JOURNAL OF VIROLOGY, Sept. 1993, p. 5635–5639
0022-538X/93/095635-05$02.00/0

Vol. 67, No. 9

NOTES

Evidence that the Structural Conformation of Envelope gp120 Affects Human Immunodeficiency Virus Type 1 Infectivity, Host Range, and Syncytium-Forming Ability

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Received 2 February 1993/Accepted 17 May 1993

We investigated how amino acid changes within and outside the V3 loop of the envelope glycoprotein of human immunodeficiency virus type 1 influence the infectivity, host range, and syncytium-forming ability of the virus. Our studies show that on the genomic backgrounds of the human immunodeficiency virus type 1 strains SF2 and SF13, a reciprocal exchange of full-loop sequences does not alter the syncytium-forming ability of the viruses, indicating that a determinant(s) for this biological property maps outside the loop. However, specific amino acid substitutions, both within and outside the V3 loop, resulted in loss of infectivity, host range, and syncytium-forming potential of the virus. Furthermore, it appears that a functional interaction of the V3 loop with regions in the C2 domain of envelope gp120 plays a role in determining these biological properties. Structural studies of mutant glycoproteins show that the mutations introduced affect the proper association of gp120 with the transmembrane glycoprotein gp41. Our results suggest that mutations that alter the structure of the V3 loop can affect the overall conformation of gp120 and that, reciprocally, the structure of the V3 loop is influenced by the conformation of other regions of gp120. Since the changes in the replicative potential, host range, and fusogenic ability of the mutant viruses correlate well with the changes in gp120 conformation, as monitored by the association of gp120 with gp41, our results support a close relationship between envelope gp120 structural conformation and the biological phenotype of the virus.

Human immunodeficiency virus type 1 (HIV-1) isolates display a high degree of biological heterogeneity in vitro. The viruses differ with respect to their replicative kinetics, susceptibility to serum neutralization, and ability to infect different cell types and to induce syncytia in the infected cells (for reviews, see references 4, 5, and 9). Some of these properties have been shown to correlate with pathogenicity of the virus in vivo (1, 6, 29). To define the minimal amino acid changes that confer differential cellular tropism as well as replicative and cytopathic properties of HIV-1, recombinant viruses were generated between two HIV-1 strains, SF2 and SF13 (HIV-1_{SF2} and HIV-1_{SF13}), which were recovered over time from the same individual. These two isolates are highly related at the nucleotide and amino acid sequence levels, but they display distinct biological properties. Compared with HIV-1_{SF2} (the early isolate), HIV-1_{SF13} (the late isolate) replicates faster and to higher titers in peripheral blood mononuclear cells (PBMCs) and several T-cell lines and is highly cytopathic (6, 7). Our prior characterization of recombinant viruses generated between HIV-1_{SF2} and HIV-1_{SF13} showed that a 0.49-kb *Stu*I-*Mst*II fragment of *env* gp120, encompassing the V3 loop, contained a major determinant(s) of host range and syncytium-forming ability of these HIV-1 isolates (7). A comparison of the predicted amino acid sequences of HIV-1_{SF2} and HIV-1_{SF13} reveals 10 amino acid differences within this region of gp120, 4 of which are located in the V3 hypervariable region.

The V3 loop of gp120 does not seem to play an important role in CD4 binding, since neither alteration in the V3 sequences (10, 11, 17, 23) nor V3-specific antibodies (19, 27) affect the attachment of gp120 to CD4. This region does, however, appear to be involved in the postbinding fusion process necessary for viral entry (11, 15, 23, 24) and helps determine cellular host range (8, 14, 26, 28, 33). The exact mechanism by which the V3 loop mediates these events is presently unclear. However, recent data accumulated in several laboratories suggest that the V3 loop participates in a process involving envelope conformational change (20, 25, 35) that leads to postbinding fusion with the cell membrane (21).

To examine the role of V3 loop sequences of HIV-1_{SF2} and HIV-1_{SF13} in viral infectivity, host range, and syncytium-forming ability, and the mechanism(s) by which amino acid changes within and outside the V3 loop affect these biological properties, mutant viruses were generated on the genetic backgrounds of HIV-1_{SF2} and HIV-1_{SF13} by site-directed mutagenesis. The replicative ability, host range, and cytopathic potential of the mutant viruses in vitro were then determined. Furthermore, wild-type and mutant envelope glycoproteins were expressed in transient expression systems. The expression and processing of these envelope products, the association of gp120 with gp41, and the ability to induce syncytia were examined.

Effects of amino acid composition of the V3 loop on viral infectivity and syncytium-forming ability. The four amino acid differences in the V3 loops of HIV-1_{SF2} and HIV-1_{SF13} are located in the variable regions flanking the conserved GPG tip; two of these amino acid differences (at positions

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TABLE 1. Infectivity, host range, and syncytium-forming ability of V3 loop mutant viruses

Strain	V3 loop sequence[a]	Replication kinetics[b] in:		Syncytium formation in HUT 78 cells[c]
		PBMCs	HUT 78 cells	
HIV-1_{SF13}	308 320			
WT[d]	KGIHIGPGRAVYT	++	++	++
Mu1	-S-Y------FH-	++	++	++
Mu2	---Y-------H-	+	−	NA
Mu3	----------H-	−	−	NA
Mu4	---Y---------	+	+	++
HIV-1_{SF2}	308 320			
WT	KSIYIGPGRAFHT	+	+	+
Mu1	-G-H------VY-	±	±	+
Mu2	---H-------Y-	+	±	+
Mu3	---H---------	+	−	NA
Mu4	----------Y-	+	+	+

[a] Amino acids of interest are shown in boldface type.
[b] ++, peak reverse transcriptase (13) activity (>10^6 cpm/ml) occurred within 10 days postinfection; +, peak reverse transcriptase activity was delayed for <7 days; ±, peak reverse transcriptase activity was delayed for >10 days; −, no reverse transcriptase activity above background level (~2,000 cpm/ml) was detected for at least 30 days.
[c] ++, >70% of the cells showed syncytia at time of peak reverse transcriptase activity; +, ~20 to 30% of the cells showed syncytia at time of peak reverse transcriptase activity; NA, not applicable.
[d] WT, wild type.

311 and 319) are nonconserved (Table 1). Interestingly, the amino acid substitutions at these positions are reciprocal; i.e., a tyrosine-to-histidine (Y→H) change at position 311 of HIV-1_{SF13} gp120 is accompanied by a histidine-to-tyrosine (H→Y) change at position 319. To evaluate the effects of amino acid composition of the V3 loop on viral infectivity, host range, and syncytium-forming ability, mutant viruses were generated by site-directed mutagenesis (18) and then RD-4 cells were cotransfected as described elsewhere (31, 36). The abilities of the mutant viruses to infect PBMCs and HUT 78 cells and to induce syncytia in the infected HUT 78 cells were examined. The results are summarized in Table 1.

On the genomic background of HIV-1_{SF13}, we observed that the first mutant, Mu1, which has the V3 loop amino acid composition of HIV-1_{SF2}, displays a biological phenotype identical to that of wild-type HIV-1_{SF13}. HIV-1_{SF13} Mu2, which has amino acid substitutions at positions 311 and 319, replicates with much slower kinetics in PBMCs and is unable to productively infect HUT 78 cells. A more dramatic effect was observed with Mu3, which has a single amino acid substitution at position 319 (Y→H). This mutant virus is not infectious for either PBMCs or HUT 78 cells. A single amino acid substitution at position 311 (H→Y) to generate Mu4, however, only moderately affected the ability of the virus to infect PBMCs and HUT 78 cells.

When mutant viruses were generated on the HIV-1_{SF2} genomic background, Mu1, which has the V3 loop of HIV-1_{SF13}, was found to replicate with slower kinetics than wild-type HIV-1_{SF2} in PBMCs and HUT 78 cells. This mutant virus also did not induce syncytia in infected HUT 78 cells. Mu2, which has amino acid substitutions at positions 311 and 319, was impaired only in its ability to infect HUT 78 cells. Mu3, which has a single amino acid substitution at position 311 (Y→H), replicated with delayed kinetics in PBMCs and did not productively infect HUT 78 cells. Mu4, the virus with a single amino acid substitution at position 319 (H→Y), displayed a biological phenotype indistinguishable from that of wild-type HIV-1_{SF2}.

Effects of amino acid substitutions outside the V3 loop on viral infectivity, host range, and syncytium-forming ability. The observations made with HIV-1_{SF2} Mu1 and HIV-1_{SF13} Mu1 suggest that regions outside the V3 loop, but within the *Stu*I-*Mst*II region of *env* gp120, can affect the biological properties of these viral strains. Six amino acid differences are located outside the V3 loop, only one of which is nonconserved: an asparagine-to-aspartic acid (N→D) substitution at position 282 in the HIV-1_{SF13} envelope gp120. To examine whether this change affects the infectivity and syncytium-forming ability of the virus, site-directed mutants were generated on the HIV-1_{SF2} and HIV-1_{SF13} genomic backgrounds

TABLE 2. Infectivity, host range, and syncytium-inducing ability of HIV-1_{SF2mc} and HIV-1_{SF13mc} viruses with mutations outside as well as inside the V3 loop

Strain	Amino acid sequences[a]:		Replication kinetics[b] in:		Syncytium formation in HUT 78 cells[c]
	Outside V3 loop	Inside V3 loop	PBMCs	HUT 78 cells	
HIV-1_{SF13}	280 285	309 319			
WT[d]	FTDNAK	GIHIGPGRAVY	++	++	++
Mu3	------	----------H	−	−	NA
Mu6	--N---	----------	−	−	NA
Mu7	--N---	----------H	+	+	+
	327 341				
WT	DIRKAHCNISRAQWN		++	++	++
Mu5	-------V--T---		++	++	++
HIV1_{SF2}	280 285	309 319			
WT	FTNNAK	SIYIGPGRAFH	+	+	+
Mu7	--D---	----------	+	+	++

[a] Amino acids of interest are shown in boldface type.
[b] For explanations of symbols, see Table 1, footnote *b*.
[c] For explanations of symbols, see Table 1, footnote *c*.
[d] WT, wild type.

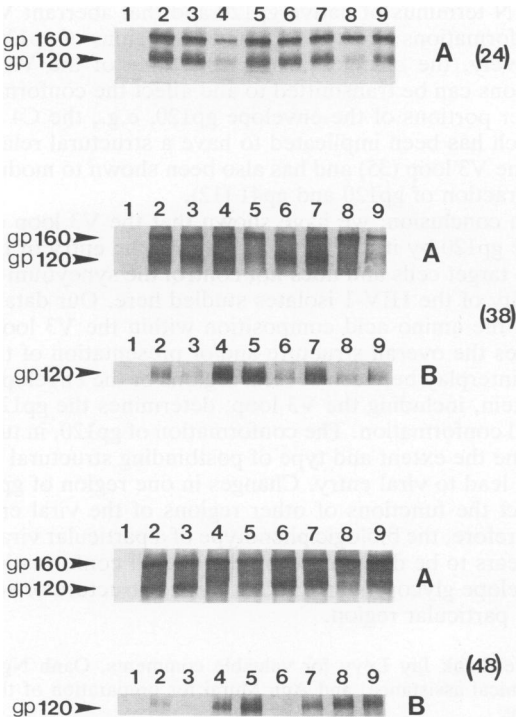

FIG. 1. Synthesis and processing of HIV-1_{SF13} wild-type and mutant envelope glycoproteins in COS-7 cells. Viral envelope glycoproteins were detected by immunoprecipitation of the cell lysates (A) and of the cell culture medium (B). Lanes: 1, control COS-7 cells; 2 to 9, COS-7 cells transfected with HIV-1_{SF13} wild type, Mu1, Mu2, Mu3, Mu4, Mu5, Mu6, and Mu7, respectively. Numbers in parentheses indicate hours posttransfection.

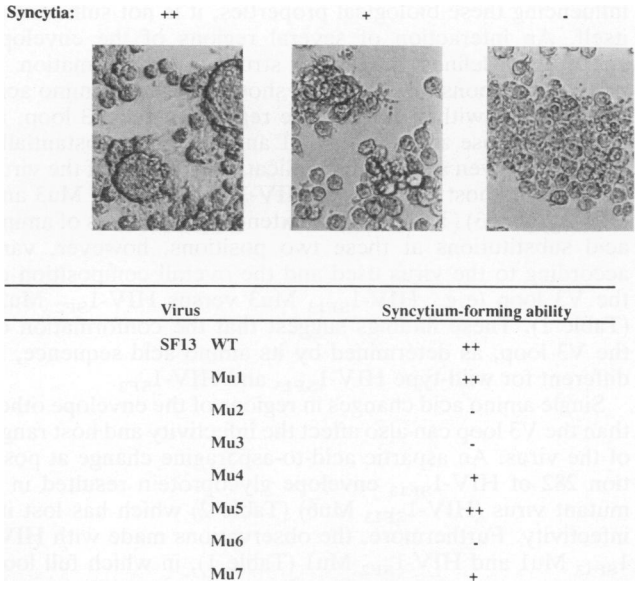

FIG. 2. Syncytium-forming ability of HIV-1_{SF13} wild-type (WT) and mutant envelope glycoproteins. At 24 h posttransfection, 10^4 COS-7 cells were coincubated with 10^5 Sup-T cells, and syncytia were evaluated 18 to 20 h later. Similar results were obtained with COS-7 cells at 38 and 48 h posttransfection. ++, Large, numerous syncytia; +, smaller, less numerous syncytia; −, no syncytia.

and the biological properties of the mutant viruses were examined. The results are summarized in Table 2.

On the HIV-1_{SF13} background, a single D→N substitution at amino acid 282 of the envelope gp120 resulted in a virus that is noninfectious (Mu6). However, the effect of this mutation can be partially compensated by an additional change at amino acid 319 within the V3 loop (Y→H) to generate Mu7. Furthermore, Mu7 was found to be less cytopathic than wild-type HIV_{SF13} in infected HUT 78 cells. The Y→H change alone at position 319, as noted above, resulted in a noninfectious virus (Mu3). Mu5, which has amino acid substitutions at positions 335 and 338, displayed a phenotype identical to that of wild-type HIV-1_{SF13}.

On the HIV-1_{SF2} genomic background, a single N→D substitution at amino acid 282 of envelope gp120 did not affect the virus's ability to infect PBMCs or HUT 78 cells, but the mutant virus induced large syncytia in infected HUT 78 cells.

Processing and syncytium-forming ability of wild-type and mutant envelope glycoproteins. To determine whether the loss of infectivity and host cell tropism of mutant viruses HIV-1_{SF13} Mu2, Mu3, and Mu6 is due to a direct effect of the mutations on envelope glycoprotein processing and structure, a 3.46-kb *Eco*RI-*Eco*RV fragment of the wild-type or mutant genome encompassing the envelope glycoprotein gene was introduced into the polylinker of the pSM vector (3) at the *Eco*RI-*Sma*I site and transiently expressed in COS-7 cells. Expression of mutant glycoproteins, processing of glycoprotein precursors, and association of gp41 and gp120 glycoproteins were then examined by Western immunoblotting (32) and immunoprecipitation with a polyclonal goat anti-gp120 antibody (Chiron Corp., Emeryville, Calif.). Results for HIV-1_{SF13} wild-type and mutant glycoproteins are presented in Fig. 1.

At 24 h posttransfection, we observed comparable levels of gp160 expression and processing in mutant- and wild-type-transfected cells with no gp120 detected in the cell supernatant. The exceptions were cells transfected with Mu2, which expresses less gp160 and gp120, and those transfected with Mu6, in which the processing of the envelope gp160 appeared to be slower. At 38 and 48 h posttransfection, decreasing amounts of cell-associated gp120 were noted in cells transfected with HIV-1_{SF13} Mu2, Mu3, and Mu6, concomitant with an increase in the amounts of gp120 found in cell supernatants. Increasing amounts of gp120 were also found in the supernatant of Mu7-transfected cells at these time points, but more cell-associated gp120 was present than in Mu3- and Mu6-transfected cells. HIV-1_{SF13} Mu1, Mu4, and Mu5, in contrast, were expressed and processed at levels similar to that of the wild type at these time points, although more gp120 was consistently found in the supernatant of Mu5-transfected cells.

When the syncytium-forming ability of mutant glycoproteins was assessed at 24, 38, or 48 h posttransfection by cocultivating transfected cells with Sup-T1 cells, the ability of Mu4 and Mu7 glycoproteins to form syncytia was found to be decreased compared with that of the envelope of the wild-type virus, whereas Mu2, Mu3, and Mu6 glycoproteins were unable to form syncytia (Fig. 2). In contrast, Mu1 and Mu5 glycoproteins displayed full fusogenic capacity.

Conclusions. Our studies of HIV-1_{SF2} and HIV-1_{SF13} mutant viruses reveal certain features of the envelope glycoprotein gp120 that are important in determining the infectivity, host range, and syncytium-forming ability of HIV-1. We observed that although the V3 loop of gp120 is involved in

influencing these biological properties, it is not sufficient in itself. An interaction of several regions of the envelope gp120 that defines the gp120 structural conformation is primarily responsible. Our data show that single amino acid substitutions within the variable regions of the V3 loop, in particular those at positions 311 and 319, can substantially reduce and even abolish the replicative potential of the virus and limit its host range (e.g., HIV-1_{SF13} Mu2 and Mu3 and HIV-1_{SF2} Mu3) (Table 1). The extents of the effects of amino acid substitutions at these two positions, however, vary according to the virus used and the overall composition of the V3 loop (e.g., HIV-1_{SF13} Mu3 versus HIV-1_{SF2} Mu3) (Table 1). These findings suggest that the conformation of the V3 loop, as determined by its amino acid sequence, is different for wild-type HIV-1_{SF13} and HIV-1_{SF2}.

Single amino acid changes in regions of the envelope other than the V3 loop can also affect the infectivity and host range of the virus. An aspartic acid-to-asparagine change at position 282 of HIV-1_{SF13} envelope glycoprotein resulted in a mutant virus (HIV-1_{SF13} Mu6) (Table 2) which has lost its infectivity. Furthermore, the observations made with HIV-1_{SF13} Mu1 and HIV-1_{SF2} Mu1 (Table 1), in which full loop sequences are exchanged, indicate that on the genomic backgrounds of the two parental isolates, a determinant(s) for syncytium formation maps outside the V3 loop.

The effects of mutations within (e.g., HIV-1_{SF13} Mu3) (Table 1) and outside (e.g., HIV-1_{SF13} Mu6) (Table 2) the V3 loop, however, can compensate each other and partially restore the functions of the envelope gp120. In contrast to these two single-site mutants, HIV-1_{SF13} Mu7, which contains mutations at both positions 311 and 282, replicates in PBMCs and HUT 78 cells (Table 2) and induces a moderate degree of syncytium formation (Fig. 2). Therefore, in agreement with our findings with the Mu1 HIV-1_{SF2} and HIV-1_{SF13} V3 loop mutant viruses, an interplay of the V3 loop with other regions of envelope gp120 is implicated. Similar observations have been reported by others (22, 34, 35). In particular, a functional interaction of the V3 domain with the second conserved region of gp120 has previously been reported by Willey et al. (34) to determine HIV-1 infectivity. Whether the changes introduced in these other regions of gp120 alter the structure or presentation of the V3 loop requires further investigation.

Structural studies of mutant glycoproteins showed that the mutations introduced at positions 311, 319, and 282 (HIV-1_{SF13} Mu2, Mu3, and Mu6, respectively) (Tables 1 and 2) alter the association of gp120 with gp41. Furthermore, these mutant glycoproteins are defective in their syncytium-forming ability (Fig. 2). These observations indicate a close relationship between changes in gp120 conformation and the biological phenotype of HIV-1. Nevertheless, the finding that Mu5 displayed properties identical to those of wild-type HIV-1_{SF13} (Table 2 and Fig. 2) yet released more gp120 into the supernatant of transfected cells (Fig. 1) suggests that gp120 dissociation is indicative of a gross structural change in the envelope gp120 which can be easily monitored but which may not be related to other more subtle conformational changes that are critical for viral infectivity, host range, and syncytium-forming ability. This conclusion is in agreement with data reported by others regarding sCD4-induced shedding of gp120 (2, 30).

Amino acids 311, 319, and 282 are located away from the C-terminal and N-terminal domains of gp120, regions that are reported to be involved in the association of gp120 with gp41 (12, 16). It is possible that either the V3 loop or the C2 domain is in close proximity with either the C terminus or the N terminus of native gp120 and that aberrant V3 or C2 conformations affect the C1 and C5 regions of gp120. Alternatively, the changes in the structures of the V3 and C2 regions can be transmitted to and affect the conformation of other portions of the envelope gp120, e.g., the C4 domain, which has been implicated to have a structural relationship to the V3 loop (35) and has also been shown to modulate the interaction of gp120 and gp41 (12).

In conclusion, we have shown that the V3 loop of envelope gp120 by itself does not regulate the entry of the virus into target cells and does not control the syncytium-forming ability of the HIV-1 isolates studied here. Our data suggest that the amino acid composition within the V3 loop determines the overall structure and/or presentation of the loop. An interplay between several regions of the envelope glycoprotein, including the V3 loop, determines the gp120 structural conformation. The conformation of gp120, in turn, may define the extent and type of postbinding structural changes that lead to viral entry. Changes in one region of gp120 can affect the functions of other regions of the viral envelope. Therefore, the biologic phenotype of a particular viral isolate appears to be determined by the overall conformation of its envelope glycoprotein and not by the structure or sequence of a particular region.

We thank Jay Levy for valuable comments, Oanh Nguyen for technical assistance, and Ann Murai for preparation of the manuscript.

This work was supported by a grant from the National Institutes of Health (AI 29857) and a postdoctoral fellowship (F91UP001) to L.S. from the California Universitywide AIDS Research Program.

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