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The Viral Envelope Gene Is Involved in Macrophage Tropism of a Human Immunodeficiency Virus Type 1 Strain Isolated from Brain Tissue

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Human immunodeficiency virus type 1 (HIV-1) strains isolated from the central nervous system (CNS) may represent a subgroup that displays a host cell tropism different from those isolated from peripheral blood and lymph nodes. One CNS-derived isolate, HIV-1_{SF128A}, which can be propagated efficiently in primary macrophage culture but not in any T-cell lines, was molecularly cloned and characterized. Recombinant viruses between HIV-1_{SF128A} and the peripheral blood isolate HIV-1_{SF2} were generated in order to map the viral gene(s) responsible for the macrophage tropism. The *env* gene sequences of the two isolates are about 91.1% homologous, with variations scattered mainly in the hypervariable regions of gp120. Recombinant viruses that have acquired the HIV-1_{SF128A} *env* gene display HIV-1_{SF128A} tropism for macrophages. Furthermore, the gp120 variable domains, V₁, V₂, V₄, and V₅, the CD4-binding domain, and the gp41 fusion domain are not directly involved in determining macrophage tropism.

Acquired immunodeficiency syndrome (AIDS) is a disease caused by the human immunodeficiency virus (HIV). Infected individuals often develop neurological disorders in addition to severe immune dysfunctions (23, 29). These diseases include encephalopathy, dementia, and vacuolar degeneration of the spinal cord (8). In many cases, infectious HIV has been isolated from brain tissues or cerebrospinal fluid of patients (15, 22), and the types of brain cells that have been shown to be infected by HIV include macrophages (16, 34), endothelial cells, and glial cells (20, 26, 34). Furthermore, HIV strains recovered from brain tissue can be distinguished from peripheral blood isolates by their ability to infect different cell types and in their sensitivity to serum neutralization (4, 6). These findings suggest that brain-derived isolates represent a distinct subgroup of HIV with differential properties, particularly cellular tropism.

An HIV type 1 isolate (HIV-1_{SF128A}) recovered at autopsy from the spinal cord of an HIV-1-infected individual with dementia displays biologic and serologic properties characteristic of brain-derived isolates (6). HIV-1_{SF128A} does not grow in any cultured T-cell lines, such as HUT 78, CEM, or Jurkat, or in the monocytic cell line U937 but can be propagated efficiently in peripheral blood mononuclear cells (PBMC) or primary macrophages. The viral gene(s) responsible for these distinct biological properties and host-range specificities of HIV-1_{SF128A} has not been defined. Thus, a detailed molecular characterization of this isolate should generate valuable information concerning the regulation of viral replication and pathogenesis.

MATERIALS AND METHODS

Cell cultures. Phytohemagglutinin (3 μ g/ml)-stimulated PBMC from HIV-1-seronegative individuals were prepared on Ficoll-Hypaque gradients as described elsewhere (10) and propagated in RPMI 1640 medium containing 10% heat-inactivated (56°C for 30 min) fetal calf serum, glutamine (2

mM), penicillin (100 U/ml), streptomycin (100 μ g/ml), and 5% interleukin-2 (Pharmacia ENI Diagnostic, Inc., Silver Spring, Md.). Purified CD4⁺ cells were prepared by the panning procedure with Leu 3a monoclonal antibodies (Becton Dickinson, Mountain View, Calif.) as described previously (35) and maintained in the same medium. The HUT 78 cell line (obtained from the American Type Culture Collection, Rockville, Md.) was maintained in the same medium without interleukin-2. Primary monocytes were obtained from Ficoll-Hypaque gradient-purified PBMC by the plastic adherent technique (6, 13). Adherent cells were cultured for 10 to 12 days in RPMI 1640 medium supplemented with 10% fetal calf serum, 5% heat-inactivated human serum, and 1% antibiotics to allow differentiation into macrophages. Human rhabdomyosarcoma (RD-4) cells were obtained from the American Type Culture Collection and maintained as monolayer cultures in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and antibiotics.

Virus. HIV-1_{SF2}, our prototype peripheral blood isolate (formerly called AIDS-associated retrovirus ARV-2), was recovered by cocultivation of mitogen-stimulated PBMC from seronegative donors with PBMC from a patient with oral candidiasis (21). HIV-1_{SF2} had been molecularly cloned and sequenced (25, 33); the molecular clone is biologically active (19). HIV-1_{SF128A} was obtained by cocultivation of PBMC from seronegative donors with spinal cord tissue obtained at autopsy from an HIV-1-positive patient with dementia (6, 20). Both isolates were grown to high titers in PBMC, reaching levels of reverse transcriptase (RT) activity of >10⁶ cpm/ml. Culture fluids were then filtered through 0.45- μ m-pore-size filters and frozen at -70°C in 1-ml aliquots.

HIV infection and transfection. Human PBMC, CD4⁺ cells, HUT 78 cells, and primary macrophages were infected with HIV-1 inocula of equal RT level (10⁶cpm/ml) as described previously (6, 10). Transfection of HIV-1 DNA into human PBMC and the RD-4 cell line by the DEAE-dextran

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TABLE 1. Comparison of host range properties of HIV-1_{SF2mc} and HIV-1_{SF128A}

Strain or clone	Initial source	Replication ^a in:		
		PBMC	HUT 78 cells	Mφ
HIV-1 _{SF2mc} ^b	PBMC	1,150.0	1,155.0	1.0
HIV-1 _{SF128A}	Spinal cord tissue	797.0	1.0	616.0
RHIV-1		521.0	2.0	121.0
RHIV-2		224.0 ^c	1.0	116.0
RHIV-3		463.0	1.0	309.0
RHIV-4		738.0	609.0	2.0
RHIV-5		328.0 ^c	2.0	125.0
RHIV-6		585.0	48.0	1.0

^a Replication of recombinant viruses was determined as described in Materials and Methods. Virus replication was measured by levels of RT activity (10³ cpm/ml) detected in culture supernatants 3- to 4-day intervals. For replication in PBMC, RT activity at 10 days postinfection is shown. For replication in HUT 78 cells and primary macrophages (Mφ), RT values at 15 days postinfection are shown. All data shown are representative of three independent experiments.

^b mc, Molecular clone.

^c Replication of RHIV-2 and RHIV-5 in PBMC reached RT titers of >5 × 10⁵ cpm/ml at 12 days postinfection.

and calcium phosphate precipitation methods, respectively, was performed as described previously (19).

Cloning of HIV-1_{SF128A} provirus. In order to clone the proviral genome, PBMC from a seronegative donor were infected with HIV-1_{SF128A}. At 10 days postinfection, about 10⁸ cells from infected cultures were collected, washed, and disrupted, and the viral DNA was isolated by the Hirt extraction procedure (14). The Hirt method-extracted DNA was then digested with *Sac*I and fractionated on a 10 to 40% sucrose gradient, and each fraction was then tested for hybridization with an HIV-1_{SF2} probe. Positive fractions were then isolated and cloned into a *Sac*I-cut lambda Wes vector (18). A recombinant library of about 5 × 10⁵ phages was then screened with the HIV-1_{SF2} probe (25) for positive clones by standard techniques (32).

Nucleotide sequence accession number. The sequence data presented in this article have been submitted to the GenBank and EMBL data bases under the accession number M38673.

RESULTS

Biologic properties of HIV-1_{SF128A}. In contrast to the peripheral blood HIV-1 isolate, HIV-1_{SF2}, the brain-derived HIV-1_{SF128A} strain does not productively infect the HUT 78 T-cell line but replicates efficiently in primary macrophages (Table 1). High levels of RT activity were detected in supernatants of infected macrophages for over 35 days in culture. Furthermore, HIV-1_{SF128A} is not highly cytopathic for CD4⁺ lymphocytes and does not down-modulate the CD4 receptor molecule upon infection of these cells (6). These biologic features of HIV-1_{SF128A} are characteristic of brain-derived HIV-1 isolates (6).

Characterization of the HIV-1_{SF128A} clone. Several positive clones were identified from the recombinant library, and one of them, which encompasses a 9-kb viral insert, was then characterized further. This clone contains the entire viral genome except for part of the long terminal repeat (LTR). Thus, this clone is not biologically active. Restriction endonuclease digestion mapping of the HIV-1_{SF128A} was performed and compared with mapping of HIV-1_{SF2}. The two viruses share many restriction enzyme sites. Both HIV-

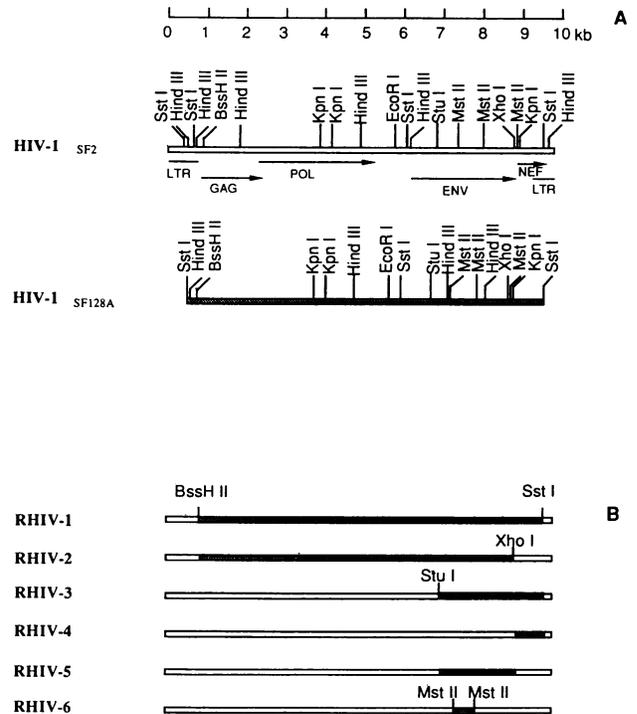


FIG. 1. Schematic representation of the genomes of HIV-1_{SF2}, HIV-1_{SF128A}, and their recombinant clones. (A) Restriction enzyme map of HIV-1_{SF128A} in comparison with the map of HIV-1_{SF2}. The restriction map of HIV-1_{SF2} has been determined previously (33). (B) Structures of HIV-1_{SF2}/HIV-1_{SF128A} recombinant clones. These clones were constructed by using specific restriction enzymes as described in the text.

1_{SF128A} and HIV-1_{SF2} have common unique *Eco*RI, *Bss*HII, *Stu*I, and *Xho*I sites (Fig. 1A). The coding sequences across the *Bss*HII, *Stu*I, and *Xho*I sites were all conserved. These sites were then subsequently used for generation of recombinant clones between HIV-1_{SF2} and HIV-1_{SF128A} (Fig. 1B). The minor restriction enzyme site differences between the two strains included two extra *Hind*III sites in the *env* gene of HIV-1_{SF128A} that were not present in HIV-1_{SF2}. Conversely, *Hind*III sites in the *gag* gene and 5' end of the *env* gene of HIV-1_{SF2} were not found in HIV-1_{SF128A}. Differences were also observed with other enzyme sites such as *Bg*II and *Pvu*II (data not shown). However, no substantial changes in genomic organization between HIV-1_{SF128A} and HIV-1_{SF2} were found.

Sequence analysis of HIV-1_{SF128A} *env* and *nef* genes. Since the 3' half of the viral genome encompassing the *env* and *nef* genes could be the critical region that affects viral growth and cellular tropism (5, 11, 24, 36), we determined the genomic sequence of this region. The nucleotides and the predicted amino acid sequences of the *env* and *nef* genes were then compared with those of HIV-1_{SF2}. The differences in sequence between the two isolates are summarized in Table 2. The *env* genes show an overall homology in nucleotide sequence of 91.1% and an amino acid homology of 85.1%. As expected, the variations are much greater in gp120 than in gp41. Most of the changes in gp120 reside in the hypervariable V₁, V₂, and V₄ domains, as well as in the CD4-binding domain (amino acids 411 to 464) (Fig. 2). Substitutions, deletions, and insertions were also observed, especially in the beginning of the CD4-binding domain,

TABLE 2. Sequence homology between HIV-1_{SF2mc} and HIV-1_{SF128A}

Gene or gene segment	% Homology	
	Nucleotides	Amino acids
Total <i>env</i>	91.1	85.1
gp120	88.7	80.9
gp41	94.7	91.3
<i>nef</i>	96.0	90.4

where there is a stretch of nine amino acid insertions. In addition, changes in the putative gp41 fusion domain were noted (amino acids 519 to 546). Three independent *env* gene clones of HIV-1_{SF128A} were sequenced, and all of these changes in the variable, CD4-binding, and fusion domains were conserved among all three clones (data not shown).

Comparison of the *nef* sequences revealed a 96% nucleotide homology and a 90.4% amino acid homology between the two isolates. However, a stretch of 45 duplicated amino acids is found in the HIV-1_{SF128A} *nef* sequence at the beginning of the LTR in the U₃ region (Fig. 3). This duplication makes the HIV-1_{SF128A} *nef* protein 251 amino acids long, whereas the HIV-1_{SF2} *nef* protein is only 210

amino acids long. This *nef* duplication could have occurred during viral replication or during cloning. However, *nef* duplication has also been observed in another brain isolate of HIV-1 (2). The biological significance of this duplication is currently not known.

Generation of recombinant clones between HIV-1_{SF128A} and HIV-1_{SF2}. In order to locate the gene(s) that is responsible for the specific cellular tropism for HIV-1_{SF128A}, a series of recombinants were generated between HIV-1_{SF128A} and HIV-1_{SF2} (Fig. 1B). These clones were made by using the *Bss*HII, *Stu*I, *Xho*I, and *Mst*II restriction enzyme sites. The RHIV-1 clone expresses all of the HIV-1_{SF128A} genes by using the HIV-1_{SF2} LTR. This clone was made by exchanging the complete genomes, except the LTRs, of the two viruses. A second recombinant clone, RHIV-2, was generated by replacing the *Bss*HII-*Xho*I fragment of HIV-1_{SF2} with that of HIV-1_{SF128A}. This fragment extends from the end of the 5' LTR to the 5' region of *nef* and contains complete *gag*, *pol*, *vif*, *env*, *tat*, *vpr*, *vpu*, and *rev* genes of HIV-1_{SF128A}. The resulting clone expresses all HIV-1_{SF128A} genes except the *nef* gene which encodes a hybrid protein with the first 34 amino acids from HIV-1_{SF128A} and the rest of the protein from HIV-1_{SF2}. A third clone, RHIV-3, was generated by replacing a 2.8-kb *Stu*I-*Sst*I fragment of HIV-

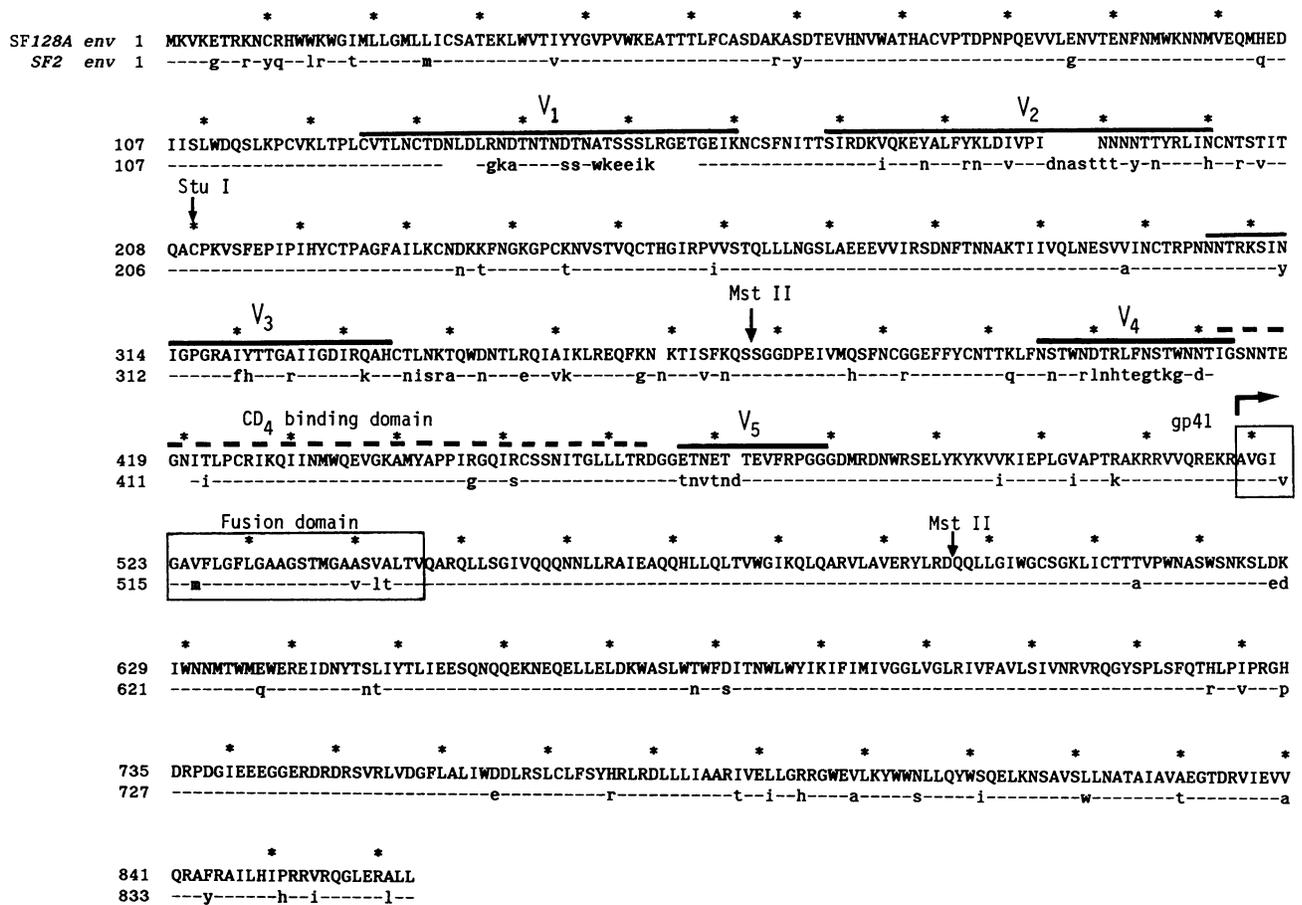


FIG. 2. Alignment of the *env* proteins of HIV-1_{SF2} and HIV-1_{SF128A}. The translated amino acid sequences of HIV-1_{SF128A} gp160 are shown on the upper line of each pair of lines, and the sequences of HIV-1_{SF2} are shown on the lower line of each pair. Dashes represent sequence homology between the two sequences, and differences are shown with lowercase letters. Hypervariable regions (27) are marked with heavy lines, the putative CD4-binding domain (9, 17) is marked by heavy dotted lines, and the gp41 fusion domain (3, 12) is boxed. Restriction enzyme sites that were used to generate HIV-1_{SF2}/HIV-1_{SF128A} recombinant clones are indicated by arrows.

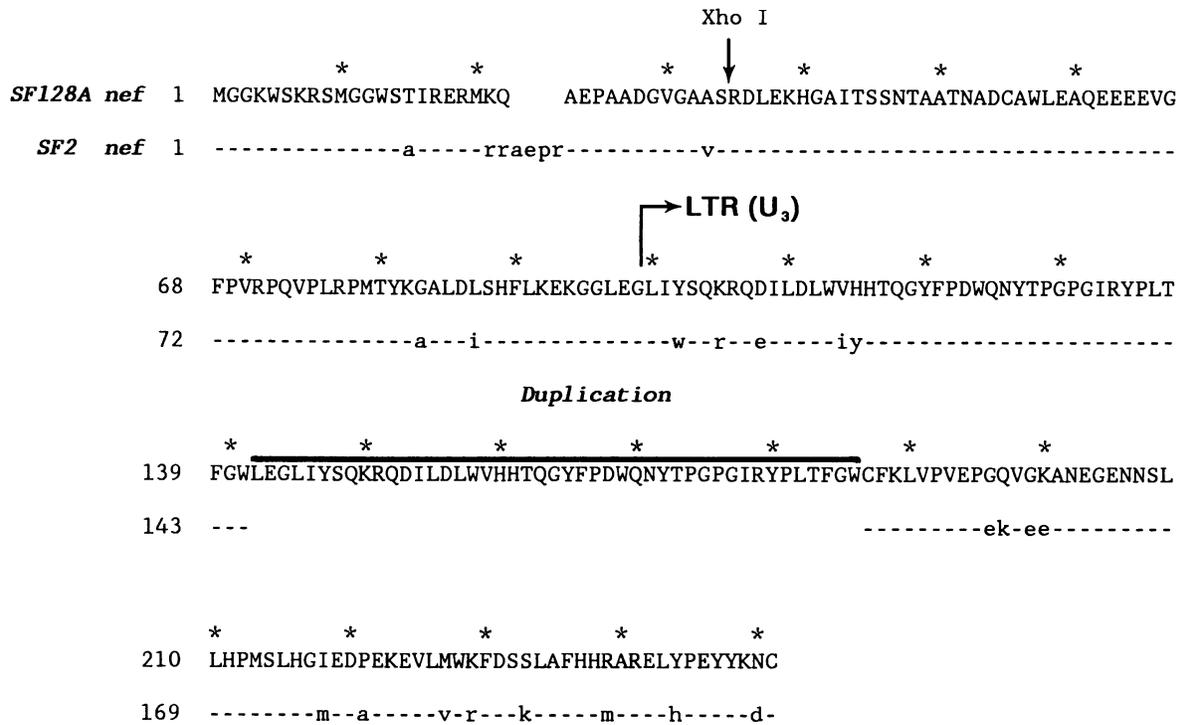


FIG. 3. Alignment of the *nef* proteins of HIV-1_{SF2} and HIV-1_{SF128A}. The translated amino acid sequence of HIV-1_{SF128A} *nef* is shown on the upper line of each pair of lines, and the sequence of HIV-1_{SF2} is shown on the lower line of each pair. Dashes represent sequence homology, and differences are shown with lowercase letters. The arrow indicates the beginning of the LTR sequence. The heavy line indicates a stretch of 45 amino acids representing a direct repeat which is found only in HIV-1_{SF128A}. The *Xho*I site that was used to generate HIV-1_{SF2}/HIV-1_{SF128A} recombinant clones is indicated by an arrow.

1_{SF2} with that of HIV-1_{SF128A}. This clone expresses all the genes of HIV-1_{SF2} origin except the *nef* gene, which is from HIV-1_{SF128A}, and a hybrid *env* gene. The resulting envelope glycoprotein consists of 210 amino acids at the amino terminus encoded by the HIV-1_{SF2} sequence and the rest of the protein encoded by the HIV-1_{SF128A} sequence. A fourth clone, RHIV-4, was generated by replacing the HIV-1_{SF2} *nef* *Xho*I-*Sst*I fragment with that of HIV-1_{SF128A}. This clone expresses a hybrid *nef* protein with the first 34 amino acids from HIV-1_{SF2} and the rest from HIV-1_{SF128A}. A fifth clone, RHIV-5, was generated by exchanging a *Stu*I-*Xho*I fragment between the two viruses. The resulting clone expresses a hybrid *env* and a hybrid *nef* protein. The hybrid envelope glycoprotein is identical to the one expressed by RHIV-3, with the HIV-1_{SF2} sequence encoding the first 210 amino acids and the rest of the protein encoded by HIV-1_{SF128A}. The hybrid *nef* protein is also identical to the one expressed by RHIV-2, with the first 34 amino acids from HIV-1_{SF128A} and the rest from HIV-1_{SF2}. Finally, a sixth clone that involved the replacement of a 658-bp *Mst*II *env* fragment from HIV-1_{SF2} with the same fragment from HIV-1_{SF128A}, was constructed. This region includes the V₄ and V₅ domains of gp120 (27), the CD4-binding domain (9, 17), and the fusion domain of gp41 (3, 12).

Replicative properties of HIV-1_{SF128A} and HIV-1_{SF2} recombinant viruses. Plasmid DNA containing the various recombinant clones was transfected into normal human PBMC or the human rhabdomyosarcoma RD-4 cell line (19). All clones yielded viruses upon transfection, and the progeny HIV-1 strains produced were then tested for their biologic properties by infection of HUT 78 T cells or primary peripheral

blood macrophages. Infection of PBMC served as a control, and all recombinant viruses grew to comparable titers in human PBMC within 10 to 12 days postinfection. As expected, HIV-1_{SF2} replicated well and produced high-titered progeny virus in HUT 78 cells; moreover, as reported previously (4, 6), it did not productively infect peripheral blood macrophages (Table 1). In contrast, RHIV-1, RHIV-2, RHIV-3, and RHIV-5 all infected and replicated well to high titers in peripheral blood macrophages, but none of these recombinant viruses could infect HUT 78 cells. These clones thus reflect the biological properties of the parental virus, HIV-1_{SF128A}, particularly its macrophage tropism. The results indicate that clones that had acquired the HIV-1_{SF128A} *env* gene sequence encoding amino acids 211 onward as well as sequences encoding the first 34 amino acids of the *nef* protein produced viruses that were macrophage tropic. The only clones that retained the HIV-1_{SF2} T-cell-tropic phenotype were RHIV-4 and RHIV-6. RHIV-4 contains an HIV-1_{SF128A} fragment coding for only the *nef* gene, and RHIV-6 contains the C terminus of gp120 and the fusion domain of gp41 from HIV-1_{SF128A}. RHIV-6, however, replicates with slower kinetics in HUT 78 cells (Table 1). In contrast to HIV-1_{SF2}, which replicated rapidly in HUT 78 cells (peak RT activity at 12 days postinfection), this recombinant virus reached peak RT activity (>500 × 10³ cpm/ml) at 20 to 25 days postinfection.

DISCUSSION

Our results demonstrate that no genomic structural differences between HIV-1_{SF128A} and HIV-1_{SF2} are responsible

for the different cellular tropisms displayed by the two HIV-1 isolates. The replicative properties of our recombinant clones indicate that the *env* gene product from amino acid 211 onward and the first 34 amino acids of the *nef* protein contain determinants responsible for T-cell and/or macrophage tropism. Much of the variation in *env* between HIV-1_{SF128A} and HIV-1_{SF2} is located within the hypervariable regions and the CD4-binding domain. Within the first 34 amino acids of the *nef* protein, there is a deletion of 4 amino acids in HIV-1_{SF128A} (alanine, glutamic acid, proline, and arginine), in addition to 4 amino acid changes (alanine, valine, and two arginines). The four amino acid deletions occur in a region reported to be duplicated in other HIV-1 strains recovered from brain tissue (2; W. O'Brien, Y. Koyanagi, J. Zack, and I. S. Y. Chen, VIth Int. Conf. AIDS, abstr. no. SA1, 1990). Since none of these molecularly cloned brain-derived isolates have been found to be biologically active, the functional significance of genomic alterations in this particular region of the *nef* gene cannot be addressed directly. The *nef* protein has been reported to be a negative regulatory protein (1, 24, 28), but its functional domains have not been defined. In view of the observation that there are no differences in viral production between HIV-1_{SF2} and HIV-1_{SF128A} upon transfection into PBMC, we assume that the functions contained within the first 34 amino acids of the *nef* protein of HIV-1_{SF128A} and HIV-1_{SF2} are similar. We conclude, therefore, that the *env* gene contains the major determinants of T-cell and/or macrophage tropism.

Previous studies with recombinant viruses generated between HIV-1 strains with different biologic properties have implied a role for the *env* gene in determining host cell tropism (5, 11, 36). However, the exchanged regions contain other viral genes, such as *tat*, *rev*, and *vpu*. The present study was conducted with recombinant viruses by exchanging only the *env-nef* region. Our finding that the RHIV-3 and RHIV-5 viruses, which contain the V₁ and V₂ regions of the *env* gene from HIV-1_{SF2}, still display the macrophage tropism characteristic of HIV-1_{SF128A} excludes the possibility that these regions play a role in determining this cellular tropism. Furthermore, the observation that RHIV-6 is unable to productively infect primary macrophages indicates that the V₄ and V₅ domains of gp120, the CD4-binding domain, and the fusion domain of gp41 do not contain the viral determinants of macrophage tropism. The region encoding these domains, however, is necessary for efficient infection of the HUT 78 T-cell line. Substitution of this region (*MstII-MstII* fragment) in HIV-1_{SF2} with corresponding sequences from HIV-1_{SF128A} resulted in a recombinant virus (RHIV-6) that replicates with slower kinetics in HUT 78 cells (Table 1). This observation could reflect the fact that multiple determinants are involved in infection of T-cell lines. An earlier report by Cordonnier et al. (7) had indicated that a single amino acid substitution at three positions in the CD4-binding domain can eliminate the infectivity of the U937 monocytic cell line but retain the infectivity of a T-cell line. However, our sequence analysis of the CD4 domain, especially at those three positions, of HIV-1_{SF128A} indicated that the isoleucine at position 427, lysine at 528, and glutamine at 429 are identical to the corresponding amino acids found in HIV-1_{SF2}. Thus, some other portion of the envelope gene must be involved in efficient replication of HIV-1.

In conclusion, our studies with recombinant viruses further demonstrate that a *StuI-MstII* fragment of the envelope glycoprotein gene of HIV-1_{SF128A} encoding the V₃ domain (30, 31) or the *MstII-XhoI* fragment encoding the C terminus

of gp41 helps to determine the macrophage tropism of HIV-1_{SF128A}. A *StuI-XhoI* fragment encoding the envelope glycoprotein of HIV-1_{SF2} from amino acid 211 onward contains determinants for T-cell tropism. Further fine mapping with recombinants between HIV-1_{SF128A} and HIV-1_{SF2} in the *env* gene should define the specific domain(s) responsible for the differential infectivity of macrophage and T-cell lines by HIV-1.

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