Journal of Virology

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*J. Virol.* 1997, 71(12):9808.

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Journal of Virology, Dec. 1997, p. 9808–9812 Vol. 71, No. 12
0022-538X/97/$04.00+0
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# Replication and Neutralization of Human Immunodeficiency Virus Type 1 Lacking the V1 and V2 Variable Loops of the gp120 Envelope Glycoprotein

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Received 11 April 1997/Accepted 28 August 1997

**A human immunodeficiency virus type 1 (HIV-1) mutant lacking the V1 and V2 variable loops in the gp120 exterior envelope glycoprotein replicated in Jurkat lymphocytes with only modest delays compared with the wild-type virus. Revertants that replicated with wild-type efficiency rapidly emerged and contained only a few amino acid changes in the envelope glycoproteins compared with the parent virus. Both the parent and revertant viruses exhibited increased sensitivity to neutralization by antibodies directed against the V3 loop or a CD4-induced epitope on gp120 but not by soluble CD4 or an antibody against the CD4 binding site. This result demonstrates the role of the gp120 V1 and V2 loops in protecting HIV-1 from some subsets of neutralizing antibodies.**

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (3, 18). Like that of other retroviruses, the entry of HIV-1 into target cells is mediated by the viral envelope glycoproteins. The gp120 exterior envelope glycoprotein binds to the viral receptors on the target cell, while the gp41 transmembrane envelope glycoprotein serves to anchor the oligomeric envelope glycoprotein complex in the viral membrane (2, 43). HIV-1 binds to the primary viral receptor, CD4 (10, 26, 32, 33), and then the gp120-CD4 complex binds to one of the family of chemokine receptors (1, 8, 12–15, 29, 59, 62). Following receptor binding, the envelope glycoproteins mediate the fusion of viral and target cell membranes, which is critical for successful entry of the viral core into the cell (19, 53).

Most of the surface-exposed elements of the mature envelope glycoprotein complex are contained on the gp120 exterior envelope glycoprotein (36, 45, 46). When the gp120 glycoproteins derived from different HIV-1 isolates are compared, five conserved regions (C1 to C5) and five variable regions (V1 to V5) can be identified (38, 51). Efficient CD4 binding is dependent on discontinuous elements derived from the third (aspartic acid 368 and glutamic acid 370) and fourth (tryptophan 427 and aspartic acid 457) conserved regions (9, 27, 30, 39). Conserved, discontinuous epitopes overlapping the CD4 binding site serve as targets for neutralizing antibodies (21, 24, 41, 52, 55, 56, 58).

Intramolecular disulfide bonds in the gp120 glycoprotein result in the inclusion of the first four variable regions (V1 to V4) in large, looplike structures (31). Antibody mapping studies indicate that of the linear epitopes on the gp120 glycoprotein, those located in the V2 and V3 regions constitute the most exposed elements on the HIV-1 multimeric envelope glycoprotein complex (36, 45). Both V2 and V3 loops can serve as targets for neutralizing antibodies (17, 20, 23, 34, 35, 42, 44, 48, 54). Changes in V2 and V3 loop amino acids can influence the tropism of HIV-1 isolates for primary macrophages and T-lymphocyte lines (4–7, 22, 47, 60, 61), probably by influencing chemokine receptor preference (8, 8a).

Consistent with the proposed exposed conformation of the V1-V2 and V3 variable loops, these regions can be removed from the HIV-1 gp120 glycoprotein without globally disrupting the conformation of the protein (40, 55, 56, 63, 64). Several of the discontinuous conserved gp120 epitopes overlapping the CD4 binding site or exposed upon CD4 binding (the CD4BS and CD4-induced [CD4i] epitopes, respectively) are more accessible to antibodies following variable-loop removal (57, 63, 64). This observation has led to the proposal that one of the functions of the major gp120 variable loops is to protect conserved gp120 epitopes from neutralizing antibodies. Here we examine the replicative ability and sensitivity to neutralization of HIV-1 variants containing deletions in the major variable loops.

Deletions affecting the major gp120 variable loops (64) were introduced into the pSVHXBc2 plasmid, which contains an infectious HIV-1 provirus (16). The ΔV1/2 envelope glycoprotein contains a deletion of gp120 residues 128 to 194. The ΔV3 envelope glycoprotein contains a deletion of gp120 residues 303 to 323. The ΔV1/2/3 envelope glycoprotein contains both of the above deletions. The pSVHXBc2ΔKS plasmid, which contains an HIV-1 provirus with a large deletion in *env*, was used as a negative control. Jurkat lymphocytes were transfected with these provirus-containing plasmids. Viral replication was monitored by measuring reverse transcriptase activity in pelleted culture supernatants, and viral cytopathic effects were measured by counting the number of syncytia and trypan blue-positive single cells in the culture (28). Figure 1 shows that the ΔV1/2 virus replicated with a delay in kinetics compared with the wild-type virus. By contrast, the ΔV3, ΔV1/2/3, and ΔKS proviruses did not give rise to detectably replicating viruses, even after long-term culture of the transfected cells (Fig. 1 and data not shown).

Supernatants of the transfected Jurkat cells were used to infect fresh Jurkat cells. The replication phenotypes of the

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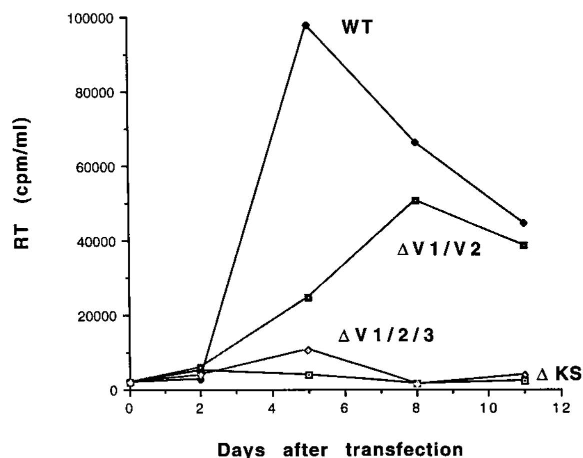


FIG. 1. Replication of HIV-1 with envelope glycoproteins with variable loops deleted. Jurkat lymphocytes were transfected with proviral DNA specifying HIV-1 with the wild-type (WT), ΔV1/2, ΔV1/2/3, or ΔKS envelope glycoprotein. The ΔKS provirus has a large deletion in *env* and served as a negative control. RT, reverse transcriptase.

viruses following infection were similar to those observed after transfection. Compared with the wild-type virus, the ΔV1/2 virus exhibited a mild delay in replication and in the induction of cytopathic effects (Fig. 2A to C). The ΔV1/2/3 and ΔKS variants apparently did not replicate (Fig. 2A to C and data not shown).

The ΔV1/2 virus obtained at 2 weeks after infection of Jurkat cells was used to infect fresh Jurkat cells, and this process was repeated until, after 2 months of passage, an increase in virus replication and cytopathic effect was noted. Infection of Jurkat cells with an equivalent number of reverse transcriptase units of the wild-type and putative revertant viruses resulted in comparable levels of virus replication and cytopathic effects (Fig. 2D to F).

To characterize the revertant virus, the *Kpn*I-*Bam*HI *env* fragments (nucleotides 5928 to 8054) were amplified by PCR from genomic DNA of infected Jurkat cells. Six *env* clones were sequenced in their entirety, and three clones containing representative sequence changes compared with the parental ΔV1/2 *env* gene were selected for detailed analysis. All of the clones retained the V1-V2 loop deletion. Clone 4.4 encoded a single additional amino acid change at aspartate 107 (107 D/R), clone 3.2 encoded two changes (128 G/D and 281 A/S), and clone 4.2 encoded the two changes observed in clone 3.2 plus two additional changes (440 S/N and 734 E/K). To determine if these amino acid changes were sufficient to account for the observed reversion in the replicative and syncytium-forming abilities of the ΔV1/2 mutant, the clone 4.4, 3.2, and 4.2 *env* sequences were cloned into an infectious HXBc2 HIV-1 provirus (16) and into plasmid pSVIIIenv (19). Direct transfection of Jurkat cells with the proviral clones indicated that the viruses with the clone 4.4, 3.2, and 4.2 *env* genes replicated faster and achieved higher levels of virus production than did the parental ΔV1/2 virus (data not shown). The replicative ability of the parental and revertant envelope glycoproteins was directly examined by using an *env* complementation assay previously described (19). Briefly, HIV-1 recombinants encoding chloramphenicol acetyltransferase (CAT) and containing the different envelope glycoproteins were produced in HeLa cells. Recombinant viruses were incubated with Jurkat cells, and CAT activity was measured. The results indicate that the clone 4.4, 3.2, and 4.2 envelope glycoproteins complemented HIV-1 entry into Jurkat cells at least as well as the wild-type envelope glycoproteins and more efficiently than the parental ΔV1/2 envelope glycoproteins (Table 1). The syncytia observed in Jurkat cultures infected with the clone 4.4, 3.2, and 4.2 viruses were greater in size and number than those seen in the culture infected with the ΔV1/2 parental virus (Table 1), and the efficiencies of single-cell lysis were similar for the parental and revertant viruses (data not shown). Immunoprecipitation of $^{35}$S-labeled envelope glycoproteins from cell lysates and supernatants of these infected cultures confirmed that the revertant virus envelope glycoproteins migrated comparably to those of the ΔV1/2 parental virus (data not shown), consistent with the retention of the V1-V2 deletion in the revertant viruses. These results indicate that only a few amino acid changes in the envelope glycoproteins are sufficient to allow functional reversion in the absence of the V1-V2 gp120 loop.

To study the neutralization sensitivity of the parental and revertant viruses with V1 and V2 deleted, recombinant CAT-encoding viruses containing the wild-type, parental ΔV1/2, and clone 4.4, 3.2, and 4.2 envelope glycoproteins were produced in HeLa cells. These viruses were incubated with soluble CD4 (sCD4) (11) or monoclonal antibodies prior to the infection of Jurkat lymphocytes. Table 2 shows that compared with the wild-type virus, the parental ΔV1/2 and revertant viruses were more sensitive to neutralization by antibody 17b, which recognizes a CD4i gp120 epitope (57), and by V3-directed monoclonal antibodies 1121, 9284, and 110.4 (25, 49). Increased neutralization sensitivity was also seen with regard to a single-chain sFv fragment of antibody 17b (65). Some of the revertant viruses were neutralized slightly more efficiently by these antibodies than was the parental ΔV1/2 virus. Compared with the wild-type virus, the viruses with V1 and V2 deleted did not exhibit increased sensitivity to neutralization by sCD4 or by F105, a monoclonal antibody directed against the CD4 binding site.

The ability of HIV-1 to replicate efficiently with an envelope glycoprotein containing a 66-residue deletion is remarkable. The HXBc2 envelope glycoproteins lacking the V1 and V2 variable loops exhibit 17 and 30% of the ability of the wild-type envelope glycoproteins to support a single round of virus entry into human peripheral blood mononuclear cells and Jurkat lymphocytes, respectively (64; data not shown). These observations support the concept that the V1 and V2 variable loops are exposed domains extrinsic to the folded core of the gp120 glycoprotein. This concept is also supported by previous observations that the gp120 glycoproteins with V1 and V2 deleted were recognized by sCD4 and a number of monoclonal antibodies directed against conformation-dependent structures (40, 55, 56, 63, 64). As has been seen for other HIV-1 envelope glycoproteins with partial fusion defects (28), the syncytium-forming ability of the ΔV1/2 virus was more attenuated than was its replicative ability.

A small number of amino acid changes are able to allow functional reversion of the ΔV1/2 mutant, consistent with the rather mild nature of the replication defect observed. The mechanistic basis for the reversion is uncertain. Deletion of the V1 and V2 loops has been shown to increase the exposure of the CD4i epitopes on the monomeric HIV-1 gp120 glycoprotein (64). The increased sensitivity of the viruses with V1 and V2 deleted to neutralization by an antibody, 17b, directed against these epitopes (57) is consistent with such an increased exposure on the oligomeric envelope glycoprotein complex. Increased sensitivity of the viruses with V1 and V2 deleted to V3-specific neutralizing antibodies was also seen. Since the V3 loop and the 17b epitope have been suggested to be proximal to each other (37, 64) and to the chemokine receptor binding

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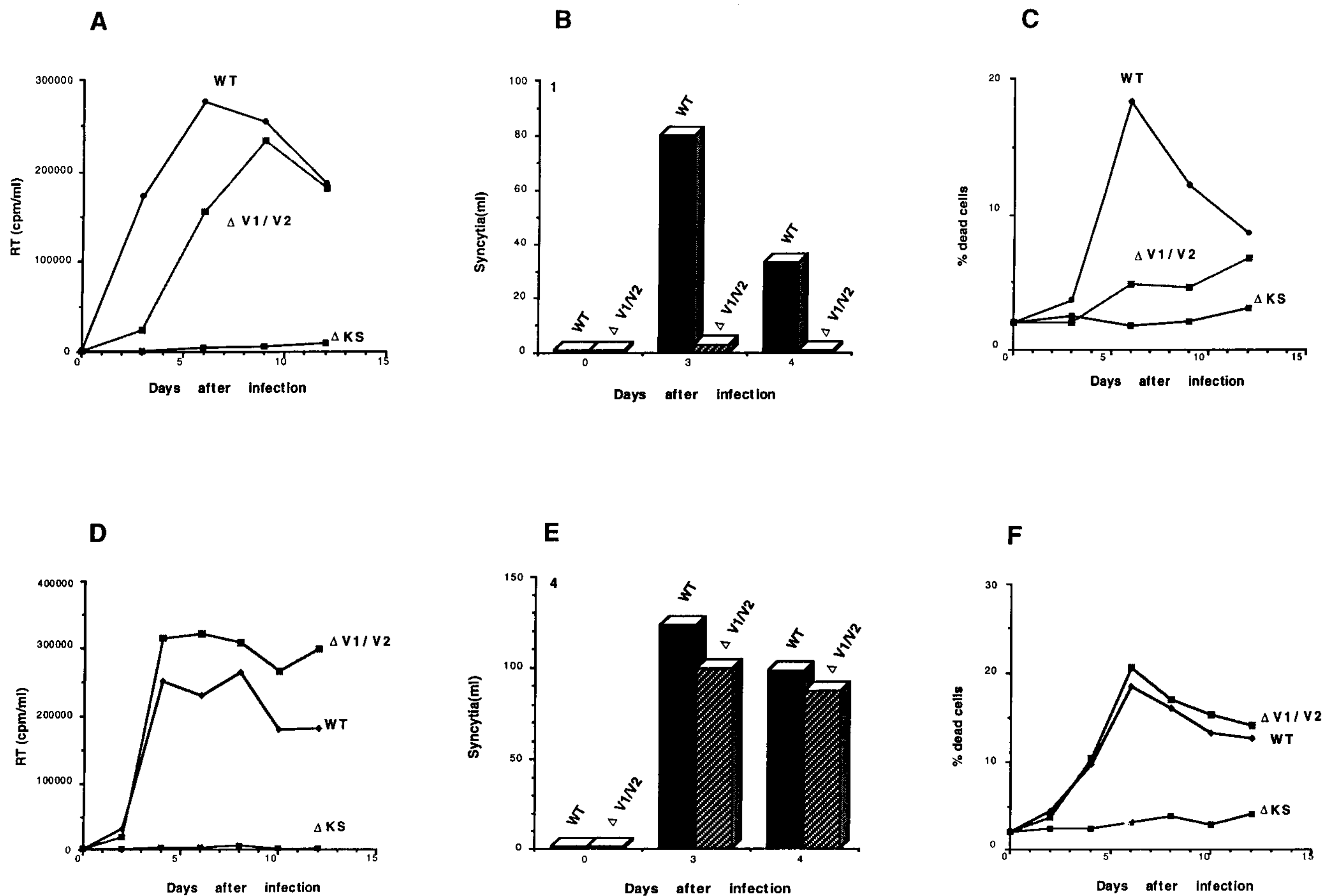


FIG. 2. Infection of Jurkat lymphocytes with HIV-1 with envelope glycoproteins with variable loops deleted. Supernatants of Jurkat lymphocytes transfected with proviral DNAs specifying HIV-1 with different envelope glycoproteins were used to infect fresh Jurkat lymphocytes (A to C). Virus replication in the Jurkat cultures was monitored by reverse transcriptase (RT) production, syncytium formation, and the amount of single-cell lysis. In D to F, equivalent numbers of reverse transcriptase units of the wild-type (WT) and ΔV1/2 revertant viruses were used to infect fresh Jurkat lymphocytes and virus replication was monitored.

region of gp120 (59, 62), this increased exposure may require some adjustments of gp120 sequences related to chemokine receptor interaction. The sensitivity patterns of the wild-type, ΔV1/2 parent, and revertant viruses provide some insight into the nature of these adjustments. The revertants (clones 3.2 and 4.2) with the phenotypes closest to that of the wild-type virus were less sensitive to neutralization by the 17b antibody and the 17b sFv fragment than was the parental ΔV1/2 virus. In addition, the revertants exhibited slight decreases in sensitivity to neutralization by the 110.4 V3 antibody compared with the ΔV1/2 parental virus. The reversions may involve a movement of the V3 loop resulting in decreased exposure of the CD4i and some V3 epitopes. In light of this model, it is interesting that two of the gp120 regions involved with reversion-associated changes have previously been suggested to interact with the V3 loop (5, 50). Alanine 281 is proximal to aspartate 279, changes in which affect the phenotype of V3 loops altered at valine 318 (50). Serine 440 has been suggested to interact with the V3 loop residue, lysine 322, which specifies HIV-1 tropism and chemokine receptor choice (5). Further studies should clarify the structural basis for the revertant phenotypes.

TABLE 1. Functional properties of HIV-1 envelope glycoproteins

| Envelope glycoprotein | Virus entry[a] | Syncytium-forming ability[b] |
|---|---|---|
| ΔKS | 0 | 0 |
| Wild type | 100 | 100 |
| ΔV1/2 | 71 | 4 |
| Clone 4.4 | 111 | 52 |
| Clone 3.2 | 128 | 74 |
| Clone 4.2 | 109 | 93 |

[a] The ability of the envelope glycoproteins to complement the single-round infection of a CAT-expressing HIV-1 recombinant with *env* deleted was determined as previously described (19). ΔKS represents a negative control envelope glycoprotein containing a large deletion in gp120. Values were normalized to a wild-type value of 100.

[b] Syncytium-forming ability was determined 3 to 4 days after transfection of Jurkat cells with HXBc2 proviruses containing the different *env* genes. Values were normalized to a wild-type value of 100.

Our data are consistent with the notion that elements of the V3 loop and the CD4i epitopes are apparently masked by the V1 and V2 loops, suggesting an in vivo function of these loops. The requirement that a persistent virus must evade neutralizing antibodies may have selected for a large, flexible, and variable structure superimposed on an extant, functional envelope glycoprotein core. Even if these exposed structures elicit neutralizing antibodies, as has been seen for the V1 and V2 loops (17, 34, 35), there may be a net survival advantage if variation in the exposed elements can be tolerated and if more conserved structures are masked. Previous studies suggested that the V3 loop contributes to masking of the CD4 binding

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TABLE 2. Neutralization of recombinant HIV-1 with different envelope glycoproteins

| gp120 ligand[a] | gp120 epitope[b] | Amt or dilution[c] | % CAT activity[d] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Wild type | ΔV1/2 | Clone 4.4 | Clone 3.2 | Clone 4.2 |
| sCD4 | CD4 | 0.5 | 10 | 5 | 6 | 7 | 11 |
| F105 | CD4BS | 5 | 40 | 39 | 38 | 30 | 52 |
| F105 | CD4BS | 10 | 29 | 28 | 32 | 25 | 39 |
| 17b | CD4i | 5 | 100 | 34 | 31 | 51 | 69 |
| 17b | CD4i | 10 | 86 | 15 | 7 | 24 | 31 |
| 17b (s.c.) | CD4i | 1 | 97 | 35 | 57 | 72 | 87 |
| 17b (s.c.) | CD4i | 5 | 94 | 16 | 13 | 16 | 21 |
| 1121 | V3 | 0.5 | 69 | 5 | 14 | 7 | 11 |
| 1121 | V3 | 5 | 11 | 5 | 6 | 4 | 9 |
| 9284 | V3 | 5 | 115 | 43 | 50 | 67 | 26 |
| 110.4 | V3 | $10^{-6}$ | 98 | 46 | 97 | 87 | 95 |
| 110.4 | V3 | $10^{-5}$ | 97 | 8 | 26 | 31 | 33 |
| 110.4 | V3 | $10^{-4}$ | 3 | 3 | 4 | 2 | 4 |

[a] 17b (s.c.) represents a single-chain sFv fragment of monoclonal antibody 17b produced in *Drosophila* cells (65).
[b] CD4BS, CD4 binding site epitope; CD4i, CD4-induced epitope.
[c] All sCD4 and antibody concentrations are in micrograms per milliliter, except for antibody 110.4, which was used at the indicated dilutions. sCD4 and antibodies were incubated with recombinant HIV-1 and Jurkat cells in 1 ml at the indicated concentrations for 1 h at 37°C prior to the addition of 0.5 ml of fresh medium without additional antibody or sCD4. After 24 h, 1 ml of fresh medium was added and the virus-cell mixture was incubated at 37°C for an additional day prior to determination of CAT activity.
[d] The values shown are percentages of the target cell CAT activity measured in the presence of sCD4 or antibody relative to that measured in the absence of sCD4 or antibody. Thus, 100% indicates no neutralization and 0% indicates complete neutralization.

site (63), consistent with the observations here that removal of the V1 and V2 loops alone does not increase the sensitivity of the virus to neutralization by sCD4 or a CD4BS antibody. Future studies should help to clarify the molecular strategies employed by HIV-1 to evade host immune responses.

We thank Marshall Posner and Raymond Sweet for gifts of monoclonal antibodies and sCD4. We thank Lorraine Rabb and Yvette McLaughlin for manuscript preparation and Amy Emmert for artwork.

R.W. was supported by a fellowship from the American Foundation for AIDS Research. This work was supported by grants from the National Institutes of Health (AI 24755 and AI 31783) and by a Center for AIDS Research grant to the Dana-Farber Cancer Institute (AI 28691). The Dana-Farber Cancer Institute is also the recipient of a Cancer Center grant from the National Institutes of Health (CA 06516). This work was made possible by gifts from the late William McCarty-Cooper, from the G. Harold and Leila Y. Mathers Charitable Foundation, and from the Friends 10.

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