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A Single Amino Acid Substitution in the V1 Loop of Human Immunodeficiency Virus Type 1 gp120 Alters Cellular Tropism

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Specific point mutations which affect viral tropism have been identified in both the V3 loop and in the CD4-binding region of the human immunodeficiency virus type 1 surface glycoprotein gp120. Here we report that a single point mutation in the first variable region (V1) of human immunodeficiency virus type 1 strain JRCSF is responsible for a change in viral tropism.

A primary requirement for human immunodeficiency virus type 1 (HIV-1) infection of most susceptible cells is the expression on the cell surface of the CD4 molecule (6, 13, 16). This molecule is expressed on the surface of a variety of cell types, including certain T lymphocytes and cells of the monocyte-macrophage lineage (24). It is clear that while cells which express CD4 may be susceptible to infection by HIV-1, some cells are not infectable by all isolates of HIV-1 despite expressing high levels of CD4, leading to speculation that a second component molecule may be required for infection by HIV-1 (7, 15).

HIV-1 isolates can be broadly placed into phenotypic groups according to their host range and/or cytopathogenicity in vitro. One such classification identifies viruses which are able to infect only primary cells, e.g., $CD4^+$ lymphocytes, macrophages, and dendritic cells, and separates these from viruses which can also infect immortalized $CD4^+$ T-cell lines. In some cases this block to infection has been shown to be at the level of virus entry (4, 12). Often these classifications are ambiguous, with virus replication occurring in both cell groups but at different rates. Yet virus isolates with distinct tropisms have been identified. This has enabled the construction of chimeric viruses for which genetic determinants of tropism have been mapped (3, 9, 10, 25, 26, 28). By using these approaches to map tropism-determining regions it has been shown that both the V3 loop and the primary CD4-binding domain of the HIV-1 SU protein are capable of determining viral tropism (3, 5, 9, 10, 25, 26, 28). In addition, Westervelt et al. have identified a region of 700 bp encompassing V1, V2, and C2 which is required to determine macrophage tropism (28). Finally, it has also been shown that regions in gp41 can affect the tropism of HIV-1 isolates (1, 9). In the mapped regions of chimeric viruses there are many amino acid differences, making it difficult to identify which amino acids confer the phenotypes.

To overcome the problems encountered in mapping tropism-determining regions between different virus isolates, we have studied the tropism of HIV-1_{JRCSF} (14). This virus infects primary cells but does not infect $CD4^+$ T-cell lines. Using the infectious molecular clone of this virus, pUCΔCSF, we selected for a variant of HIV-1_{JRCSF} which could replicate in $CD4^+$ T-cell lines.

Viral stocks were produced by transfection of phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMC) with Lipofectin (GIBCO-BRL). Prior to transfection circularly permuted plasmids were concatemerized to reconstruct the viral DNA into an infectious configuration (2). PBMC were prepared from whole blood by using Ficoll-Paque (Pharmacia) and were stimulated with 1 μg of phytohemagglutinin per ml for 3 days prior to transfection. PBMC were maintained in RPMI medium supplemented with 20% fetal calf serum and 20 U of interleukin 2 (Boehringer Corp. Ltd.) per ml. T-cell lines were maintained in RPMI medium supplemented with 10% fetal calf serum. Supernatants from transfected PBMC were harvested and used to infect 10^6 cells in 5- to 10-ml cultures. Equivalent amounts of p24 antigen (5 ng/ml) were used to infect cells to determine virus tropism. Input virus was removed by washing cells after 1 h, and virus production was determined by p24 enzyme-linked immunosorbent assay (ELISA) (24).

HIV-1_{JRCSF} was propagated in PBMC with repeated rounds of new infection. After 12 passages (approximately 3 months) a variant isolate was detected by its ability to infect

TABLE 1. HIV-1 replication[a]

Virus	Growth in[b]:			
	PBMC	Molt-4/8 cells	SupT1 cells	Other[c]
HIV-1_{JRCSF}	+	−	−	−
M1	+	+	−	−
M2	+	+	+	−
pC3	+	+	+	−
m4	+	+	+	−
m5	+	+	+	−

[a] HIV-1 replication was assessed by p24 protein levels detected by ELISA; results shown were consistent from three separate experiments (23). Supernatants were taken at 4, 7, 10, 17, 20, and 27 days postinfection to monitor p24 production. The detection limit for the ELISA used was 0.6 ng/ml. To confirm that de novo mutations did not occur, virus supernatants were used to reinfect cells to confirm that no change had occurred in the virus kinetics.

[b] −, value below the threshold for all samples tested during the time course; +, value of >40 ng/ml.

[c] T-cell lines CEMx174, C8166, H9, and MT4 and the monocytic cell line U937.

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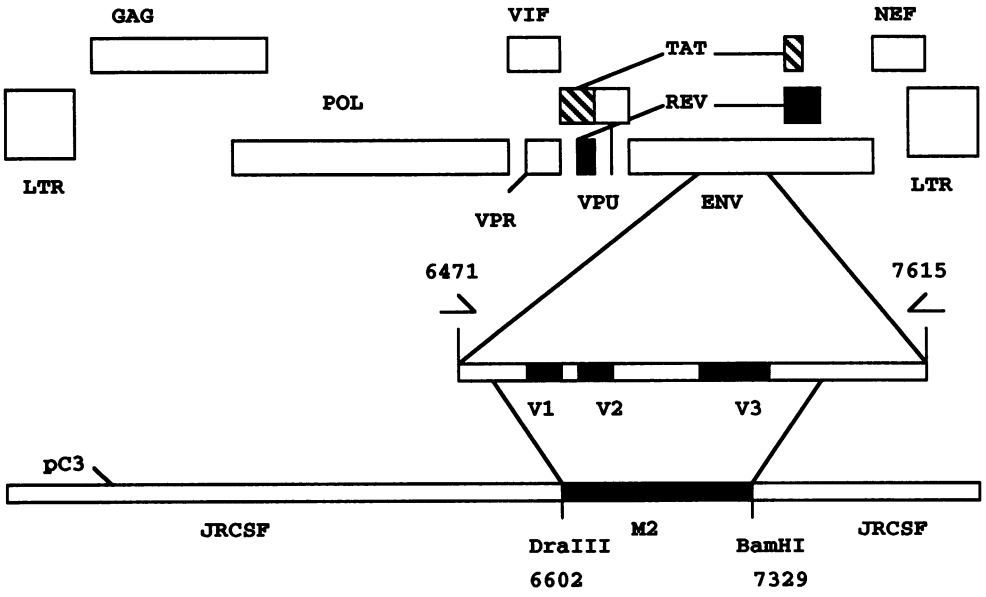

V1-loop nucleotide sequences nt 6623-6691.

HIV-1(JRCSF) tgcaaagatgtgaatgctactaataccactagtagtagtgagggaatgatggagagaggagaaataaaa

pC3 ----------a----------

V1-loop amino acid sequence aa:130-152

HIV-1(JRCSF) C K D V N A T N T T S S S E G M M E R G E I K

pC3 - - - - - - - - - - - - N - - - - - - - - - - - -

FIG. 1. Comparison of mutant virus pC3 with HIV-1_{JRCSF}. PCR was used to amplify the region from nucleotides 6471 to 7615 from M2-infected Molt-4/8 cells. This fragment was digested with *Dra*III and *Bam*HI and subcloned into the same unique sites of pUCΔCSF to produce pC3, and the entire fragment was sequenced. The change at nucleotide 6657 of guanosine in HIV-1_{JRCSF} for an adenosine in pC3 results in the replacement of a serine at position 141 in HIV-1_{JRCSF} by an asparagine in pC3. Thus, the chimeric plasmid pC3 contains the *Dra*III-to-*Bam*HI fragment of M2 and the flanking sequences of HIV-1_{JRCSF}. The V1-loop glycosylation sites are underlined.

the $CD4^+$ T-cell line Molt-4 clone 8 (Molt-4/8) (20). This variant of HIV-1_{JRCSF}, M1, was then passaged for 6 more months in Molt-4/8 cells until a further variant, M2, with a tropism extended to SupT1 cells was obtained. The growth characteristics of HIV-1_{JRCSF}, M1, and M2 are given in Table 1 and Fig. 2. The viruses were tested for their ability to replicate in a variety of other T-cell and monocytic lines (CEMx174, C8166, U937, MT4, and H9). Replication of HIV-1_{JRCSF}, M1, and M2 could not be detected in these cell lines by p24 antigen or by reverse transcriptase activity (Table 1). Infectious molecular clones were obtained from M2-infected cells, all of which displayed growth characteristics identical to those of the M2 isolate.

Polymerase chain reaction (PCR) amplification of the envelope region (bp 6471 to 7615) from M2-infected cells, prepared at an earlier time than the infectious molecular clones, was performed. PCR primers used were 5′ CCA ACC CAC AAG AAG TAG TAT TGG 3′ and 5′ ACC ATC TCT TGT TAA TAG CAG CCC 3′. The *Dra*III-to-*Bam*HI fragment derived from the PCR product was substituted into pUCΔCSF to create the plasmid pC3 (Fig. 1). This fragment has a single nucleotide difference at position 6657 (G→A), resulting in a Ser→Asn change at position 141 in the V1 loop region of gp120. Transfection of pC3 into PBMC led to the production of viable virus. Infection of $CD4^+$ T-cell lines with this virus demonstrated the same extended tropism (Table 1). To confirm that the single nucleotide change found in pC3 was responsible for the mutant phenotype, we mutagenized pUCΔCSF at position 6657 (from G to A) to produce the independent yet identical mutants m4 and m5. Site-directed mutagenesis was performed with an oligonucleotide corresponding to nucleotides 6648 to 6665 (5′ CCA CTA GTA ATA GTG AGG G 3′) which introduces the G-to-A substitution at position 6657. Viral stocks of m4 and m5 generated by transfection into PBMC demonstrated that the point mutation at 6657 was sufficient to confer the broader viral tropism observed for pC3 (Table 1). The time course of virus growth for each of the mutants in comparison with that for HIV-1_{JRCSF} was studied. Figure 2 shows that pC3, m4, m5, and M1 exhibited comparable growth rates. It should also be noted that the M2 virus grows faster than any of the single point mutation mutants; this suggests that there are other changes in this virus which enhance the replicative capacity of this virus.

Our results show that a single mutation in the V1 loop of HIV-1_{JRCSF} confers the ability to propagate in Molt-4/8 and

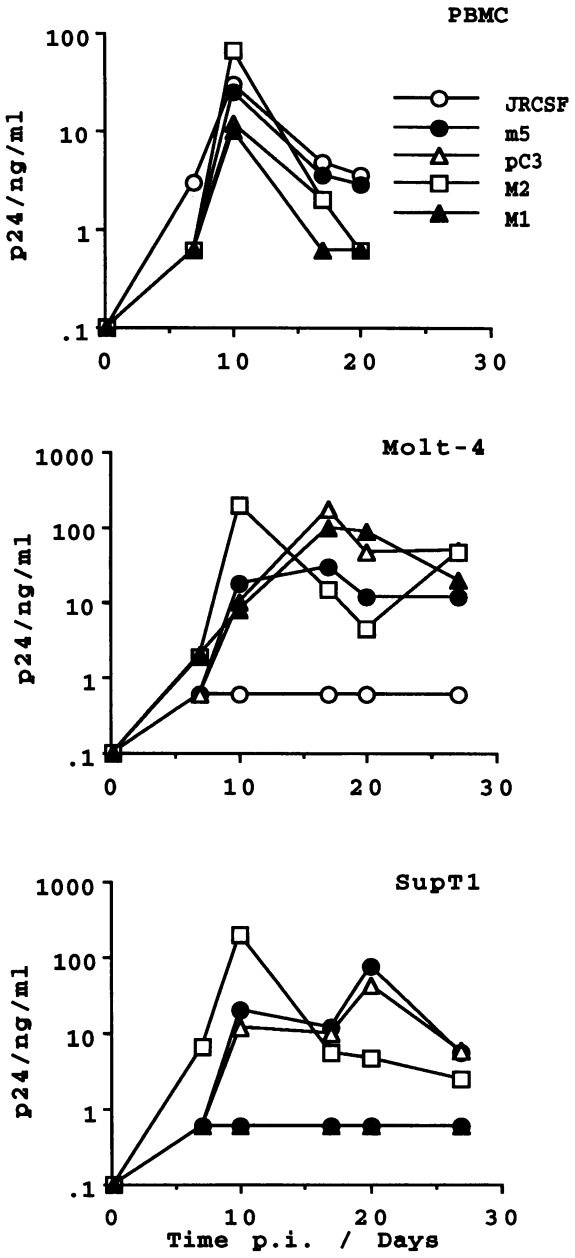

FIG. 2. Growth curves for HIV-1_{JRCSF} and mutant viruses. Note that HIV-1_{JRCSF} grows only in PBMC and that the baseline shown is the limit for detection of p24 by our ELISA. Mutant m4 results are not shown for clarity, but the growth curves for m5 and m4 are identical. p.i., postinfection.

SupT1 cells but not in the other T-cell and monocytic cell lines tested. We do not know the mutation that allows the M1 variant to grow solely in Molt-4/8 cells. The subtlety of such differences in sensitivity to infection of different T-cell lines by HIV strains is extraordinary.

Little is known about the events which take place in the HIV-1 membrane fusion reaction after the binding of virion gp120 to CD4 (18). The initial step is mediated by the CD4-binding domain of gp120 which has been shown to consist of at least three discontinuous regions of the SU protein, centered on amino acids 368 to 370, 427, and 457 (mutations at position 257 only weakly affect CD4-gp120 interaction) (17, 27). While mutations in any of these three regions can affect the interaction of CD4 with gp120, it is not clear how such changes in CD4 binding affect tropism (5).

Most efforts to study molecular determinants of HIV-1 tropism have focused on regions of the envelope gene containing the third hypervariable domain (3, 9, 25, 26). This region of the SU protein has no known function, although it is one of the principle targets of neutralizing antibodies (11). Takeuchi and colleagues reported that a single amino acid substitution in the V3 loop of HIV-1_{GUNwt} alters the tropism of this virus, enabling growth in both $CD4^+$ lymphocytic cell lines and BT (fibroblastlike, possibly astroglial) brain cells (26). However, this substitution does not have the same effects on the tropism of other isolates (22). These results suggest that the effects of mutations can be correctly interpreted only in the context of their original envelope. The importance of the interaction of different regions of the envelope with one another has been demonstrated in studies of virus neutralization, where some escape mutants to anti-V3 loop neutralizing monoclonal antibodies have been shown to map outside of the V3 loop (16).

The primary structure of the V1 loop is more variable than that of V3. The only conserved features are two glycosylation sites (at amino acids 134 and 137 in HIV-1_{JRCSF}). One of these is conserved in approximately 50% of all sequenced isolates, and the other is conserved in approximately 90% (19). Neither of these sites is directly affected by the mutation at position 141. It is not clear how an amino acid substitution in the V1 loop, a hypervariable region of the HIV-1 envelope, can affect viral tropism. Recent studies of the simian immunodeficiency virus V1 loop have shown that this hypervariability may be the result of immune selection (21). This begs the question of whether the V1 loop of HIV-1 is a target for neutralization. Either the V1 loop itself has a role in virus infection or changes in the loop may affect other regions of gp120. This has been shown in studies of HIV-1_{NL4-3} in which a mutation of amino acid 267 (the second conserved region) which resulted in noninfectious virus was spontaneously compensated for by a mutation at position 128 (the V1 hypervariable region—though not in the loop) (29). Other studies have shown that regions of gp120 interact correctly only with other regions of the same isolate (8).

The precise mechanisms by which HIV causes depletion of $CD4^+$ lymphocytes and AIDS are not known; indeed, it is not known which of the many cell types infected in vivo are principally involved in pathogenesis by HIV. Studies of tropism determination in HIV may help us to understand the mechanisms by which HIV infects cells.

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