

Human Immunodeficiency Virus Type 1 Tropism for T-Lymphoid Cell Lines: Role of the V3 Loop and C4 Envelope Determinants

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A functional analysis of chimeric proviral clones generated from the T-cell line-tropic isolate HXB2 and the non-T-cell line-tropic isolate YU-2 identified the minimal determinant for infection of T-lymphoid cell lines to include two interacting domains: the V3 loop and a small region of the C4 domain.

The entry of human immunodeficiency virus type 1 (HIV-1) into target cells is mediated by envelope glycoproteins on the surface of the viral particle. Although most strains of HIV-1 are able to infect primary lymphocytes, most molecularly cloned viruses are capable of infecting either primary macrophages or T-lymphoid cell lines, but not both cell types (64, 67). T-cell line-tropic strains are able to induce syncytia in primary lymphocytes and are designated syncytium-inducing strains, whereas most macrophage-tropic isolates display the non-syncytium-inducing phenotype. Additionally, isolates able to infect T-cell lines replicate more rapidly and to higher levels in primary lymphocytes (“rapid, high” phenotype) than macrophage-tropic isolates, which replicate more slowly and to lower levels (“slow, low” phenotype) (65).

The emergence of T-cell line-tropic syncytium-inducing isolates in infected patients correlates with the progression of HIV-1 infection as characterized by declining CD4⁺ lymphocyte counts and the appearance of clinical symptoms of AIDS (63, 72, 73), whereas macrophage-tropic isolates that display the non-syncytium-inducing phenotype are generally found within the first few months after infection and persist throughout the course of infection (4, 22, 35, 43, 58, 63). It appears that the uncompromised immune system suppresses high-replicating SI variants present in the inoculum, but slow-replicating non-syncytium-inducing variants avoid eliciting an effective immune response by their ability to infect macrophages. However, it is unclear whether the emergence of T-cell line-tropic isolates is a result of host immune system deterioration or whether these isolates are the causative agent of immune dysfunction.

Many studies have examined the viral determinants necessary for infection of primary macrophages (13, 30, 54, 66, 67, 78–80). By analysis of full-length chimeric proviral clones between strains of HIV-1 either capable or incapable of infecting macrophages, a region of the Env protein containing the third hypervariable region (V3) has been identified as a minimal macrophage-tropic determinant (30), although additional viral sequences within the Env protein (67, 80) or accessory proteins Vpr and Vpu (79) are required for maximum infection. The V3 loop is a well-characterized domain of 34 to 37 amino acids that is formed by a disulfide bond between cysteine residues

296 and 331 (38). This region of the Env protein serves as a primary target for neutralizing antibodies, functions as a fusion domain, and regulates the sensitivity of HIV-1 to soluble CD4 (sCD4) (17, 19, 25, 30–32, 52, 59, 77, 83), although it is distinct from the domain of the envelope protein important for the initial interaction with the cellular receptor CD4 (55).

The V3 loop plays an important role in regulating HIV-1 infection of other cell types in addition to macrophages, such as T-lymphoid cell lines. However, the V3 loop by itself is insufficient for conferring infection of T-cell lines. Nevertheless, substitution of a T-cell line-tropic V3 loop with a macrophage-tropic V3 loop results in loss of infection of T-cell lines (9, 30, 67). This implies that interaction of the V3 loop with other portions of the Env protein is required to establish the proper conformation necessary for infection. Previous work has suggested a functional interaction between the variable region (V3) and the fourth conserved domain (C4) lying between the fourth and fifth hypervariable regions (2, 49, 50, 86). The C4 domain constitutes part of the CD4-binding site and has also been implicated as important for association with the gp41 transmembrane glycoprotein (16, 27, 33, 34, 37, 55). In the present study, we have identified two amino acid residues lying within the C4 domain that are required in combination with the V3 loop to establish infection in T-cell lines.

Identification of a minimal T-lymphoid cell line infection determinant. In order to examine the molecular determinants necessary for T-lymphoid cell line tropism, chimeric proviruses were constructed between two HIV-1 strains differing in their ability to infect T-cell lines. Both strains are capable of productively infecting peripheral blood lymphocytes (PBLs) (Fig. 1A). The following procedure was used.

Chimeric HIV-1 clones were constructed from the T-cell line-tropic clone NLHX and the macrophage-tropic clone YU-2 (39). NLHX was derived from the NL4-3 clone by subcloning of the 2.7-kb *SalI-BamHI* fragment (nucleotides 5785 to 8474) from HXB2 into the full-length NL4-3 clone, which corrects a *vpr* defect and results in an intact *vpr* open reading frame (1). This construct is designated as HX. A 3.2-kb *EcoRI-XhoI* fragment (nucleotides 5742 to 8896) from the macrophage-tropic YU-2 proviral clone was inserted into NL4-3 and is designated as YU. Construct YH-GG was generated by exchanging the 0.6-kb *BglII-BglII* fragment (nucleotides 7040 to 7620) from HXB2 into construct YU, and constructs YH-GP and YH-PG were generated by reciprocal exchanges of 1.1-kb *PpuMI-PpuMI* fragments (nucleotides 7323 to 8396) between constructs YU

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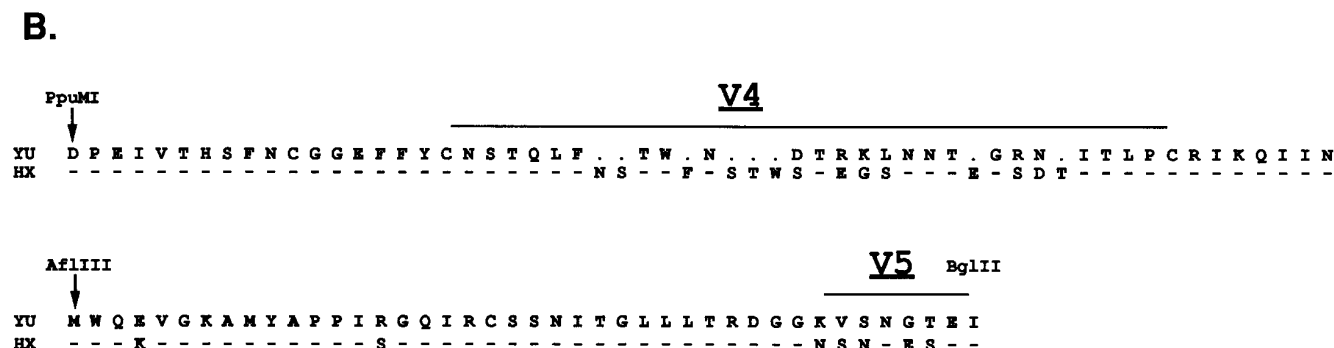
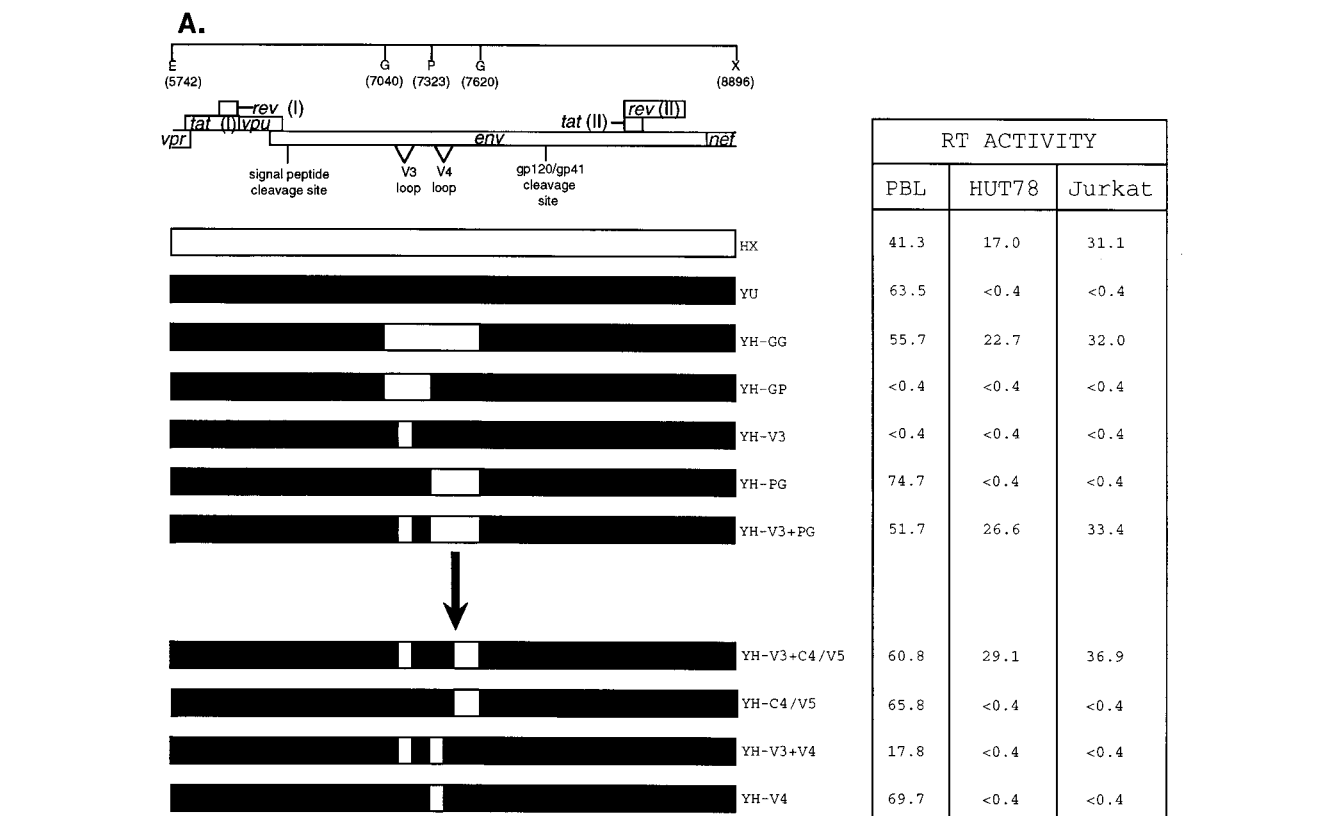


FIG. 1. Identification of a minimal T-lymphoid cell line infection determinant. (A) Chimeric proviral clones were constructed between the T-cell line-tropic virus HXB2 (white) and the macrophage-tropic virus YU-2 (black), which is incapable of conferring infection in a wide range of T-lymphoid cell lines. The top of the figure shows a schematic diagram of the HIV-1 genome between the *EcoRI* (E) and *XhoI* (X) restriction enzyme sites (showing the relative positions of the *vpr*, *tat*, *vpu*, *env*, *rev*, and *nef* genes). Additional restriction sites used in clone construction (G, *BglII*; P, *PpuMI*), with nucleotide positions listed in parentheses, are also indicated. 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 18 to 22 days postinfection for PBLs and two T-cell lines, HUT78 and Jurkat, is shown to the right of the figure and is expressed here and in all other figures as 10⁵ cpm/ml. Similar results were obtained in at least three independent experiments. (B) Sequence alignment of the 100-amino-acid *PpuMI*-*BglII* (PG) region, including V4, C4, and the N terminus of V5. The YU-2 sequence is shown on the top line, and any changes present within the HXB2 sequence are shown underneath. (C and D) Replication kinetics for several constructs in the HUT78 (C) and Jurkat (D) T-cell lines. RT activity is plotted versus days postinfection. Similar replication kinetic profiles were obtained from at least three experiments. The symbol key shown in panel C also applies to panel D.

and YH-GG. The YH-V3 construct was generated by site-directed mutagenesis (36). Construct YH-V3+PG was then generated by insertion of the *PpuMI*-*PpuMI* fragment from YH-PG into YH-V3. Constructs YH-V3+V4 and YH-C4/V5 were made by reciprocal exchanges of 1.0-kb *AflIII*-*AflIII* fragments (nucleotides 6504 to 7497) between YH-V3+PG and a YU construct containing an *AflIII* site at nucleotide 7497. Insertion of the 1.0-kb *PpuMI*-*PpuMI* fragment (nucleotides 7323 to 8398) from YH-V3+V4 into YU and from YH-C4/V5 into YH-V3 generated constructs YH-V4 and YH-V3+C4/V5, respectively.

PBLs were purified from normal human leukocytes by centrifugation onto Ficoll. After 3 days of stimulation with phytohemagglutinin (15 μg/ml; Sigma), PBLs were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, recombinant interleukin 2 (50 U/ml; Cetus), penicillin (100 U/ml), and streptomycin (100 μg/ml). Jurkat and HUT78 T-cell lines were maintained in RPMI medium supplemented the same way but lacking recombinant interleukin 2.

COS-7 cells at 50 to 60% confluence on 60-mm-diameter tissue culture plates were maintained in Dulbecco's modified

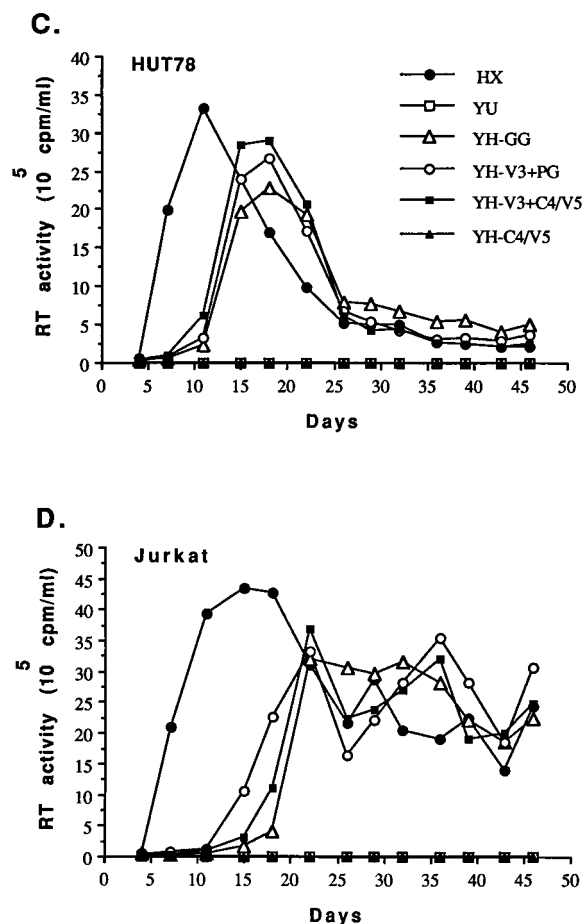


FIG. 1—Continued.

Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, 1 mM pyruvate, penicillin (100 U/ml), and streptomycin (100 μ g/ml). Cells were transfected by DEAE-dextran (Sigma Chemical Co.) with 5 μ g of double cesium chloride-purified proviral DNA and 1.25 μ g of the *tat* expression vector pCV1 (3). Five hours after the addition of DNA, the cells were shocked for 2 min with 10% dimethyl sulfoxide and then washed twice with phosphate-buffered saline (PBS) before refeeding with 5 ml of fresh medium. Culture supernatants were harvested 72 h posttransfection and filtered through 0.2- μ m-pore-size filters (Millipore). Viral stocks were standardized either by RT activity (57) or by a p24 antigen assay (Dupont) and used to infect 5×10^5 PBLs, HUT78 cells, and Jurkat cells plated in 1 ml of medium in 24-well plates. Viral replication was monitored by determination of supernatant RT activity sampled two to three times per week. Mock-infected cultures were exposed to filtered culture supernatants from COS-7 cells transfected with pCV1 and were maintained throughout the course of the experiment.

Our previous work (12) demonstrated that insertion of a 580-bp *Bgl*II-*Bgl*II *env* determinant, encoding the V3 and V4 loops and C4 domain, from the T-cell line-tropic strain HXB2 into the macrophage-tropic YU-2 backbone (YH-GG) was sufficient to confer infectivity for several T-cell lines, including HUT78 and Jurkat cells (Fig. 1A).

The GG region was further defined by dividing it into two domains: an N-terminal 93-amino-acid *Bgl*II-*Ppu*MI (GP) re-

gion which includes the V3 loop and a C-terminal 100-amino-acid *Ppu*MI-*Bgl*II (PG) region which includes the V4 loop and the C4 domain. Substitution of T-cell line-tropic sequences for the entire N-terminal GP region (YH-GP) or just the V3 loop (YH-V3) resulted in nonfunctional isolates incapable of replicating in any cell type, including PBLs (Fig. 1A). Upon substitution of the C-terminal PG region (YH-PG), no changes in replication properties from those of the parental YU-2 strain were observed. However, a chimera containing the T-cell line-tropic V3 loop and the C-terminal PG region (YH-V3+PG) replicated to levels similar to those of the parental HX isolate (Fig. 1A), although the kinetics of replication were slightly delayed and more similar to those seen with the YH-GG construct (Fig. 1C and D).

Subdivision of the V4-C4-V5 region. To assess the importance of specific amino acids within the PG region in conferring T-cell line tropism, the amino acid sequences of the two parental strains were compared. Most of the differences within the PG region were limited to the V4 loop, although there was a cluster of differences at the N terminus of the V5 loop as well as two single-amino-acid changes in the C4 domain (Fig. 1B). An *Afl*III site was introduced by site-directed mutagenesis at nucleotide 7497, which allowed the 100-amino-acid PG region to be divided into two domains containing either the V4 loop or the C4/V5 region. The presence of a T-cell line-tropic V3 loop and C4/V5 region (YH-V3+C4/V5) resulted in a chimera having replication kinetics almost identical to those of the chimeras containing larger T-cell line-tropic domains (YH-GG and YH-V3+PG) in both HUT78 (Fig. 1C) and Jurkat (Fig. 1D) T-cell lines, whereas expression of the T-cell line-tropic C4/V5 domain in the absence of a T-cell line-tropic V3 loop (YH-C4/V5) resulted in a chimera unable to replicate in either T-cell line (Fig. 1A). This suggests that the C4/V5 domain contains critical residues that function as one of two important elements necessary for the infectivity of T-cell lines. The combination of T-cell line-tropic V3 and V4 loops (YH-V3+V4) resulted in a chimera also able to replicate in both T-cell lines, but peak reverse transcriptase (RT) levels were significantly delayed. Results from the latter construct are described in the accompanying paper (11).

Site-directed mutagenesis of the C4/V5 region. The C4/V5 region was further analyzed by introduction of site-directed mutations at the positions which differed between the HXB2 and YU-2 isolates. YU-2-to-HXB2 changes were introduced at either of the two positions which differed in the C4 domain (positions 1 and 2) or at the N terminus of the V5 loop (position 3). The three possible double mutations involving these positions were also introduced. These mutations were introduced in the context of the parental YU-2 backbone (Fig. 2A, top), or into the YH-V3 construct, which contains a T-cell line-tropic V3 loop (Fig. 2A, bottom). In the absence or presence of a T-cell line-tropic V3 loop, three of the six mutations (mut1, mut1,3, and mut2,3) displayed a nonfunctional phenotype.

None of the remaining mutations (mut2, mut3, and mut1,2) was sufficient for infection of HUT78 or Jurkat T cells in the absence of a T-cell line-tropic V3 loop. In the presence of a T-cell line-tropic V3 loop, however, the double mutant containing E-429 \rightarrow K and R-440 \rightarrow S changes in the C4 domain (YH-V3+mut1,2) replicated almost identically to the YH-V3+C4/V5 construct in both the HUT78 (Fig. 2B) and Jurkat (Fig. 2C) T-cell lines, whereas changes at the N terminus of the V5 loop (YH-V3+mut3) did not contribute to infection of either cell line. The presence of just the R-440 \rightarrow S change in the C4 domain, in combination with a T-cell line-tropic V3 loop (YH-V3+mut2), also resulted in a high level of replication in both T-cell lines, although

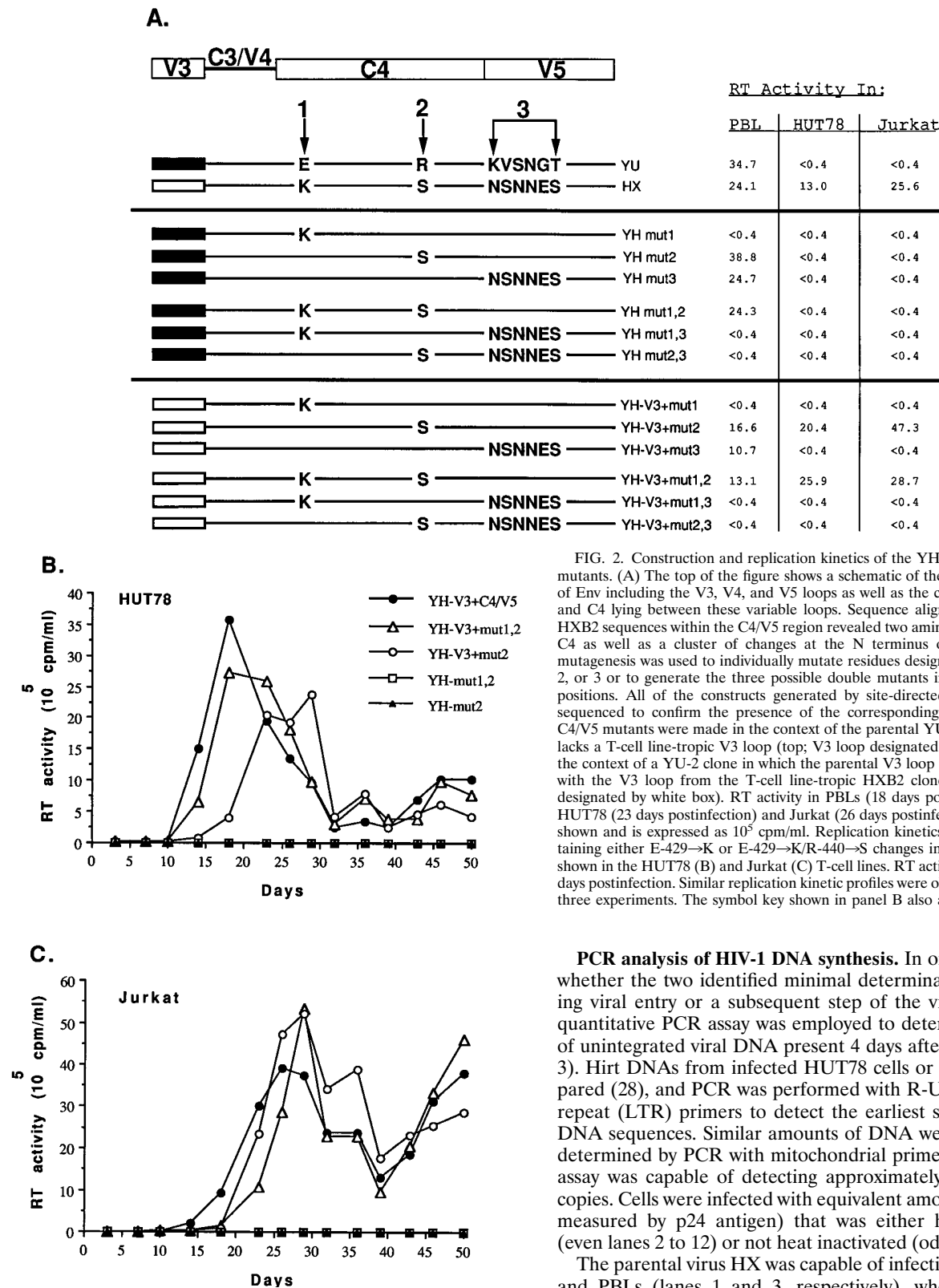


FIG. 2. Construction and replication kinetics of the YH-C4/V5 site-directed mutants. (A) The top of the figure shows a schematic of the C-terminal portion of Env including the V3, V4, and V5 loops as well as the constant domains C3 and C4 lying between these variable loops. Sequence alignment of YU-2 and HXB2 sequences within the C4/V5 region revealed two amino acid differences in C4 as well as a cluster of changes at the N terminus of V5. Site-directed mutagenesis was used to individually mutate residues designated by positions 1, 2, or 3 or to generate the three possible double mutants involving these three positions. All of the constructs generated by site-directed mutagenesis were sequenced to confirm the presence of the corresponding mutations. The six C4/V5 mutants were made in the context of the parental YU-2 backbone, which lacks a T-cell line-tropic V3 loop (top; V3 loop designated by black box), or in the context of a YU-2 clone in which the parental V3 loop had been exchanged with the V3 loop from the T-cell line-tropic HXB2 clone (bottom; V3 loop designated by white box). RT activity in PBLs (18 days postinfection) and the HUT78 (23 days postinfection) and Jurkat (26 days postinfection) T-cell lines is shown and is expressed as 10^5 cpm/ml. Replication kinetics for constructs containing either E-429→K or E-429→K/R-440→S changes in the C4 domain 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. The symbol key shown in panel B also applies to panel C.

the absence of the E-429→K change led to slightly delayed replication in the HUT78 cell line (Fig. 2B). These results suggest that a functional interaction between the V3 loop and at least one amino acid (S-440) in the C4 domain is required for establishing infection of the HUT78 and Jurkat T-cell lines.

PCR analysis of HIV-1 DNA synthesis. In order to examine whether the two identified minimal determinants were affecting viral entry or a subsequent step of the viral life cycle, a quantitative PCR assay was employed to determine the levels of unintegrated viral DNA present 4 days after infection (Fig. 3). Hirt DNAs from infected HUT78 cells or PBLs were prepared (28), and PCR was performed with R-U5 long terminal repeat (LTR) primers to detect the earliest synthesized viral DNA sequences. Similar amounts of DNA were amplified, as determined by PCR with mitochondrial primers (85), and the assay was capable of detecting approximately three provirus copies. Cells were infected with equivalent amounts of virus (as measured by p24 antigen) that was either heat inactivated (even lanes 2 to 12) or not heat inactivated (odd lanes 1 to 11).

The parental virus HX was capable of infecting both HUT78 and PBLs (lanes 1 and 3, respectively), whereas the heat-inactivated HX control gave rise to no significant viral DNA synthesis (lanes 2 and 4). Similarly, infection with virus YH-V3+mut1,2 containing the minimal identified T-cell line-tropic determinant also gave rise to viral DNA synthesis in both cell types (lanes 5 and 7), although the level of viral DNA

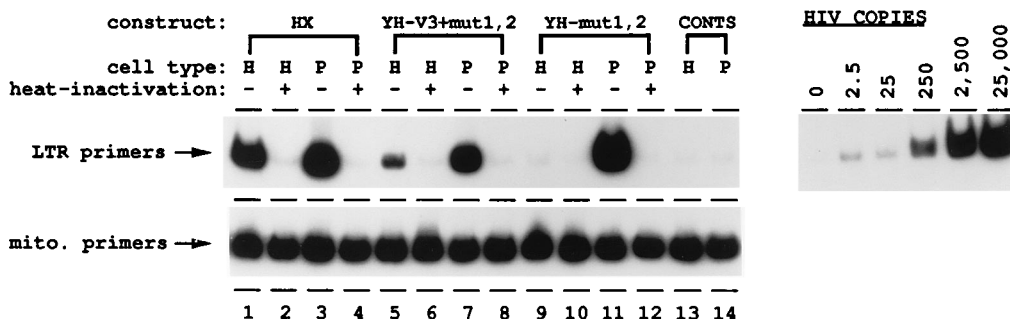


FIG. 3. PCR analysis of HIV-1 DNA synthesis. HUT78 cells (H) or PBLs (P) were infected with equivalent amounts (as measured by p24 antigen) of HX (lanes 1 to 4), YH-V3+mut1,2 (lanes 5 to 8), YH-mut1,2 (lanes 9 to 12), or no virus (lanes 13 and 14). Each virus sample was used in duplicate, with (even lanes 2 to 12) or without (odd lanes 1 to 11) heat inactivation. Virus stocks for this experiment were generated from infected PBLs. Virus was heat inactivated by incubation at 65°C for 1 h. Four days after infection, unintegrated DNA was prepared (28), and PCR was performed with end-labelled R-U5 LTR primers (nucleotides 1 to 145) or mitochondrial (mito.) primers (85). PCR was performed for 1 min at 91°C and 2 min at 65°C (LTR primers) or 2 min at 94°C, 2 min at 56°C, and 2 min at 72°C (mitochondrial primers) for 30 cycles. Standards included 0.05, 0.5, 5, 50, or 500 fg (2.5 to 25,000 copies) of HXB2 proviral DNA. PCR products were analyzed with 6% polyacrylamide gel electrophoresis and exposed to X-ray film.

was lower than that seen with the parental HX virus. This correlates with a lowered infectivity of this virus versus the HX virus as measured by 50% tissue culture infective doses (not shown). In contrast, virus YH-mut1,2 which lacks a T-cell line-tropic V3 loop, produced comparable levels of viral DNA in PBLs (lane 11) but did not give rise to detectable levels of viral DNA in HUT78 cells (lane 9). These findings are consistent with those from other groups (10, 13, 22, 44, 54, 62) and suggest that regulation of HIV-1 infection of T-lymphoid cell lines occurs at an early step in the virus replication cycle such as virus binding, uptake, uncoating, or reverse transcription.

Vaccinia virus expression of Env proteins. In order to further elucidate how the minimal T-cell line-tropic determinant might mediate infectivity for T-cell lines, recombinant Env proteins were expressed in BSC-40 cells with the vaccinia virus/T7 hybrid system. Using HIV-1 patient antiserum, immunoprecipitation of ³⁵S-*trans*-labelled cell lysates (not shown) or cell-free supernatants (Fig. 4) revealed that all of the expression constructs produced comparable levels of protein.

The following procedure was used. An *Nco*I site was introduced by site-directed mutagenesis into construct HX at nucleotide 6222 representing the *env* initiation codon. The 2.7-kb *Nco*I-*Xho*I fragment (nucleotides 6222 to 8896) was then inserted into the polylinker region of plasmid pTM3- Δ BglII, which was made from pTM3 (21, 51) by destroying the *Bgl*II site normally present at nucleotide 2035 to facilitate subsequent cloning steps. The 2.6-kb *Kpn*I-*Xho*I fragment (nucleotides 6346 to 8896) from construct YU was then inserted into this plasmid and used as the parental vector for insertion of 0.6-kb *Bgl*II-*Bgl*II fragments (nucleotides 7040 to 7620) from the corresponding full-length HIV-1 clones.

BSC-40 cells at 90% confluence on 60-mm-diameter tissue culture plates were transfected with 5 μ g of double cesium chloride-purified pTM3 vector DNAs with lipofectin (GIBCO BRL) as described by the manufacturer. Five hours after the addition of DNA, infection with the recombinant vaccinia virus vTF7-3 (21, 52), which expresses T₇ polymerase, was performed with an inoculum of 10 PFU per cell at room temperature for 1 h. The cells were then washed once with PBS and either refed with 5 ml of complete medium or labelled with 1 ml of cysteine-free, methionine-free Dulbecco's modified Eagle's medium containing 100 μ Ci of ³⁵S-*trans* label for 15 h BSC-40. Cell-free supernatants were immunoprecipitated with HIV-1 patient antiserum, and proteins were run on sodium dodecyl sulfate-8% polyacrylamide gels.

Proteins were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8% polyacrylamide). A vaccinia virus-based reporter gene activation assay (53) was used to measure gp120/CD4-mediated cell fusion. The degree of cell fusion was monitored by the level of reporter gene activation (β -galactosidase) selectively produced in fused cells (Table 1).

In cell lysates, Env was expressed primarily in the form of the gp160 precursor protein, whereas gp120 was the predominant Env protein detected in cell-free supernatants, suggesting that all of the chimeric proteins were correctly processed to

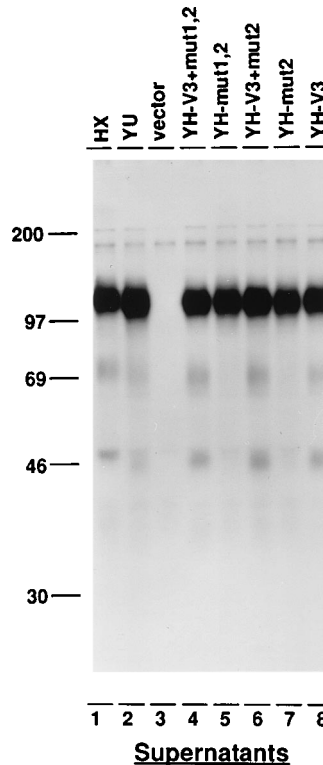


FIG. 4. Analysis of vaccinia virus-expressed Env proteins. Cell-free supernatants were immunoprecipitated with HIV-1 patient antiserum, showing similar expression in all cases. For details of the methods used, see the text. Proteins were run on sodium dodecyl sulfate-8% polyacrylamide gels, and molecular mass markers from 30 to 200 kDa are shown.

TABLE 1. β -Galactosidase activity of cell lysates after Env-mediated cell fusion of BSC-40 and HeLa T4⁺ cells^a

Construct	Replication in:		β -Galactosidase activity (% HX activity) ^b
	PBLs	T-cell lines	
HX	+	+	100
YU	+	–	<1
YH-mut1,2	+	–	<1
YH-V3	–	–	5.9 \pm 0.5
YH-V3+mut1,2	+	+	21.4 \pm 0.3

^a A vaccinia virus-based reporter gene activation assay (53) was used to measure gp120/CD4-mediated cell fusion. The degree of cell fusion was monitored by the level of reporter gene activation (β -galactosidase) selectively produced in fused cells (see text for details). Results are representative of three separate experiments, with each experiment done at least in duplicate.

^b Mean values (\pm standard error) after subtraction of background β -galactosidase activity seen with vector DNA alone.

form the mature surface (SU) gp120 and transmembrane (TM) gp41 proteins. No differences in electrophoretic mobility were seen, suggesting that the glycosylation patterns of all of the recombinant Env proteins were similar, using this expression system.

Figure 4 reveals that the constructs containing a T-cell line-tropic V3 loop (lanes 1, 4, 6, and 8) also displayed enhanced expression of two protein species having molecular masses of approximately 70 and 50 kDa (presumably generated by an endogenous cleavage of gp120). Incubation with trypsin showed that gp120 proteins lacking a T-cell line-tropic V3 loop remained resistant to cleavage, whereas the constructs containing a T-cell line-tropic V3 loop were almost completely cleaved (not shown). The presence or absence of residues in the C4 domain that are required for T-cell line infection did not alter V3 loop proteolytic susceptibility.

The labelled gp120 proteins were also incubated with OKT4/sCD4 beads, in the presence or absence of unlabelled gp120 competitor. No differences in gp120 binding to sCD4 could be detected with V3 and/or C4 mutations (not shown), although the semiquantitative nature of this assay does not rule out subtle differences in binding affinity. It should also be noted that sCD4 binding of only the monomeric form and not the oligomeric form of gp120 was assessed with this assay.

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 with the β -galactosidase reporter gene activation assay (53). gp120/CD4-mediated cell fusion was monitored by the level of reporter gene activation (β -galactosidase) selectively produced in fused cells (Table 1).

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. A second population of cells, a HeLa T4⁺ monolayer (40), was transfected with a plasmid containing the *lacZ* gene linked to the T7 promoter and then infected with the recombinant vaccinia virus vCB-3 (6) 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 μ l. After the infection of the HeLa T4⁺ cells, the cells were washed twice with PBS and refed. Both populations of cells were incubated overnight to allow for accumulation of the recombinant proteins. Sixteen hours later, the HeLa T4⁺ cells were trypsinized and 5×10^4 cells per 50 μ l were added to each well containing BSC-40 cells for a total volume of 150 μ l per well. Fusion was allowed to

proceed for 3 to 3.5 h, after which 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 μ l of each lysate with 50 μ l of 2 \times substrate solution containing 16 mM chlorophenol red- β -D-galactopyranoside (Boehringer Mannheim). A_{563} was monitored with a microtiter plate absorbance reader (Bio-Tek Instruments, Winooski, Vt.).

Neither the parental YU construct nor the YH-mut1,2 construct displayed β -galactosidase activity above the levels seen with vector DNA alone. The YH-V3+mut1,2 construct displayed β -galactosidase activity that was 21% of the level seen with HX, whereas the YH-V3 construct displayed 6% wild-type activity. Even though the YH-V3+mut1,2 construct was capable of efficient replication in the HUT78 and Jurkat T-cell lines (Fig. 2B and C), 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 protein for the HX construct is derived from the HXB2 strain, while the YH-V3+mut1,2 construct contains a gp41 protein derived from the YU-2 strain (Fig. 1A). Nevertheless, the presence of both V3 and C4 T-cell line-tropic domains resulted in significantly increased β -galactosidase activity compared with constructs containing either domain alone (3.5-fold).

Defective viruses. A difficulty in this study has been the inability to express T-cell line-tropic sequences spanning the V3 loop in the context of gp120 sequences from a macrophage-tropic isolate. Since HXB2 is derived from an extensively passaged laboratory isolate, we attempted to overcome this problem by insertion of V3 loop sequences from the T-cell line-tropic strain SF₂ or from patient isolates obtained late in disease into different macrophage backbones (YU-2 and ADA). However, only nonfunctional clones were generated in all cases (not shown). On the basis of sucrose sedimentation analysis, viral particles displaying the proper density were generated after transfection of COS-7 cells with these proviral clones (not shown), but it is unclear whether the envelope protein is expressed on these particles. In contrast, reciprocal constructs generated by insertion of macrophage-tropic V3 domains in the context of T-cell line-tropic backbones are able to replicate in primary lymphocytes and macrophages (13, 29, 54, 67, 78, 80), suggesting that most T-cell line-tropic Env backbones are more tolerant than macrophage-tropic Env backbones for insertion of foreign sequences that disrupt the native conformation of the protein.

V3-C4 domain interactions. In spite of the difficulties in generating functional constructs expressing T-cell line-tropic V3 domains, this study identifies the V3 loop and the C4 domain (residues K-429 and S-440) as two determinants of the envelope protein that functionally interact to mediate infectivity for the HUT78 and Jurkat T-cell lines. The presence of just a T-cell line-tropic V3 loop or C4 domain in the context of a macrophage-tropic backbone resulted in a chimera that was either nonfunctional and unable to replicate in any cell type (YH-V3) or one whose ability to enter T-cell lines was restricted at an early step (YH-mut1,2). Expression of chimeric envelope proteins with the vaccinia virus/T7 hybrid system revealed that while chimeras expressing either domain retained an ability to bind to sCD4 comparable to wild-type levels, the presence of both domains resulted in a 3.5-fold increase in the efficiency of Env-mediated fusion. These results suggest that subsequent to the initial gp120-CD4 binding event, a functional interaction between the T-cell line-tropic V3 loop and C4 domain is required for infection of T-cell lines.

Previous studies have suggested a structural relationship between the V3 loop and the C4 domain. Morrison et al. (50)

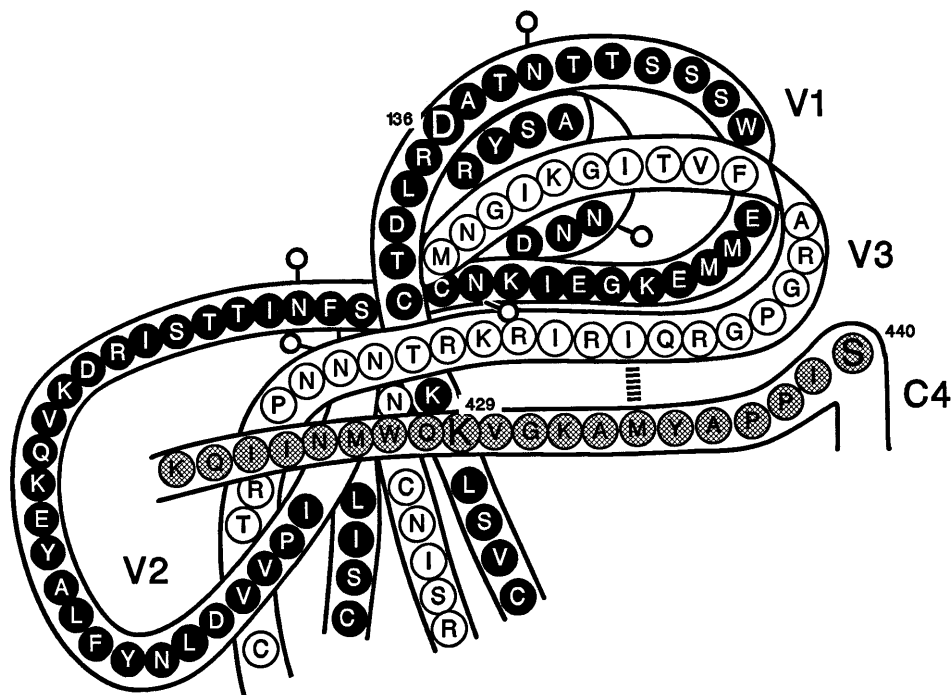


FIG. 5. Proposed relationship between the HIV-1 gp120 V1, V3, and C4 domains. The relative positions of K-429 and S-440 mutations within C4 (shaded residues) are shown with a model of critical domains of gp120, modified from that of Choe and Sodroski (14). Mutation of residue 136 in the V1/V2 domain (black residues) may also modify HIV-1 infection of T cells (11). Potential N-linked glycosylation sites are indicated by the open ball and rod symbols.

have shown that a simian immunodeficiency virus strain (SIV-mac239) strain containing a mutation in the C4 domain that renders it defective for viral replication can be corrected by a compensatory mutation in the V3 loop (Fig. 5). Furthermore, additional studies have reported that changes in the V3 loop can affect binding of antibodies directed against the C4 region and vice versa (7, 41, 48, 49, 56, 60, 74–76, 84, 86).

Our results are consistent with those from these earlier studies and suggest that the V3-C4 interaction is required for viral infection of T-cell lines. The precise step of viral entry affected by these determinants has not yet been defined. Several studies have suggested that infection of T-cell lines may be regulated by the affinity or kinetics of CD4 binding (82, 84). However, data from the current study and other previous studies suggest that infection is regulated subsequent to CD4 binding. Since envelope proteins from T-cell line-tropic isolates can be cleaved within the V3 loop by exogenous proteases, it was proposed that infection by these strains is dependent on proteolytic cleavage of the envelope by a cell-surface protease (15, 18, 24, 70), although proteolytic cleavage has not been proven to be required for HIV infection of any cell type. In this study, the presence of a T-cell line-tropic V3 loop was sufficient to allow proteolytic cleavage of the chimeric envelope proteins, regardless of the presence or absence of additional residues in the C4 domain required for T-cell line infection. The failure of macrophage-tropic envelope proteins to be similarly cleaved suggests that proteolytic cleavage is not required for macrophage infection (18, 24), and another mechanism exists to explain the selective macrophage tropism of certain strains of HIV-1. The V3 loops from T-cell line-tropic and macrophage-tropic envelope proteins may adopt distinct conformations (18, 69) which allow the native gp120 structure to interact with alternative cell-specific cofactor molecules in addition to CD4.

The gp120 C4 region has previously been implicated as an

important domain for association with the gp41 transmembrane glycoprotein (16, 27, 37, 55). It is likely that conformational changes induced in the Env protein subsequent to CD4 binding and resulting in the proposed V3-C4 interaction affect the affinity of the noncovalent gp120-gp41 interaction. As a result, the N terminus of gp41 may become exposed because of complete shedding of gp120 from the glycoprotein complex, as has been suggested by several studies (8, 26, 42, 46, 47), or alternatively, because of further conformational changes in the envelope protein (60, 68). Since recent data have questioned the relevance of gp120 shedding in the process of membrane fusion (5, 20, 23, 45, 61, 71, 74, 81, 84), we favor a model in which conformational changes induced in the envelope protein upon gp120-CD4 binding shift the initial C4-gp41 interaction to one in which the C4 domain interacts with the V3 loop, allowing exposure of the N terminus of gp41 to fuse with the target cell membrane.

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