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Cooperative Effects of the Human Immunodeficiency Virus Type 1 Envelope Variable Loops V1 and V3 in Mediating Infectivity for T Cells

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Insertion of T-cell line-tropic V3 and V4 loops from the HXB2 strain into the macrophage-tropic YU-2 envelope resulted in a virus with delayed infectivity for HUT78 and Jurkat cells compared with HXB2. Sequence analysis of viral DNA derived from long-term cultures of Jurkat cells revealed a specific mutation that changed a highly conserved Asn residue in the V1 loop of Env to an Asp residue (N-136→D). Introduction of this mutation into clones containing a T-cell line-tropic V3 loop, either with or without a T-cell line-tropic V4 loop, resulted in viruses that replicated to high levels in Jurkat cells and peripheral blood lymphocytes. The Env proteins from these constructs were expressed with the vaccinia virus/T7 hybrid system and were found to be translated, processed, and cleaved and to bind to soluble CD4 similar to the wild-type HXB2 and YU-2 Env proteins. Env-mediated fusion with HeLa T4$^+$ cells, however, was regulated by both the altered V1 loop and T-cell line-tropic V3 loop. These results suggest that subsequent to the initial gp120-CD4 binding event, a functional interaction can occur between the altered V1 loop and T-cell line-tropic V3 loop that results in infection of Jurkat cells and peripheral blood lymphocytes.

Strains of human immunodeficiency virus type 1 (HIV-1) differ markedly in their ability to infect particular cell types. Most strains of HIV-1 are capable of infecting primary lymphocytes, but the ability to infect primary macrophages versus T-lymphoid cell lines is an inversely related property of most molecularly cloned viruses (41, 43). The predominant determinants of HIV tissue specificity lie within the *env* gene (5–8, 17, 36, 42, 43, 45–47), and a critical domain is a region known as the V3 loop that is formed by a disulfide bond between cysteine residues 296 and 331 (25). In addition to its role as an important tissue tropism determinant, the V3 loop is a target for neutralizing antibodies and cytotoxic T cells, functions as a fusion domain, and regulates the sensitivity of HIV-1 to soluble CD4 (sCD4) (13, 18–20, 34, 40), but it is distinct from the domain of the envelope protein known to be important for the interaction with CD4 (37).

In spite of the importance of the V3 loop in determining tissue tropism of HIV, it is clear that infection by HIV is mediated by complex interactions between distinct regions of the Env protein. Several reports have suggested the importance of an interaction between the V1/V2 region of Env and the V3 loop (21, 43, 47), whereas others (6, 29, 31, 50) indicate that there is an interaction between the V3 loop and the conserved region C4 between the V4 and V5 loops. Yet another study by Freed and Martin (12) suggests the importance of an interaction between the V1/V2 region and C4.

In the current study, we identified a mutation from a long-term culture that resulted in the loss of a potential N-linked glycosylation site. The combination of the altered V1 loop and a T-cell line-tropic V3 resulted in a virus that was capable of replicating to wild-type levels in Jurkat cells and peripheral blood lymphocytes (PBLs). This study suggests that a functional interaction between the V1 and V3 loops of the envelope protein can occur to allow infection of certain cell types.

Identification of virus having delayed kinetics in T-cell lines. Our previous work with chimeric full-length proviral clones between the T-cell line-tropic strain HXB2 and the macrophage-tropic strain YU-2 had identified two determinants of the envelope protein that were necessary for T-cell line infection: the V3 loop and a 0.3-kb *Ppu*MI-*Bgl*II (PG) region coding for the V4 loop, C4 domain, and the N-terminal portion of the V5 loop (Fig. 1A) (6). An *Afl*III site was introduced at nucleotide 7497 by site-directed mutagenesis, which allowed the 100-amino-acid PG region to be divided into two regions encoding either the V4 loop or the C4/V5 domain. Insertion of T-cell line-tropic sequences encoding the V3 loop and C4/V5 domain into a YU-2 gp120 envelope protein (YH-V3+C4/V5) resulted in a chimera having replication kinetics almost identical to those of the chimera containing the larger T-cell line-tropic PG region (YH-V3+PG) in both HUT78 and Jurkat T-cell lines (Fig. 1B and C). The combination of T-cell line-tropic V3 and V4 loops (YH-V3+V4), however, resulted in a chimera having significantly delayed kinetics in both T-cell lines, with peak reverse transcriptase (RT) levels not attained until at least 40 days postinfection (Fig. 1B and C). This chimera also displayed decreased replication kinetics in PBLs (Fig. 1A). Expression of either the T-cell line-tropic V4 loop or C4/V5 domain in the absence of a T-cell line-tropic V3 loop (YH-V4 and YH-C4/V5, respectively) resulted in chimeras unable to replicate in either T-cell line (Fig. 1B and C), although both viruses were competent for replication in PBLs (Fig. 1A).

Molecular cloning and characterization of amino acid changes in the revertant virus *env* gene sequences. The emergence of RT activity late after infection with the YH-V3+V4 chimera suggested that the original virus had undergone a mutation that allowed it to replicate in Jurkat cells and that virus emerging late from the infected cultures would replicate with wild-type kinetics. To test this hypothesis, virus emerging from Jurkat cells 36 days after infection with the chimeric

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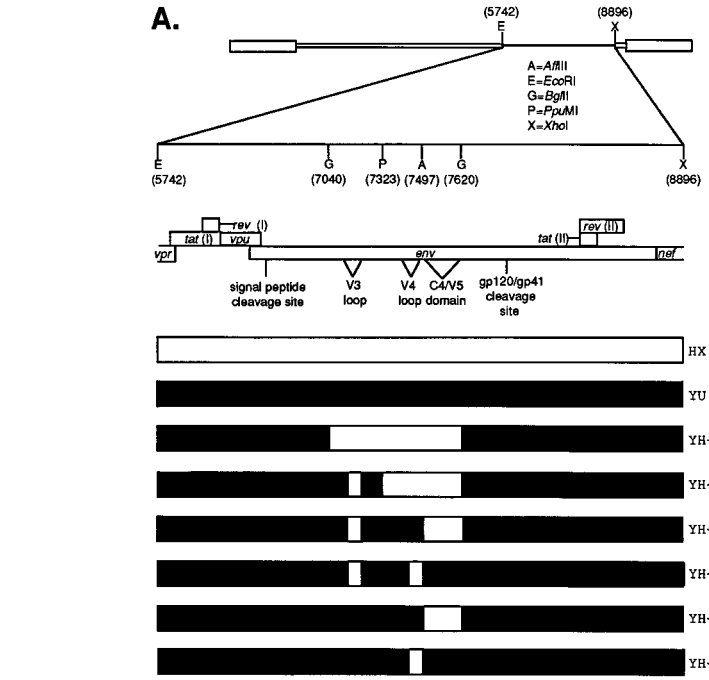
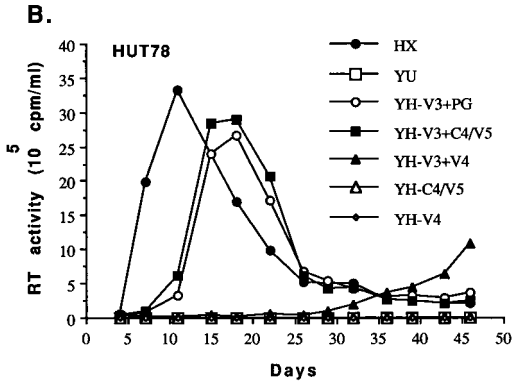
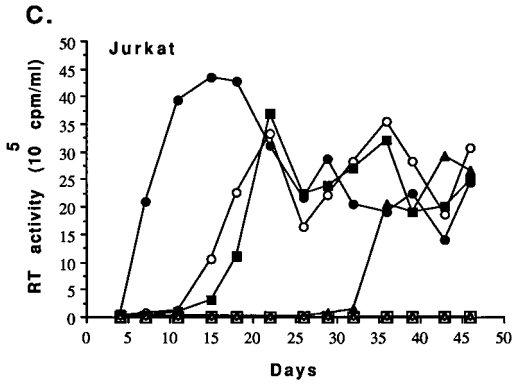


RT Activity In:

	PBL	HUT78		Jurkat	
	d18	d18	d46	d22	d46
HX	41.3	17.0	2.1	31.1	24.3
YU	63.5	<0.4	<0.4	<0.4	<0.4
YH-GG	55.7	22.7	4.9	32.0	22.4
YH-V3+PG	51.7	26.6	3.7	33.4	30.6
YH-V3+C4/V5	60.8	29.1	2.6	36.9	24.9
YH-V3+V4	17.8	<0.4	10.9	<0.4	26.9
YH-C4/V5	65.8	<0.4	<0.4	<0.4	<0.4
YH-V4	69.7	<0.4	<0.4	<0.4	<0.4





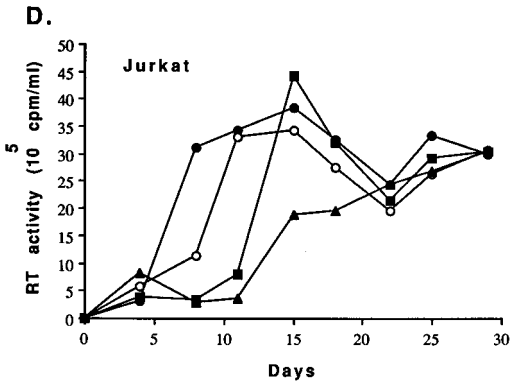


FIG. 1. Identification of virus displaying delayed replication kinetics in T-cell lines. (A) Chimeric proviral clones were constructed between the T-cell line-tropic virus HXB2 (white) and the macrophage-tropic virus YU-2 (black), which is incapable of conferring infection in a wide range of T-lymphoid cell lines. The top of the figure shows a schematic diagram of the HIV-1 genome, with the position of each long terminal repeat designated by an open box. The portion between the *Eco*RI (E) and *Xho*I (X) restriction enzyme sites is expanded below, illustrating additional restriction sites used in clone construction (A, *Afl*III; G, *Bgl*II; P, *Ppu*MI), with their nucleotide positions listed in parentheses, and relative positions of the *vpr*, *tat*, *vpu*, *env*, *rev*, and *nef* genes. Sequences encoding the signal peptide cleavage site, the gp120/gp41 cleavage site, and the V3 and V4 loops are indicated below the schematic for the *env* gene. RT activity for PBLs (18 days postinfection) and two T-cell lines, HUT78 (18 and 46 days postinfection) and Jurkat (22 and 46 days postinfection), is shown to the right of the figure and is expressed here and in all other figures as 10^5 cpm/ml. Similar results were obtained in at least three independent experiments. Replication kinetics for constructs are shown in the HUT78 (B) and Jurkat (C) T-cell lines. RT activity is plotted versus days postinfection. Similar replication kinetic profiles were obtained from at least three experiments. Virus emerging from Jurkat cells 36 days after infection (C) was passaged and used to reinfect fresh Jurkat cells. The replication kinetics of these passaged viruses are shown in panel D. The key shown in panel B also applies to panels C and D.

TABLE 1. Sequence analysis of *Kpn*I-*Bgl*II changes in YH-V3+V4-passaged virus

No. of clones[a]	Nucleotide mutation	Amino acid change
1	A-6515→G	N-98→D
1	G-6559→A	W-112→STOP
9	A-6629→G	N-136→D
1	A-6721→G	R-166 (silent)
1	A-6805→G	I-192→M
1	T-6994→C	T-257 (silent)

[a] Number of clones containing the specific mutation. Ten different clones were sequenced.

constructs was used to reinfect fresh Jurkat cells as shown in Fig. 1D. The reinfection kinetics with the HX, YH-V3+PG, and YH-V3+C4/V5 chimeras were similar to those obtained previously (Fig. 1C and D), although slightly accelerated because of a higher starting inoculum. However, reinfection with the YH-V3+V4 chimera resulted in significantly accelerated replication kinetics (Fig. 1C and D), suggesting that a mutation of the original virus had occurred.

To assess the nature of the putative mutation, viral DNA derived from Jurkat cells 15 days after reinfection was PCR amplified. gp120 sequences from the 0.6-kb *Bgl*II-*Bgl*II region (nucleotides 7040 to 7620) spanning the V3, V4, and N-terminal portion of the V5 loops, or from the 0.7-kb *Kpn*I-*Bgl*II region (nucleotides 6346 to 7040) spanning the V1 and V2 loops of Env, were cloned and sequenced. Sequencing of the entire 0.6-kb *Bgl*II-*Bgl*II region from two separate clones, representing viral DNA from Jurkat cells reinfected with the YH-V3+V4 virus, revealed no mutations in this region. The T-cell line-tropic V3 and V4 loops were maintained without any changes.

For analysis of the 0.7-kb *Kpn*I-*Bgl*II region (nucleotides 6346 to 7040), a total of 10 clones were sequenced. Nine of 10 clones contained a specific A-to-G mutation at nucleotide 6629, resulting in the change of a highly conserved Asn residue at position 136, which lies within a potential N-linked glycosylation site (consensus tripeptide sequence Asn-X-Thr/Ser), to an Asp residue in the N-terminal portion of the V1 loop of Env. This mutation was not seen in five separate clones representing viral DNA derived from Jurkat cells reinfected with the YH-V3+PG virus. A list of all of the nucleotide changes occurring within the *Kpn*I-*Bgl*II region in the 10 YH-V3+V4 clones, as well as the predicted amino acid changes and their frequency, is shown in Table 1.

The V1 loop containing the N-136→D change is able to interact with a T-cell line-tropic V3 loop. To test whether the N-136→D mutation was responsible for the more rapid reinfection kinetics of the YH-V3+V4 construct, the N-136→D change was introduced by site-directed mutagenesis into chimeras containing T-cell line-tropic V3 and/or V4 loops in the context of a YU-2 Env backbone, as well as into the parental YU clone (Fig. 2A [N-136→D mutation indicated by *]). The introduction of the V1 change did not alter the phenotype of either the parental YU clone (YU*) or a chimera containing a T-cell line-tropic V4 loop (YH-V4*). When this change was introduced into the chimera containing T-cell line-tropic V3 and V4 loops (YH-V3+V4*) from which the V1 mutation was initially characterized, the delayed replication kinetics were corrected, resulting in a virus having kinetics very similar to those of the YH-V3+PG chimera in Jurkat cells (Fig. 2B) and replicating to wild-type levels in PBLs (Fig. 2A). The replication kinetics in HUT78 cells of this virus (YH-V3+V4*) were intermediate between those of YH-V3+PG and YH-V3+V4 (not shown). Interestingly, the combination of the V1 change with just a T-cell line-tropic V3 loop (YH-V3*) was sufficient to rescue the normally nonfunctional YH-V3 construct and resulted in kinetics identical to those of YH-V3+V4*. This virus was also able to replicate in PBLs to wild-type levels (Fig. 2A).

Vaccinia virus expression of chimeric Env proteins. To determine what step of the viral life cycle might be affected by the presumed interaction between the altered V1 loop and T-cell line-tropic V3 loop, properties of recombinant Env proteins which might affect infectivity, such as sCD4 binding affinity, proteolytic cleavage susceptibility, and fusogenicity, were examined. Env proteins were expressed in BSC-40 cells by the vaccinia virus/T7 hybrid system. Analysis of ^{35}S-*trans*-labelled cell lysates (Fig. 3A) or cell-free supernatants (Fig. 3B) immunoprecipitated with HIV-1 patient antiserum revealed that all of the expression constructs produced comparable levels of protein. Whereas the gp160 precursor protein was the predom-

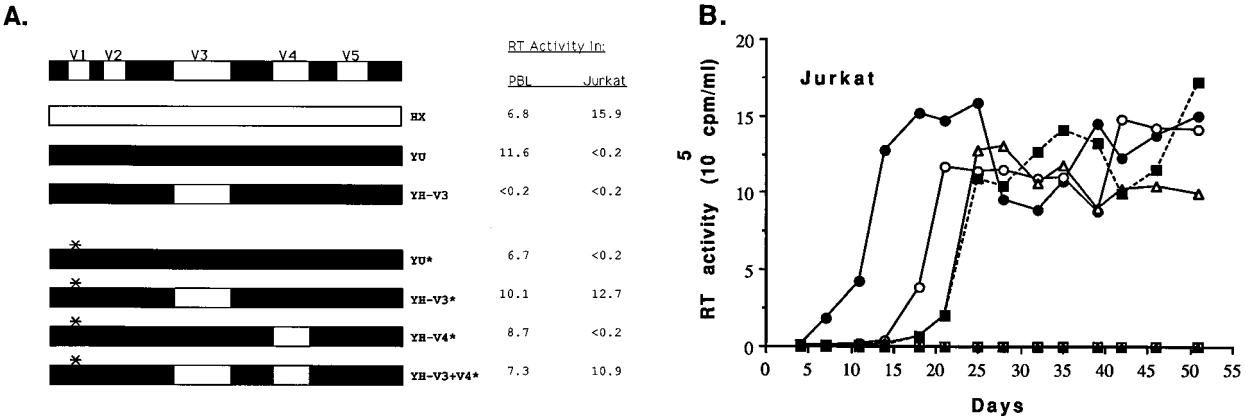


FIG. 2. Evidence of a functional interaction between the altered V1 loop and T-cell line-tropic V3 loop. (A) The top of the figure shows a schematic of the envelope protein depicting the five variable regions. The A-to-G mutation in the V1 loop was introduced by site-directed mutagenesis into the parental YU clone (black) and into clones containing T-cell line-tropic V3 and/or V4 loops from HXB2 (white). This mutation is depicted in the diagram by asterisks. RT levels in PBLs (23 days postinfection) and the Jurkat T-cell line (25 days postinfection) are shown and are expressed as 10^5 cpm/ml. (B) Replication kinetics for constructs containing the V1 loop change are shown in the Jurkat T-cell line. RT activity is plotted versus days postinfection. Similar replication kinetic profiles were obtained from at least three experiments. ●, HX; ○, YH-V3+PG; □, YU*; △, YH-V3*; ▲, YH-V4*; ■, YH-V3+V4*.

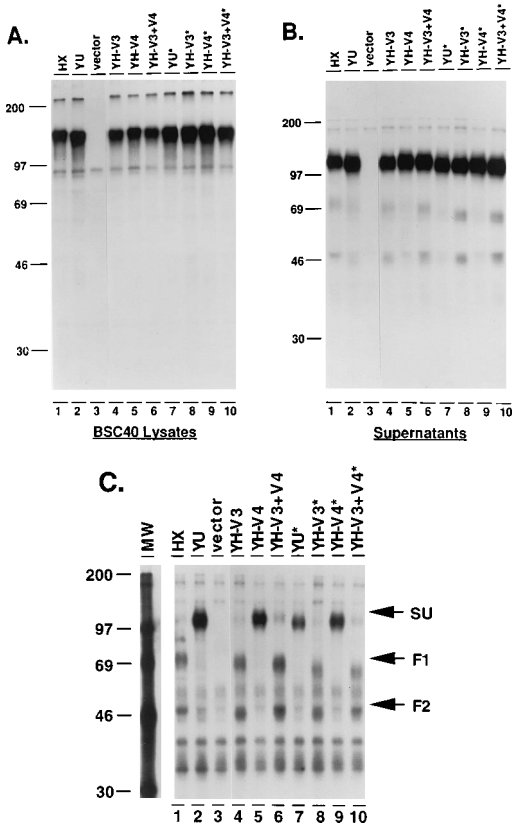


FIG. 3. Proteolytic cleavage analysis of vaccinia virus-expressed Env proteins. BSC-40 cells were transfected/infected with various Env-expressing constructs and labelled with ^{35}S-*trans* label overnight. Cell lysates (A) and cell-free supernatants (B) were immunoprecipitated with HIV-1 patient antiserum, showing similar expression in all cases. (C) gp120 proteins (SU) in supernatants were proteolytically cleaved with 100 ng of tryptase for 16 h and then immunoprecipitated with HIV-1 patient antiserum. Only constructs expressing T-cell line-tropic V3 loops were cleaved to yield 70-kDa (F1) and 50-kDa (F2) cleavage products (lanes 1, 4, 6, 8, and 10). Proteins were run on sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (8% polyacrylamide), and molecular mass markers from 30 to 200 kDa are shown.

inant Env form expressed in cell lysates, only the mature gp120 Env form was seen in cell-free supernatants. All of the recombinant Env proteins had similar electrophoretic mobility, suggesting that the overall glycosylation patterns, although not necessarily identical, were similar for all constructs by using this expression system.

To determine whether the V1 alteration or the presence or absence of a T-cell line-tropic V3 loop could affect the ability of the Env proteins to bind sCD4, OKT4/sCD4 beads were generated and used to bind supernatants containing labelled Env proteins. No significant differences in sCD4 binding were noted among the Env proteins from the parental T-cell line-tropic HX strain and the macrophage-tropic YU strain or the other Env chimeras (not shown). Moreover, no significant differences in the ability of soluble gp120 protein to compete for sCD4 binding with HX or YU envelope proteins were noted (not shown).

Proteolytic cleavage analysis of chimeric Env proteins. Analysis of Fig. 3B reveals that those constructs containing a T-cell line-tropic V3 loop (lanes 1, 4, 6, 8, and 10) also displayed enhanced expression of two protein species having molecular masses of approximately 70 and 50 kDa that were immunoprecipitated with HIV-1 patient antiserum and that were presumably generated by endogenous cleavage of gp120. Previous studies (9, 11, 15) have shown that Env proteins from T-cell line-tropic isolates may adopt a different conformation from macrophage-tropic Env proteins that renders them more susceptible to proteolytic cleavage within the V3 loop. To determine if the apparent endogenous cleavage of those constructs containing T-cell line-tropic V3 loops could be enhanced, supernatants expressing Env proteins were incubated with 100 ng of human tryptase and proteolytically digested for 16 h. Even after such an extended incubation, the constructs lacking a T-cell line-tropic V3 loop remained resistant to cleavage (Fig. 3C, lanes 2, 5, 7, and 9), whereas the constructs containing a T-cell line-tropic V3 loop were almost completely cleaved (Fig. 3C, lanes 1, 4, 6, 8, and 10). Cleavage studies done with various amounts of tryptase for a 2-h incubation period revealed that the Env proteins from all constructs containing T-cell line-tropic V3 loops were similarly susceptible to proteolytic cleavage (not shown). These studies suggest that the presence of a T-cell line-tropic V3 loop is sufficient for proteolytic cleavage of recombinant Env proteins by human tryptase and that the size of the observed cleavage products is consistent with such a cleavage event occurring within the V3 loop. However, the presence or absence of the V1 residue that allows infection in Jurkat cells did not alter V3 loop proteolytic susceptibility.

HIV-1 gp120/CD4-mediated cell fusion monitored by β-galactosidase cell fusion assay. The ability of the chimeric envelope proteins to fuse with a target T-cell line (HeLa T4$^+$) was monitored by using a β-galactosidase reporter gene activation assay (35) (Table 2). In this assay, the degree of cell fusion was monitored by the level of reporter gene activation (β-galactosidase) selectively produced in fused cells. BSC-40 cells on 60-mm-diameter tissue culture plates were transfected with the Env expression DNAs and then infected with the recombinant vaccinia virus vTF7-3 (14, 32), which expresses the T7 polymerase. HeLa T4$^+$ cells were transfected with a plasmid containing the *lacZ* gene linked to the T7 promoter and then infected with the recombinant vaccinia virus vCB-3 (4) in order to increase the level of CD4 surface expression.

After the infection of the BSC-40 cells, the cells were trypsinized and plated in 96-well flat-bottom microtiter plates at a density of 1.5×10^5 cells per 100 μl. After the infection of the HeLa T4$^+$ cells, the cells were washed twice with phosphate-buffered saline (PBS) and refed. Both populations of

TABLE 2. β-Galactosidase activity of cell lysates after Env-mediated cell fusion of BSC-40 and HeLa T4$^+$ cells

Construct	Replication in:		β-Galactosidase activity (% HX activity)[a]
	PBLs	Jurkat cells	
HX	+	+	100
YU	+	−	<1.0
YH-V3	−	−	5.9 ± 0.5
YH-V4	+	−	<1.0
YH-V3+V4	+/−[b]	+/−	4.6 ± 0.2
YU*	+	−	<1.0
YH-V3*	+	+	18.4 ± 0.7
YH-V4*	+	−	<1.0
YH-V3+V4*	+	+	27.2 ± 0.8

[a] Results are representative of three separate experiments. Each experiment was done at least in duplicate. Numbers represent mean values (± standard error) after subtraction of background β-galactosidase activity seen with vector DNA alone.

[b] +/−, delayed kinetics phenotype.

cells were incubated overnight to allow for accumulation of the recombinant proteins. After a 16-h incubation at 37°C, the cells were cocultivated and fusion was allowed to proceed for 3 to 3.5 h, after which the cells were lysed by addition of 0.5% (vol/vol) Nonidet P-40. β-Galactosidase assays were performed in 96-well plates by mixing 50 μl of each lysate with 50 μl of 2× substrate solution containing 16 mM chlorophenol red–β-D-galactopyranoside (Boehringer Mannheim), and A_{563} was monitored with a microtiter plate absorbance reader (Bio-Tek Instruments, Winooski, Vt.).

Neither the parental YU construct nor the YH-V4 construct displayed β-galactosidase activity above levels seen with vector DNA alone (Table 2). Addition of the V1 change did not alter the fusogenicity of these constructs (YU* and YH-V4*). The YH-V3 construct displayed only 6% wild-type activity, but the addition of the V1 change (YH-V3*) increased β-galactosidase activity threefold to 18% of the level seen with HX. The YH-V3+V4 construct also displayed a low level of activity (5% of that of the wild type), but this could be enhanced fivefold (27% of that of the wild type) by the introduction of the V1 change, as shown with the YH-V3+V4* construct. This suggests that the T-cell line-tropic V4 loop may interact with the V1 and V3 loops to increase the efficiency of the fusion process. Even though the YH-V3* and YH-V3+V4* constructs were capable of efficient replication in the Jurkat T-cell line (Fig. 2), the reduced ability to fuse with the HeLa $T4^+$ cell line compared with that of the HX construct may reflect intrinsic differences in the fusion capabilities of the gp41 proteins of these constructs. The gp41 domain for the HX construct is derived from the HXB2 strain, while both the YH-V3* and YH-V3+V4* constructs contain a gp41 domain derived from the YU-2 strain. Nevertheless, the presence of both the altered V1 loop and T-cell line-tropic V3 loop resulted in significantly increased β-galactosidase activity compared with constructs containing either of these domains alone (threefold).

N-linked glycosylation site mutation and T-cell line tropism. Analysis of a revertant virus emerging from extended culture of Jurkat cells enabled the identification of an amino acid change in the V1 loop of Env that results in loss of a highly conserved N-linked glycosylation site. The combination of this change with a T-cell line-tropic V3 loop led to a chimeric virus able to infect both Jurkat cells and PBLs, suggesting that the V1 and V3 loops can functionally interact to infect certain cell types. Expression of chimeric envelope proteins with the vaccinia virus/T7 hybrid system revealed that while chimeras expressing either the V1 loop change or the T-cell line-tropic V3 domain retained the ability to bind to sCD4 comparable to wild-type levels, the presence of both domains resulted in a significantly increased efficiency (threefold) of Env-mediated fusion. It is recognized, however, that the sCD4-binding studies were only semiquantitative and did not utilize oligomeric forms of gp120. These results suggest that subsequent to the initial gp120-CD4 binding event, a functional interaction can occur between the altered V1 loop and T-cell line-tropic V3 loop that results in infection of Jurkat cells and PBLs.

Several studies have shown that mutations affecting putative N-linked glycosylation sites of the gp120 envelope protein may lead to defects in CD4 binding (10, 30), Env protein transport to the cell surface (16), or infectivity (22, 24, 48). These defects depend on the strain of HIV used as well as the specific amino acid substitution and cannot in every case be solely attributed to the loss of glycosylation at a particular position. Only a subset of the putative N-linked glycosylation sites in gp120, however, is important for these activities (2, 10, 24, 30, 49).

Most, if not all, of the potential glycosylation sites of gp120 are utilized for oligosaccharide attachment (25). Mutagenesis

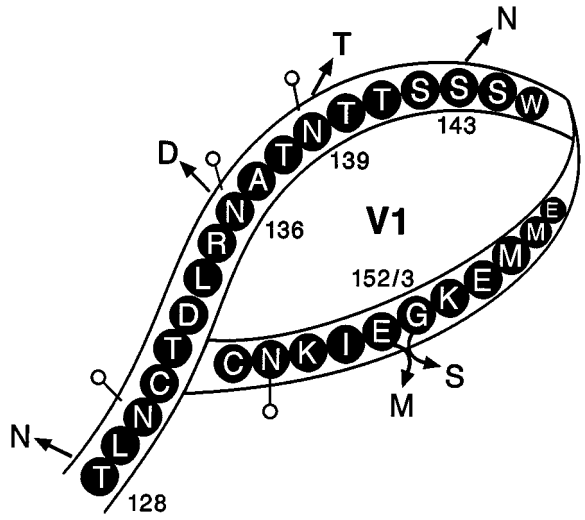


FIG. 4. Functionally important V1 loop residues. The YU-2 V1 loop is shown, and the mutation at residue 136 identified in this study is indicated. Previous studies have characterized mutations at residues 128 (48), 139 (1), 143 (3), and 152 to 153 (152/3) (44).

of individual sites in the Env protein from the T-cell line-tropic strain HXB2, however, revealed that only five consensus N-linked glycosylation sites in the N-terminal half of gp120 are important in viral infection of SupT1 cells (24). This is a surprising result given the high degree of conservation of these N-linked glycosylation sites. The authors of that study speculated that most of these sites have been conserved by HIV-1 for the purpose of masking neutralizing epitopes in order to evade the host immune response. Nevertheless, one of the five sites found to be important for infectivity in that study (amino acid 141) lies within the V1 loop of HXB2, although it is missing from the YU-2 V1 loop (Fig. 4).

The present study, unlike most previous studies, describes a mutation leading to the predicted loss of glycosylation at a residue in the V1 loop of Env which results in the gain of viral tropism for the Jurkat T-cell line and PBLs (Fig. 4). Similarly, Koito et al. (22) showed that individual mutation of two potential N-linked glycosylation sites in the V2 loop of the T-cell line-tropic strain SF2 conferred limited macrophage tropism and significantly reduced the ability of SF2 to infect HUT78 T cells. It is likely that loss of glycosylation at a particular site can expose domains of the protein previously inaccessible for interaction with other protein domains, leading to changes in the overall conformation of the Env protein. While this study has suggested an interaction between the altered V1 loop lacking an N-linked oligosaccharide at amino acid 136 with a T-cell line-tropic V3 loop, it is unclear whether a functional interaction between the V1 and V3 loops occurs in other strains of HIV.

The N-linked glycosylation site at amino acid 136 is well conserved among both T-cell line-tropic and macrophage-tropic strains of HIV-1 (33), which suggests that there has been selective pressure to maintain that site. It is unclear whether reversion from an Asn to an Asp residue is required for the observed V1-V3 interaction or whether any mutation resulting in loss of glycosylation at position 136 would suffice. Mutation of any single base in the Asn codon can result in seven different amino acids (Asp, His, Ile, Lys, Ser, Thr, and Tyr). The fact that 9 of 10 clones contained a specific Asp substitution argues for selection of this specific alteration. Alternatively, if only

loss of glycosylation at this site is critical for virus propagation in T-cell lines, the Asn-to-Asp change may represent the first alteration that occurred in which elimination of the N-linked glycosylation site resulted in replication-competent virus. Further mutational analysis at this position should distinguish between these two possibilities.

It is interesting that the same A-to-G mutation responsible for gain of viral infectivity in our revertant virus is 1 of only 24 nucleotide differences between the infectious YU-2 clone (A at position 6629) and the noninfectious YU-10 clone (G at position 6629) previously described (26, 27). This change does not appear to be the cause of the defect in YU-10, since this virus also contains a 1-bp deletion in the *pol* gene that is more than likely to be responsible for the noninfectious phenotype. Nevertheless, this finding may indicate that there is selective pressure for variation at this site.

A study of maternal-infant strains of HIV-1 by Lamers et al. (23) has found that genetic variation in the 5′ portion of the *env* gene in vivo is not random but can be localized almost exclusively to the V1 and V2 loops. In addition, Overbaugh et al. (38, 39) have shown that variation in simian immunodeficiency virus envelope sequences during progression to simian AIDS is primarily localized to the V1 and V4 loops. Interestingly, in this case, the variation in the V1 loop was associated with an increase of potential O-linked and N-linked glycosylation sites. Thus, it appears that immune selection can drive the variation observed in the V1 loop of HIV and simian immunodeficiency virus in vivo.

Role of the V1 loop in virus entry. Previous studies have shown that changes in the V1 region of Env can affect syncytium formation (44) as well as viral tropism (3, 44, 48). Boyd et al. (3) showed that a single amino acid substitution in the V1 loop seven residues C terminal to the V1 change described in the present study allowed the altered virus to infect the SupT1 T-cell line (Fig. 4). In the study by Sullivan et al. (44), a double amino acid substitution at the C-terminal end of the V1 loop resulted in decreased syncytium formation and deficient entry into Jurkat and SupT1 T-cell lines (Fig. 4). Willey et al. (48) demonstrated that long-term culture of a noninfectious HIV mutant containing a substitution in the C2 domain led to generation of a spontaneous revertant virus containing an additional amino acid substitution preceding the V1 loop (Fig. 4). None of the V1 loop alterations in these three studies, however, involved changes in the N-linked glycosylation pattern of the envelope protein. In addition, Bolmstedt et al. (1) described a mutation at the N-linked glycosylation site at residue 139 which did not affect virus replication but altered its sensitivity to neutralization by a monoclonal antibody (Fig. 4).

While the V1 loop does not appear to function alone as a major tropism-determining element, it is evident that alterations in the V1 loop can affect the cellular tropism of HIV strains. This most likely occurs as a consequence of changes in the overall conformation and structure of Env induced by interactions of the V1 loop with other Env determinants. Several studies have suggested the importance of interactions between the V1/V2 domain and the V3 loop (21, 43, 47). In these studies, the V1/V2 region appears to function as an efficiency domain which is required in addition to the V3 domain in order to attain full macrophage tropism. Furthermore, structural mapping of the HIV-1 surface envelope with an extensive panel of monoclonal antibodies directed against distinct determinants has revealed a structure in which only the V2, V3, C4, and perhaps, V1 domains are at least partially exposed in oligomeric gp120-gp41 complexes (28). Most of the conserved domains lie within the unexposed interior of the molecule. It is likely that interactions between the exposed regions are important for determining the cellular tropism of HIV-1.

Our previous work (6) identified two regions of Env which were necessary for infection of the HUT78 and Jurkat T-cell lines: the V3 loop and a small region of the C4 domain. By analysis of a revertant virus, the current study identifies a different portion of Env (V1 loop) that may interact with the V3 loop to mediate infectivity for Jurkat cells and PBLs. These findings suggest that there are multiple Env conformations which allow entry into particular cell types. Consistent with this idea, there are data suggesting that the three regions described in our studies (V1, V3, and C4 domains) are exposed on the exterior of the gp120 molecule (28), allowing perhaps for alternative interactions between these domains.

The ability of a strain of HIV to infect a particular cell type depends not only on the proper Env conformation but also on the presence of cell-specific secondary receptors able to recognize that particular Env conformation. Infection of T-cell lines probably requires a different Env conformation than that adopted by strains able to infect macrophages. Since different domains of Env are able to undergo complex interactions while the proper function of the protein is still maintained, the implication is that there may be more than one correct Env conformation that allows infection of a particular cell type. Alternatively, V1-V3 or V3-C4 interactions may provide different mechanisms of achieving the same V3 conformation required for infection of T cells. This adds a layer of complexity to the problem of blocking HIV infection at the cell surface and has obvious ramifications for vaccine strategies targeted against epitopes of the gp120 molecule.

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