular envelope glycoprotein that modify N-linked glycosylation. J. Virol. **73:**5294–5300.
7. **Chohan, B., D. Lang, M. Sagar, B. Korber, L. Lavreys, B. Richardson, and J. Overbaugh.** 2005. Selection for human immunodeficiency virus type 1 envelope glycosylation variants with shorter V1-V2 loop sequences occurs during transmission of certain genetic subtypes and may impact viral RNA levels. J. Virol. **79:**6528–6531.
8. **Derdeyn, C. A., J. M. Decker, F. Bibollet-Ruche, J. L. Mokili, M. Muldoon, S. A. Denham, M. L. Heil, F. Kasolo, R. Musonda, B. H. Hahn, G. M. Shaw, B. T. Korber, S. Allen, and E. Hunter.** 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science **303:**2019–2022.
9. **Etemad-Moghadam, B., G. B. Karlsson, M. Halloran, Y. Sun, D. Schenten, M. Fernandes, N. L. Letvin, and J. Sodroski.** 1998. Characterization of simian-human immunodeficiency virus envelope glycoprotein epitopes recognized by neutralizing antibodies from infected monkeys. J. Virol. **72:**8437–8445.
10. **Fung, M. S., C. R. Sun, W. L. Gordon, R. S. Liou, T. W. Chang, W. N. Sun, E. S. Daar, and D. D. Ho.** 1992. Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. J. Virol. **66:**848–856.
11. **Gaschen, B., J. Taylor, K. Yusim, B. Foley, F. Gao, D. Lang, V. Novitsky, B. Haynes, B. H. Hahn, T. Bhattacharya, and B. Korber.** 2002. Diversity considerations in HIV-1 vaccine selection. Science **296:**2354–2360.
12. **Gorny, M. K., J. P. Moore, A. J. Conley, S. Karwowska, J. Sodroski, C. Williams, S. Burda, L. J. Boots, and S. Zolla-Pazner.** 1994. Human anti-V2 monoclonal antibody that neutralizes primary but not laboratory isolates of human immunodeficiency virus type 1. J. Virol. **68:**8312–8320.
13. **Gorny, M. K., L. Stamatatos, B. Volsky, K. Revesz, C. Williams, X. H. Wang, S. Cohen, R. Staudinger, and S. Zolla-Pazner.** 2005. Identification of a new quaternary neutralizing epitope on human immunodeficiency virus type 1 virus particles. J. Virol. **79:**5232–5237.
14. **Gram, G. J., A. Hemming, A. Bolmstedt, B. Jansson, S. Olofsson, L. Akerblom, J. O. Nielsen, and J. E. Hansen.** 1994. Identification of an N-linked glycan in the V1-loop of HIV-1 gp120 influencing neutralization by anti-V3 antibodies and soluble CD4. Arch. Virol. **139:**253–261.
15. **Ho, D. D., M. S. Fung, Y. Z. Cao, X. L. Li, C. Sun, T. W. Chang, and N. C. Sun.** 1991. Another discontinuous epitope on glycoprotein gp120 that is important in human immunodeficiency virus type 1 neutralization is identified by a monoclonal antibody. Proc. Natl. Acad. Sci. USA **88:**8949–8952.
16. **Hunter, E.** 1997. Viral entry and receptors, p. 71–121. *In* S. H. H. John, M. Coffin, and Harold E. Varmus (ed.), Retroviruses. Cold Spring Harbor Laboratory Press, Plainview, NY.
17. **Kayman, S. C., Z. Wu, K. Revesz, H. Chen, R. Kopelman, and A. Pinter.** 1994. Presentation of native epitopes in the V1/V2 and V3 regions of human immunodeficiency virus type 1 gp120 by fusion glycoproteins containing isolated gp120 domains. J. Virol. **68:**400–410.
18. **Koito, A., G. Harrowe, J. A. Levy, and C. Cheng-Mayer.** 1994. 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. J. Virol. **68:**2253–2259.
19. **Koito, A., L. Stamatatos, and C. Cheng-Mayer.** 1995. Small amino acid sequence changes within the V2 domain can affect the function of a T-cell line-tropic human immunodeficiency virus type 1 envelope gp120. Virology **206:**878–884.
20. **Kolchinsky, P., E. Kiprilov, P. Bartley, R. Rubinstein, and J. Sodroski.** 2001. Loss of a single N-linked glycan allows CD4-independent human immuno-

deficiency virus type 1 infection by altering the position of the gp120 V1/V2 variable loops. J. Virol. **75:**3435–3443.
21. **Kolchinsky, P., E. Kiprilov, and J. Sodroski.** 2001. Increased neutralization sensitivity of CD4-independent human immunodeficiency virus variants. J. Virol. **75:**2041–2050.
22. **Korber, B., B. Gaschen, K. Yusim, R. Thakallapally, C. Kesmir, and V. Detours.** 2001. Evolutionary and immunological implications of contemporary HIV-1 variation. Br. Med. Bull. **58:**19–42.
23. **Krachmarov, C., A. Pinter, W. J. Honnen, M. K. Gorny, P. N. Nyambi, S. Zolla-Pazner, and S. C. Kayman.** 2005. Antibodies that are cross-reactive for human immunodeficiency virus type 1 clade A and clade B v3 domains are common in patient sera from Cameroon, but their neutralization activity is usually restricted by epitope masking. J. Virol. **79:**780–790.
24. **Krachmarov, C. P., W. J. Honnen, S. C. Kayman, M. K. Gorny, S. Zolla-Pazner, and A. Pinter.** 2006. Factors determining the breadth and potency of neutralization by v3-specific human monoclonal antibodies derived from subjects infected with clade A or clade B strains of human immunodeficiency virus type 1. J. Virol. **80:**7127–7135.
25. **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.
26. **Leitner, T., B. Foley, B. Hahn, P. Marx, F. McCutchan, J. Mellors, S. Wolinksy, B. Korber, et al.** 2003. HIV sequence compendium. Los Alamos National Laboratory, Los Alamos, NM.
27. **Li, B., J. M. Decker, R. W. Johnson, F. Bibollet-Ruche, X. Wei, J. Mulenga, S. Allen, E. Hunter, B. H. Hahn, G. M. Shaw, J. L. Blackwell, and C. A. Derdeyn.** 2006. Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1. J. Virol. **80:**5211–5218.
28. **Losman, B., A. Bolmstedt, K. Schonning, A. Bjorndal, C. Westin, E. M. Fenyo, and S. Olofsson.** 2001. Protection of neutralization epitopes in the V3 loop of oligomeric human immunodeficiency virus type 1 glycoprotein 120 by N-linked oligosaccharides in the V1 region. AIDS Res. Hum. Retrovir. **17:**1067–1076.
29. **Ly, A., and L. Stamatatos.** 2000. V2 loop glycosylation of the human immunodeficiency virus type 1 SF162 envelope facilitates interaction of this protein with CD4 and CCR5 receptors and protects the virus from neutralization by anti-V3 loop and anti-CD4 binding site antibodies. J. Virol. **74:**6769–6776.
30. **McCutchan, F.** 2006. Global epidemiology of HIV. J. Med. Virol. **78:**S7–S12.
31. **McKeating, J. A., C. Shotton, J. Cordell, S. Graham, P. Balfe, N. Sullivan, M. Charles, M. Page, A. Bolmstedt, S. Olofsson, et al.** 1993. Characterization of neutralizing monoclonal antibodies to linear and conformation-dependent epitopes within the first and second variable domains of human immunodeficiency virus type 1 gp120. J. Virol. **67:**4932–4944.
32. **McKenna, S. L., G. K. Muyinda, D. Roth, M. Mwali, N. Ng'andu, A. Myrick, C. Luo, F. H. Priddy, V. M. Hall, A. A. von Lieven, J. R. Sabatino, K. Mark, and S. A. Allen.** 1997. Rapid HIV testing and counseling for voluntary testing centers in Africa. AIDS **11**(Suppl. 1)**:**S103–S110.
33. **Morikita, T., Y. Maeda, S. Fujii, S. Matsushita, K. Obaru, and K. Takatsuki.** 1997. The V1/V2 region of human immunodeficiency virus type 1 modulates the sensitivity to neutralization by soluble CD4 and cellular tropism. AIDS Res. Hum. Retrovir. **13:**1291–1299.
34. **Nabatov, A. A., G. Pollakis, T. Linnemann, A. Kliphius, M. I. Chalaby, and W. A. Paxton.** 2004. Intrapatient alterations in the human immunodeficiency virus type 1 gp120 V1V2 and V3 regions differentially modulate coreceptor usage, virus inhibition by CC/CXC chemokines, soluble CD4, and the b12 and 2G12 monoclonal antibodies. J. Virol. **78:**524–530.
35. **Pastore, C., R. Nedellec, A. Ramos, S. Pontow, L. Ratner, and D. E. Mosier.** 2006. Human immunodeficiency virus type 1 coreceptor switching: V1/V2 gain-of-fitness mutations compensate for V3 loss-of-fitness mutations. J. Virol. **80:**750–758.
36. **Peeters, M.** 2000. Recombinant HIV sequences: their role in the global epidemic, p. 39–54. *In* F. B. Kuiken, B. Hahn, P. Marx, F. McCutchan, J. Mellors, J. Mullins, J. Sodroski, S. Wolinksy, and B. Korber (ed.), HIV sequence compendium 2000. Los Alamos National Laboratory, Los Alamos, NM.
37. **Pincus, S. H., K. G. Messer, P. L. Nara, W. A. Blattner, G. Colclough, and M. Reitz.** 1994. Temporal analysis of the antibody response to HIV envelope protein in HIV-infected laboratory workers. J. Clin. Investig. **93:**2505–2513.
38. **Pinter, A., W. J. Honnen, P. D'Agostino, M. K. Gorny, S. Zolla-Pazner, and S. C. Kayman.** 2005. The C108g epitope in the V2 domain of gp120 functions as a potent neutralization target when introduced into envelope proteins derived from human immunodeficiency virus type 1 primary isolates. J. Virol. **79:**6909–6917.
39. **Pinter, A., W. J. Honnen, Y. He, M. K. Gorny, S. Zolla-Pazner, and S. C. Kayman.** 2004. The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary human immunodeficiency virus type 1 isolates to neutralization by antibodies commonly induced upon infection. J. Virol. **78:**5205–5215.
40. **Pugach, P., S. E. Kuhmann, J. Taylor, A. J. Marozsan, A. Snyder, T. Ketas, S. M. Wolinsky, B. T. Korber, and J. P. Moore.** 2004. The prolonged culture of human immunodeficiency virus type 1 in primary lymphocytes increases its sensitivity to neutralization by soluble CD4. Virology **321:**8–22.
41. **Saunders, C. J., R. A. McCaffrey, I. Zharkikh, Z. Kraft, S. E. Malenbaum, B. Burke, C. Cheng-Mayer, and L. Stamatatos.** 2005. The V1, V2, and V3 regions of the human immunodeficiency virus type 1 envelope differentially affect the viral phenotype in an isolate-dependent manner. J. Virol. **79:**9069–9080.
42. **Si, Z., M. Cayabyab, and J. Sodroski.** 2001. Envelope glycoprotein determinants of neutralization resistance in a simian-human immunodeficiency virus (SHIV-HXBc2P 3.2) derived by passage in monkeys. J. Virol. **75:**4208–4218.
43. **Stamatatos, L., and C. Cheng-Mayer.** 1998. An envelope modification that renders a primary, neutralization-resistant clade B human immunodeficiency virus type 1 isolate highly susceptible to neutralization by sera from other clades. J. Virol. **72:**7840–7845.
44. **Trask, S., C. A. Derdeyn, F. Kasolo, R. Musonda, F. Gao, S. A. Allen, and B. Hahn.** 2002. Epidemiological linkage analysis in a heterosexual transmission cohort in Zambia. J. Virol. **76:**397–405.
45. **UNAIDS-WHO.** 2006. Report on the global AIDS epidemic. World Health Organization, Geneva, Switzerland.
46. **Walter, B. L., K. Wehrly, R. Swanstrom, E. Platt, D. Kabat, and B. Chesebro.** 2005. Role of low CD4 levels in the influence of human immunodeficiency virus type 1 envelope V1 and V2 regions on entry and spread in macrophages. J. Virol. **79:**4828–4837.
47. **Warrier, S. V., A. Pinter, W. J. Honnen, M. Girard, E. Muchmore, and S. A. Tilley.** 1994. A novel, glycan-dependent epitope in the V2 domain of human immunodeficiency virus type 1 gp120 is recognized by a highly potent, neutralizing chimpanzee monoclonal antibody. J. Virol. **68:**4636–4642.
48. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. Nature **422:**307–312.
49. **Wu, X., A. B. Parast, B. A. Richardson, R. Nduati, G. John-Stewart, D. Mbori-Ngacha, S. M. Rainwater, and J. Overbaugh.** 2006. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. J. Virol. **80:**835–844.
50. **Wu, Z., S. C. Kayman, W. Honnen, K. Revesz, H. Chen, S. Vijh-Warrier, S. A. Tilley, J. McKeating, C. Shotton, and A. Pinter.** 1995. Characterization of neutralization epitopes in the V2 region of human immunodeficiency virus type 1 gp120: role of glycosylation in the correct folding of the V1/V2 domain. J. Virol. **69:**2271–2278.
51. **Wyatt, R., J. Moore, M. Accola, E. Desjardin, J. Robinson, and J. Sodroski.** 1995. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. J. Virol. **69:**5723–5733.
52. **Zhang, H., F. Hoffmann, J. He, X. He, C. Kankasa, R. Ruprecht, J. T. West, G. Orti, and C. Wood.** 2005. Evolution of subtype C HIV-1 Env in a slowly progressing Zambian infant. Retrovirology **2:**67.
53. **Zwick, M. B., R. Kelleher, R. Jensen, A. F. Labrijn, M. Wang, G. V. Quinnan, Jr., P. W. Parren, and D. R. Burton.** 2003. A novel human antibody against human immunodeficiency virus type 1 gp120 is V1, V2, and V3 loop dependent and helps delimit the epitope of the broadly neutralizing antibody immunoglobulin G1 b12. J. Virol. **77:**6965–6978.