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Viral Determinants of Human Immunodeficiency Virus Type 1 T-Cell or Macrophage Tropism, Cytopathogenicity, and CD4 Antigen Modulation

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The genome of the human immunodeficiency virus type 1 (HIV-1) is highly heterogeneous. Some of this genomic variability is reflected in the biologic and serologic differences observed among various strains of HIV-1. To map the viral determinants that correlate with pathogenicity of the virus, recombinant viruses were generated between biologically active molecular clones of HIV-1 strains that show differences in T-cell or macrophage tropism, cytopathogenicity, CD4 antigen modulation, and susceptibility to serum neutralization. The results of these studies indicate that the envelope region contains the major determinants of these viral features. Further studies with sequence exchanges within this region should help identify specific domains that contribute to HIV pathogenesis.

The genomes of different human immunodeficiency virus type 1 (HIV-1) strains display a high degree of sequence variations (2, 5, 24, 49, 55, 63). Besides this well-recognized genetic heterogeneity, HIV-1 isolates vary in their host range tropism (8, 10, 16, 34), kinetics of replication (4, 11, 32, 59), and susceptibility to serum neutralization (9). Furthermore, differences in their ability to down modulate the CD4 receptor molecule and to induce cytopathology in infected cells have been observed (12, 17, 25, 58, 59). Some of these biologic properties have correlated with pathogenicity of the virus (11, 60). Results from our previous studies showed that HIV-1 isolates recovered from patients with advanced disease are more cytopathic, replicate with faster kinetics and to higher titers, and display a wider host range than isolates obtained from the same individuals when they were healthy. The later isolates infected several established T-cell lines (e.g., HUT-78 and Jurkat), some B-cell lines, and primary peripheral blood macrophages (11). In addition, we have found that isolates recovered from the central nervous system display biologic and serologic properties which distinguish them from viruses recovered from peripheral blood (12). The HIV-1 genes that control these different biologic and replicative abilities of the virus, and hence influence pathogenesis, are still largely undefined. An understanding of the functional role of each viral gene in the replicative cycle and of the mechanism by which each gene exerts its effect are important steps towards the development of antiviral drugs and a vaccine.

In this report, structure and function studies were performed to begin mapping the HIV-1 genes that control different viral properties. Two HIV-1 isolates showing differences in T-cell and macrophage tropism, cytopathogenicity, CD4 receptor downmodulation, and sensitivity to serum neutralization have been molecularly cloned. Recombinant DNAs were generated by reciprocal exchange of genetic materials between the two isolates, and the biologic and serologic properties of the recombinant viruses obtained by recombinant DNA transfection were determined. The results indicate that the envelope region contains the major determinants of the viral properties examined.

MATERIALS AND METHODS

Cells. HUT-78 and Jurkat T-cell lines (obtained from the American Type Culture Collection) were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum, 2 mM glutamine, and 1% antibiotics (100 U of penicillin per ml and 100 μg of streptomycin per ml) (16). Phytohemagglutinin (3 μg/ml)-stimulated peripheral blood mononuclear cells (PMC) were maintained in the same medium, which contained interleukin-2 (5%) (Electronucleonics, 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 (65) and maintained in the same medium. Primary monocytes were obtained from Ficoll-Hypaque-gradient-purified PMC by the plastic adherent technique (12, 21). In order to allow for differentiation into macrophages, the 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 before infection with HIV-1 (11). Human rhabdomyosarcoma (RD-4) cells were obtained from the American Type Culture Collection and maintained as monolayer cultures in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and antibiotics (31).

Virus. HIV-1_{SF2}, our prototype peripheral blood HIV-1 isolate (formerly called acquired immune deficiency syndrome-associated retrovirus, ARV-2), was recovered by cocultivation of mitogen-stimulated PMC from seronegative donors with PMC from a patient with oral candidiasis (33). HIV-1_{SF162} was obtained by cocultivation of PMC from seronegative donors with cerebrospinal cord fluid of a HIV-1-seropositive patient with toxoplasmosis (12). Both isolates were grown to high titers in PMC (i.e., reaching levels of reverse transcriptase [RT] activity of $>10^6$ cpm/ml) and then frozen in 1 ml aliquots at −70°C for future studies. HIV-1_{SF2} had been molecularly cloned and sequenced (31, 38, 50).

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HIV-1_{SF162} was molecularly cloned from a library of recombinant bacteriophages constructed from the *Eco*RI partially digested genomic DNA of HIV-1_{SF162}-infected PMC.

HIV-1 infection. T-cell lines, purified CD4+ cells, or peripheral blood macrophages were treated with Polybrene (2 μg/ml) for 30 min at 37°C and exposed to 1 ml of virus-containing fluids (RT activity, ~10^6 cpm/ml or 100 50% tissue culture infectious doses) for 1 h at 37°C. Cells were then washed three times and maintained in culture medium. Culture supernatants of these infected cells were harvested at 3- to 4-day intervals and assayed for RT activity as described previously (27). The presence of HIV-1 viral antigens in infected cells was further confirmed by immunoblot analysis (45) or by a p25 HIV-1 core antigen enzyme-linked immunosorbent assay (Du Pont Co., Wilmington, Del.). CD4 receptor molecule expression on the surface of infected and uninfected cells was measured by flow cytometry (35), and cytopathic effect (CPE) was assessed by the presence of ballooning and syncytium formation in the infected CD4+ cultures.

HIV-1 neutralization. Virus neutralization assays were performed as described previously (9, 12). Briefly, 100 μl of 10-fold dilutions of heat-inactivated (56°C for 30 min) serum was incubated with an equal volume of virus-containing fluid (RT activity, ~10^6 cpm/ml) for 1 h at room temperature. The mixture was then used for infection of PMC. Control cultures received virus incubated with sera from seronegative healthy individuals. A two-third (67%) or greater reduction in RT activity at day 7 postinfection was considered indicative of serum neutralization. The three serum specimens used came from HIV-1-antibody-positive individuals (9).

Restriction enzyme mapping and DNA sequence analysis. Restriction mapping of viral DNA obtained from recombinant phage was performed according to methods described previously (38). The orientation of *gag* and *env* genes was identified by hybridization to HIV-1 *gag*- and *env*-specific probes. For DNA sequencing, portions of the 3′ subclone DNA of HIV-1_{SF162} were subcloned into single-stranded bacteriophage M13 vectors and sequenced by the dideoxynucleotide-chain termination method (42, 51). Homologous regions of the viral genes between HIV-1_{SF2} and HIV-1_{SF162} were identified by using the GENALIGN computer program (40).

Generation of recombinant DNA and viruses. A methodology similar to the one used in the studies of integration and expression of Rous sarcoma virus (37) and described in our previous study (66) was used to generate recombinant viruses. A common internal *Eco*RI site at the midpoint of the genome was identified in the biologically active molecular clones of HIV-1_{SF2} and HIV-1_{SF162}. The 5′ and 3′ *Eco*RI viral fragments generated after digestion with *Eco*RI were subcloned into a pUC19 plasmid vector. In addition, the 3′ subclone was manipulated to generate recombinants exchanging the *env* region. A 3.2-kilobase (kb) *Eco*RI-*Xho*I fragment containing the *env* and part of the *nef* gene regions was prepared from HIV-1_{SF2} and HIV-1_{SF162} 3′ *Eco*RI subclones. This 3.2-kb fragment was reciprocally substituted and ligated into heterologous 3′ plasmid, i.e., the 3.2-kb *Eco*RI-*Xho*I fragment from HIV-1_{SF2} into 3′ subclone of HIV-1_{SF162} deleted of the *Eco*RI-*Xho*I fragment and vice versa. These plasmids are designated the 3′ recombinant subclone. Plasmid DNA was then prepared by the alkaline lysis method (39) and banded twice in cesium chloride.

Recombinant viruses were generated by transfection of RD-4 cells with plasmid DNAs by the calcium phosphate precipitation method, followed by cocultivation with PMC from seronegative donors (31). Viruses were recovered at 7 to 10 days posttransfection. No difference in time for recovery of viruses or the efficiency of recombinant virus formation with this method was observed compared with transfection of RD-4 cells with the full-length DNA clones. To generate parental viruses, 5 μg each of DNA from the 5′ and 3′ subclones of the same virus was cotransfected into RD-4 cells. For 5′-3′ interisolate recombinant viruses, 5 μg of DNA of the 5′ subclone of one isolate and 5 μg DNA of the 3′ subclone of the other isolate were cotransfected. Finally, for recombinant viruses in the *env* region, 5 μg of the 5′ subclone of one isolate was cotransfected with 5 μg of DNA of the 3′ recombinant subclone of the same isolate.

Immunoblot and Southern blot analysis. For immunoblot analysis, PMC from seronegative donors were infected with parental or recombinant viruses. At peak RT activity (~12 days postinfection), 4×10^6 cells of each infected culture were collected and washed three times with phosphate-buffered saline. The cell pellets were then disrupted in buffer (0.05 M Tris hydrochloride [pH 7.8], 0.15 mg of dithiothreitol per ml, 0.1% Triton X-100) and analyzed by immunoblot on a 10% sodium dodecyl sulfate-polyacrylamide gel with the use of a pool of HIV-1-positive sera as described previously (45). For Southern blot analysis, high-molecular-weight DNA was extracted from 10^8 HIV-infected PMC by the sodium dodecyl sulfate-phenol-chloroform procedure as described previously (11, 38). A 20-μg portion of each infected cellular DNA was then digested to completion with restriction enzymes under conditions specified by the supplier (New England BioLabs, Inc., Beverly, Mass.). The restricted DNA fragments were then separated by electrophoresis on an 0.8% agarose gel and blotted onto nitrocellulose membranes, and the viral species were detected by hybridization with a ^{32}P-labeled probe representing the entire HIV-1 genome (11).

RESULTS

Biologic properties of HIV-1_{SF2} and HIV-1_{SF162} molecularly cloned virus. The recombinant phage DNA containing HIV-1_{SF2} and HIV-1_{SF162} viral DNA sequences was found to be biologically active upon transfection into PMC from seronegative individuals, as well as when transfected into the human rhabdomyosarcoma cell line RD-4. The biologic properties of molecular clones of HIV-1_{SF2} and HIV-1_{SF162} were the same as those of the uncloned parental viruses (Table 1). HIV-1_{SF2} replicated to high titers in established T-cell lines (HUT 78 and Jurkat) but was unable to productively infect primary peripheral blood macrophages. HIV-1_{SF2} infection of T-cell lines, or purified CD4+ cells, resulted in syncytium formation and down modulation of the CD4 receptor molecule, as measured by flow cytometry. Furthermore, this isolate was highly sensitive to serum neutralization. In contrast, HIV-1_{SF162} did not replicate efficiently in T-cell lines but grew to high titers in primary peripheral blood macrophages. Thus, HIV-1_{SF162} is a macrophage-tropic HIV-1 isolate. This isolate, although growing to high titers in human peripheral blood CD4+ lymphocytes, was not very cytopathic and did not down modulate the CD4 receptor molecule. It was also resistant to serum neutralization by the three reference HIV-1-positive serum specimens used in these studies. All these properties of HIV-1_{SF162} are characteristic of HIV-1 isolates recovered from the central nervous system (12).

Genomic and amino acid sequence comparisons of HIV-1_{SF2} and HIV-1_{SF162}. The nucleotide sequences of genes in the 3′

TABLE 1. Biologic properties of molecular clones of HIV-1_{SF2} and HIV-1_{SF162}

Strain	Initial source	Replication (RT activity) in				CPE in CD4+ cells[c]	% Modulation of CD4 molecule[d]	Sensitivity to neutralization[e]
		T-cell line[a]		Macrophage[b]				
		d7	d30	d7	d30			
HIV-1_{SF2}	PMC	50.5	1,778.6	1.5	1.0	+	38	≥1:1,000
HIV-1_{SF162}	CSF[f]	1.0	0.7	217.8	153.4	−	84	≤1:10

[a] The T-cell line used was HUT 78. Similar results were obtained with the Jurkat line. Virus replication was measured by levels of reverse transcriptase (RT, $\times 10^3$ cpm/ml) activity detected in the culture supernatant at 3- to 4-day intervals for up to 35 days. All data shown are representative of three or more independent experiments.

[b] Primary peripheral blood macrophages were prepared by the plastic adherence method (12, 21). RT activity ($\times 10^3$ cpm/ml) in culture supernatant was assayed at 3- to 4-day intervals for up to 35 days.

[c] CPE was assessed by the presence of ballooning and syncytium formation in infected peripheral blood CD4+ lymphocytes. +, >50% cells showing CPE; −, <20% cells showing CPE.

[d] CD4 antigen modulation is defined as the disappearance of the surface CD4 receptor molecule upon HIV-1 infection of purified CD4+ peripheral blood lymphocytes as measured by flow cytometry (35). Data shown are percent staining with Leu 3a antibodies (Becton Dickinson) at day 14 postinfection.

[e] Serum neutralization was performed as described previously (9, 12).

[f] CSF, Cerebrospinal fluid.

region of the HIV-1_{SF162} genome are presented in Fig. 1. The predicted amino acid sequences of the viral genes in the 3′ region were also determined and compared with HIV-1_{SF2} (Table 2). The two isolates showed 87.5 to 94.8% homology on the nucleotide sequence and 82.2 to 89.5% homology on the predicted amino acid sequence in the genes compared. Unlike HIV-1_{SF162}, which encodes a functional *vpu* gene of 81 amino acids, HIV-1_{SF2} encodes a truncated *vpu* gene (66). An 89.4% nucleotide sequence homology was observed in the *env* gene, which encodes for 848 amino acids, with an overall identity of 84.6%. An alignment of the predicted amino acid sequence of the *env* glycoprotein of HIV-1_{SF2} and HIV-1_{SF162} is presented in Fig. 2. The data show that differences were clustered primarily within the five hypervariable regions of gp120 (43), including the RP135 loop region (46, 48) (amino acids 298 to 412, according to the coding sequence of HIV-1_{SF162}). The 22 cysteine residues, the CD4-binding domain (amino acids 403 to 446) (15, 30), and the gp120/gp41 cleavage site (amino acid 503) (6, 23) are highly conserved in the two isolates. Amino acid differences were observed in the putative gp41 fusion domain (amino acids 503 to 530) (7, 20, 22). These are the absence of a glycine in HIV-1_{SF162} and the substitution of a leucine and arginine in that strain for isoleucine and valine residues, respectively, in HIV-1_{SF2}.

Comparison of restriction endonuclease maps of HIV-1_{SF2} and HIV-1_{SF162} genomes. Restriction endonuclease digestion mapping of the HIV-1_{SF162} genome was performed and compared with HIV-1_{SF2}. A common internal *Eco*RI site that divided each genome into two fragments was identified. The 5′ fragment contained the *gag*, *pol*, *vif*, *vpr*, and 5′ long terminal repeat regions. The 3′ fragment contained the *env*, *tat*, *rev*, *vpu*, *nef*, and 3′ long terminal repeat regions. A comparison of the 3′ genomes of HIV-1_{SF2} and HIV-1_{SF162} revealed minor restriction site differences (Fig. 3). For example, the absence of a *Hin*dIII and *Ssp*I site and the addition of a *Pst*I site were found in HIV-1_{SF162}. Furthermore, the restriction enzyme patterns of *Sca*I and *Bgl*II reveal some differences. A common *Xho*I site was present in the *nef* region of each viral genome; therefore, a double digestion of the 3′ portion of the genome with *Eco*RI and *Xho*I could be used to release a 3.2-kb fragment containing the *env* region plus 36 or 38 amino acids from the N terminus of *nef* (Fig. 3). These two enzymes, *Eco*RI and *Xho*I, were then used for the generation of recombinant DNA reciprocally exchanging genomic materials in the 3′ region.

Generation of recombinant DNAs and viruses between HIV-1_{SF2} and HIV-1_{SF162}. Recombinant DNAs were generated between the HIV-1_{SF162} and HIV-1_{SF2} genomes for structure and function studies as described in Materials and Methods. The parental 5′ *Eco*RI and 3′ *Eco*RI plasmid DNA, as well as the recombinant *env* DNA plasmids were linearized and then used in cotransfection of the human rhabdomyosarcoma (RD-4) cells. For example, to generate the parental isolates, the 5′ *Eco*RI and the 3′ *Eco*RI fragments of the same isolate were cotransfected into RD-4 cells. These cells were then cocultivated with PMC to recover high titers of the recombinant viruses generated from in vivo ligation of the transfected fragments (37, 66). Schematic structures of the parental and interstrain genomes obtained are presented in Fig. 4. Infectious recombinant viruses recovered, designated R1 to R6, were then used for host range, cytopathology and serum neutralization studies. No substantial difference was observed in the kinetics or titers of replication of the recombinant viruses when cultured in PMC from seronegative donors (Table 3). Furthermore, the parental viruses, R1 and R2, generated by this method displayed properties identical to those of viruses recovered by transfection with the full-length viral DNA (Table 1).

Structural and genomic analyses of recombinant viruses. Immunoblot and Southern blot analyses of the recombinant viruses obtained were performed to confirm their genomic and protein structures. Immunoblot analysis of HIV-1_{SF162}- and HIV-1_{SF2}- infected cells showed that the major external envelope glycoprotein (gp120), the p55 precursor, and p17 *gag* proteins of the two viruses differ in molecular size (Fig. 5A). The size differences in these viral proteins, therefore, served as markers for the presence of the respective 5′ *gag* and 3′ *env* regions. For example, the R5 virus was shown to contain the envelope proteins of HIV-1_{SF162} and *gag* proteins of HIV-1_{SF2}; the R6 virus contains the envelope proteins of HIV-1_{SF2} and *gag* proteins of HIV-1_{SF162} (Fig. 5A). Southern blot analyses also demonstrated the characteristic restriction endonuclease fragment hybridization patterns expected for each recombinant virus upon digestion of their genomic DNAs with *Sca*I-*Xho*I. Results are shown for recombinant viruses R5 and R6 (Fig. 5B). A 2.2- and a 1.49-kb *Sca*I-*Xho*I fragment, both present in recombinant virus R5, are characteristic fragments in the *env* region of HIV-1_{SF162} (Fig. 3 and 5B). A doublet of 0.7 kb and a 2.1-kb fragment are characteristic of the *env* region of HIV-1_{SF2}; these fragments are present in recombinant virus R6.

Biologic and serologic characterizations of HIV-1_{SF2}/HIV-1_{SF162} recombinant viruses. The results of biologic and sero-

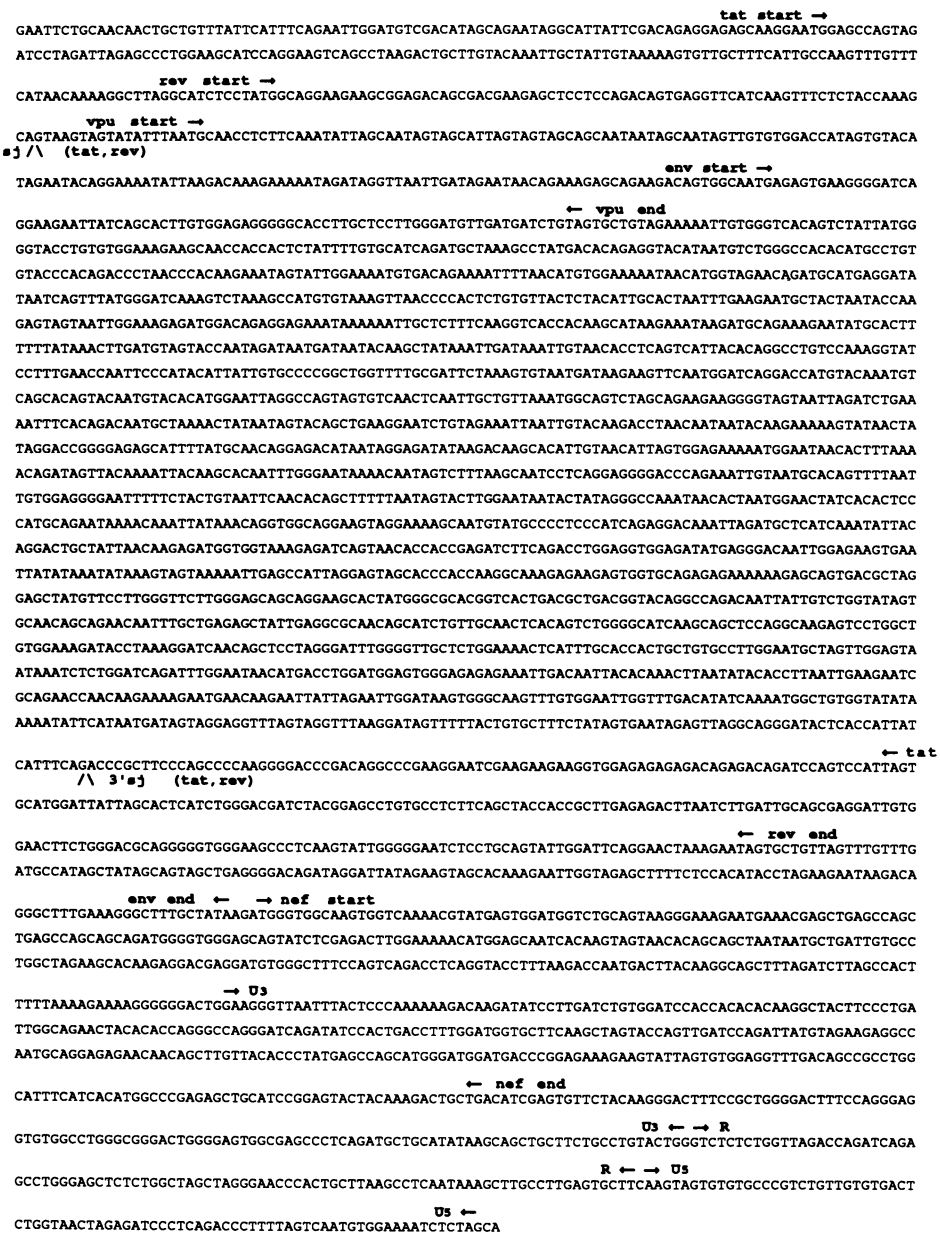

FIG. 1. Nucleotide sequence of the 3′ genomic region of the HIV-1_{SF162} molecular clone. Restriction enzyme DNA fragments of recombinant phage DNA were isolated after electophoresis in polyacrylamide or agarose gels, cloned into M13 vectors, and used as templates for DNA sequencing by the dideoxy-chain termination method (50, 51). Arrows (⟶, ⟵) indicate the start and end of each open reading frame. Splice junctions (sj) are noted, and the U3, R, and U5 regions of the long terminal repeat are also designated.

logic studies with the recombinant viruses generated are summarized in Table 3. As noted above, all viruses grew to comparable titers in PMC. Recombinant viruses R4 and R6 displayed biologic properties characteristic of those of the parental HIV-1_{SF2} strain (R1), whereas the features of the recombinant viruses R3 and R5 were similar to those of HIV-1_{SF162} (R2). No substantial difference in titers or kinetics of virus replication was observed for R4 and R6 in HUT 78 cells as compared with R1 (Table 3) or for R3 and R5 in primary macrophages compared with R2 (Table 3). Therefore, in agreement with our other studies in which recombinant viruses between HIV-1_{SF2} and HIV-1_{SF33} were used (66), the cytopathogenicity of HIV-1 was found associated with a 3.2-kb *Eco*RI-*Xho*I fragment of the 3′ region of the genome. Moreover, the other properties examined in the present studies, such as T-cell and macrophage tropism, the ability to down modulate the CD4 receptor molecule, and sensitivity to serum neutralization, also segregated with this region (Table 3).

DISCUSSION

The observations with recombinant viruses in which the HIV-1_{SF2} and HIV-1_{SF162} strains were used indicate that the sequences within nucleotides 5750 to 8914 (*Eco*RI-*Xho*I fragment) contain determinants of some major biologic prop-

TABLE 2. Sequence homologies between HIV-1_{SF2} and HIV-1_{SF162}[a]

Region	Percent identity	
	Nucleic acid	Amino acid
rev	92.0	87.2
tat	91.1	82.2
vpu	92.1	89.5
env		
Total	89.4	84.6
gp120	87.5	82.5
gp41	92.3	87.9
nef	92.5	88.6
LTR	94.8	

[a] DNA from a recombinant HIV-1_{SF162} lambda clone (clone 40-1) was digested with *Eco*RI, and the 3′ *Eco*RI genomic fragment was subcloned into pUC19 plasmid vector. Restriction enzyme DNA fragments of the insert DNA were isolated, cloned into M13 vectors, and used as templates for DNA sequencing by the dideoxynucleotide-chain terminator method as described previously (50, 51). Homologous regions of the viral genes were identified by using the GENALIGN computer program (40).

erties of the HIV-1 strains examined. This region identified has coding sequences for the *env*, *tat*, *rev*, and *vpu* genes and 36 amino acids from the N terminus of *nef* (Fig. 3). The *tat* and *rev* genes are positive regulators of HIV-1 replication (3, 18, 53, 54, 64), and the *vpu* gene has been shown to facilitate efficient production of mature infectious virions (13, 57, 61). In contrast, the *nef* gene can be a down regulator of HIV-1 replication (1, 36, 44, 62). The *vpu* gene in HIV-1_{SF2} is truncated; termination occurs after the 38th amino acid. However, similar amounts of virus production (RT activity, $>10^6$ cpm/ml within 12 days postinfection, Table 3) were made in PMC infected with the different prototype recombinant viruses. Furthermore, similar levels of viral protein and DNA (Fig. 5) were synthesized in the infected cells. These observations suggest that the *vpu* and regulatory gene (*tat* and *rev*) functions of HIV-1_{SF2} and HIV-1_{SF162} are comparable. Nevertheless, further studies comparing the activities of these regulatory genes and the effects of a truncated *vpu* protein on virus replication in other cell types will more definitively address their possible functional roles. We conclude from these studies, however, that the envelope region of HIV-1 is most likely the major determinant of T-cell or macrophage tropism, CD4 receptor down modulation, and the cytopathology observed. As expected, this region also conferred sensitivity to serum neutralization.

The association of the envelope region with HIV-1 cytopathicity and sensitivity to serum neutralization is in agreement with published observations in which recombinant proteins or synthetic peptides were used (26, 28, 29, 41, 47,

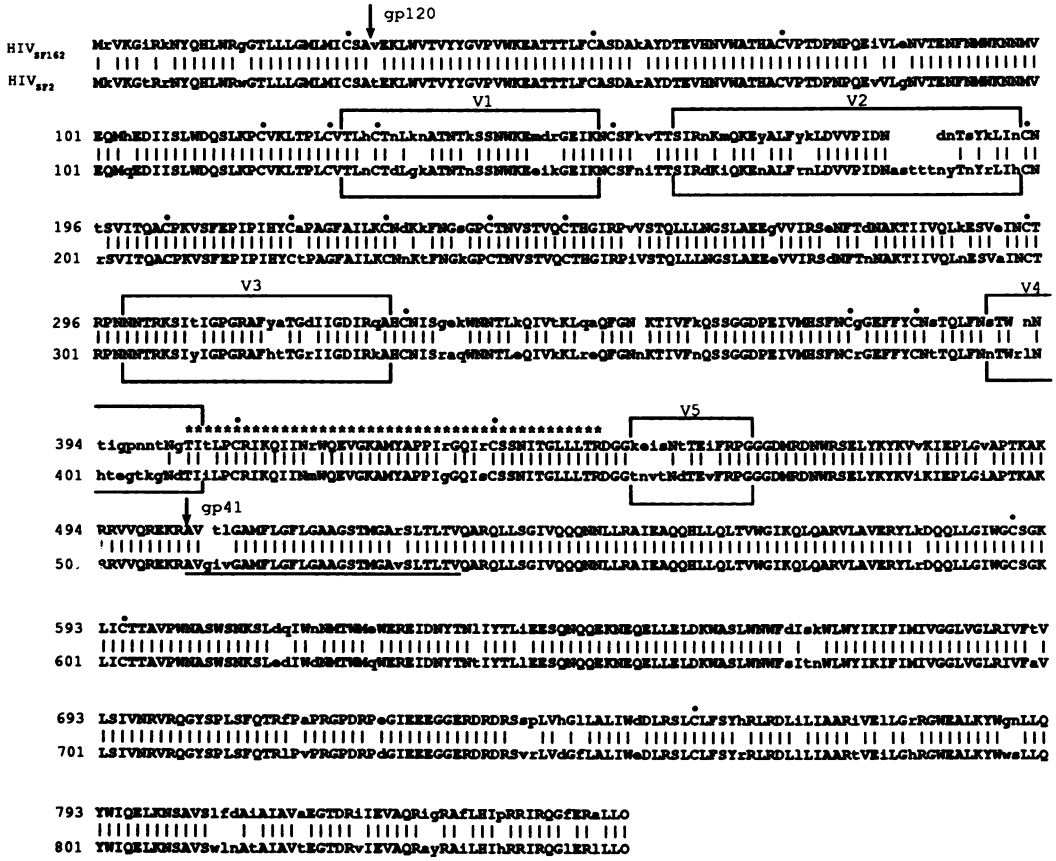

FIG. 2. Alignment of the *env* proteins of HIV-1_{SF2} and HIV-1_{SF162}. Alignment of the predicted amino acid sequences of the gp160 proteins of HIV-1_{SF162} (upper line) and HIV-1_{SF2} (bottom line) by using the GENALIGN program is presented in 1-letter amino acid code. Vertical bars and capital letters indicate amino acid identity. Hypervariable regions (43) are boxed. Potential cleavage sites (6, 23) are indicated by arrows. Dots show cysteine residues. The CD4-binding domain (15, 30) is shown with asterisk, and the fusion peptide domain (7, 20, 22) is underlined.

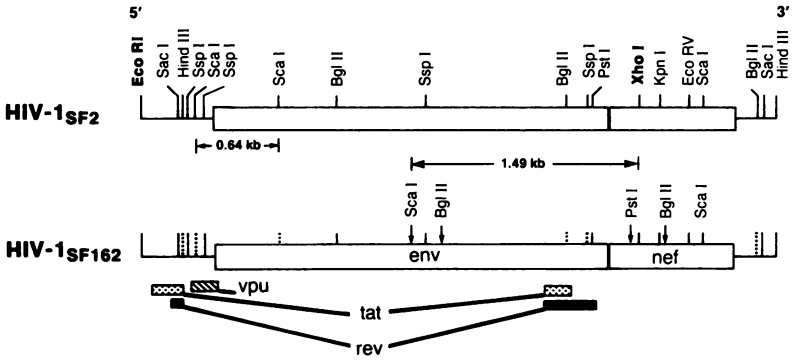

FIG. 3. Comparison of restriction endonuclease sites in the 3′ genomic regions of HIV_{SF2} and HIV_{SF162}. The restriction endonuclease map of HIV-1_{SF2} has been determined previously (38). For HIV-1_{SF162}, DNA from a recombinant lambda clone (clone 40-1) containing the full-length genome was prepared and digested with *Eco*RI. The 4.5-kb *Eco*RI fragment containing the *env* region, as identified by hybridization to a radioactive *env*-specific probe, was subcloned into pUC19. Restriction mapping of the 3′ subcloned viral DNA was performed as described previously (39). Differences in the restriction enzyme maps of both clones are indicated by arrows pointing to addition of restriction sites and by dotted bars denoting absence of restriction sites. Boldface letters denote enzymes (*Eco*RI and *Xho*I) used to generate fragments in the construction of *env* recombinant plasmids.

52, 56) and recently with recombinant viruses (66). Moreover, a previous study with chimeric viruses also indicated that host range tropism is in part determined at the level of the viral envelope (19). This study used hybrid genomes in which the envelope region of six viral clones obtained within the same individual were separately substituted into a biologically active prototype HIV-1 genome. Thus, this approach measures only the effect of the *env* gene. However, the chimeric viruses obtained replicated with different kinetics (19), and this raises the possibility that alterations in other regions of the HIV-1 genome (e.g., regulatory elements) might have contributed to the biological differences observed. The finding that recombinant viruses generated in our studies replicated with similar kinetics and titers (Tables 1 and 3) strongly implicate, but do not yet definitively identify, the envelope gene as a major determinant of host range tropism.

A comparison of the predicted amino acid sequence for the envelope glycoprotein of HIV-1_{SF2} and HIV-1_{SF162} reveals a high degree of homology (40 out of 44 amino acids) in the CD4-binding domain (Fig. 2), including the amino acids implicated in tropism for the monocytic U937 cells (14). Furthermore, HIV-1_{SF162} is biologically active upon transfection into HUT-78 cells (data not shown). These findings agree with previous studies showing that the binding to T-cell lines is comparable between HIV-1 isolates that differ in their ability to productively infect these target cells (16) and suggest that a postbinding entry process, perhaps fusion, is responsible for the difference in host range tropisms observed with HIV-1_{SF2} and HIV-1_{SF162}. Amino acid differences were observed in the putative gp41 fusion domain of HIV-1_{SF162} and HIV-1_{SF2} (Fig. 2) and in the 24-amino-acid

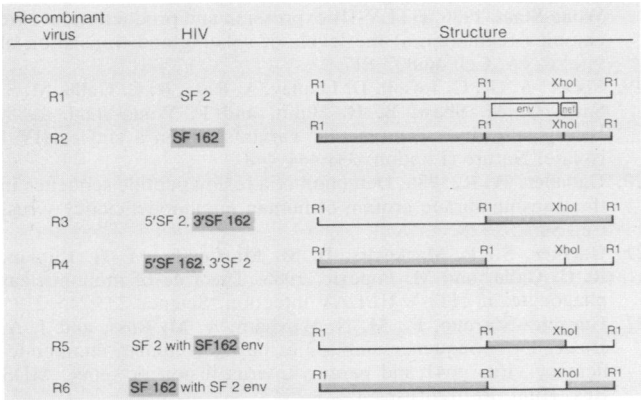

FIG. 4. Schematic structures of HIV-1_{SF2}-HIV-1_{SF162} recombinant DNA. Recombinant DNAs were constructed as described in the text and transfected into RD-4 cells by the calcium phosphate precipitation method (31).

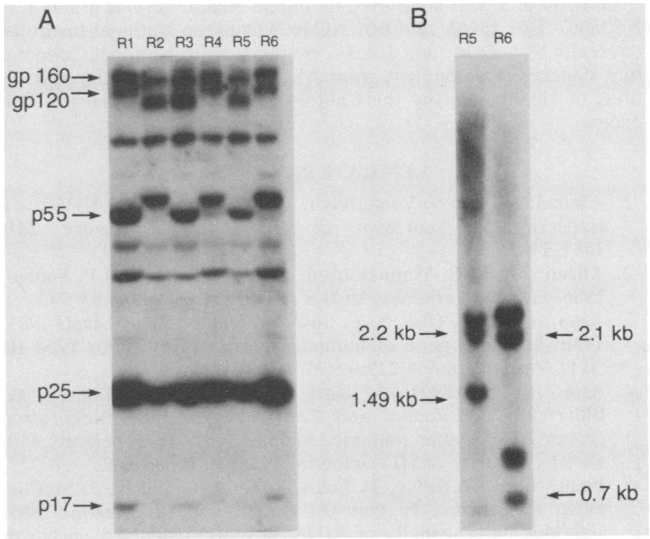

FIG. 5. Immunoblot and Southern blot analyses of HIV-1_{SF2} and HIV-1_{SF162} recombinant viruses. (A) Cell lysates from normal PMC infected with recombinant viruses R1 to R6 were prepared and analyzed by immunoblot on a 10% sodium dodecyl sulfate-polyacrylamide gel as described previously (45). The positions of the major viral proteins are noted. (B) A 20-μg portion of high-molecular-weight whole-cell DNA from recombinant virus R5- and R6-infected PMC was prepared, digested with *Sca*I-*Xho*I restriction enzymes, and subjected to electrophoresis on 0.8% agarose gel. Southern blot analysis of restricted DNA was performed, and viral species were detected with a radioactive probe representing the entire HIV-1 genome.

TABLE 3. Biologic and serologic properties of HIV-1_{SF2} and HIV-1_{SF162} recombinant viruses[a]

Recombinant viruses	Replication (RT activity) in					CPE	% CD4 modulation	Sensitivity to serum neutralization
	PMC (RT activity)	HUT 78		Macrophage				
		d7	d30	d7	d30			
R1	2,783.1	56.8	1,306.9	3.7	4.7	+	46	≥1:1,000
R2	2,732.1	2.2	1.2	1,851.8	1,330.1	−	85	≤1:10
R3	3,234.3	2.7	3.3	892.4	1,264.7	−	82	≤1:10
R4	2,121.2	17.9	1,945.5	4.2	8.4	+	54	≥1:1,000
R5	1,980.5	2.9	1.0	1,099.7	1,501.9	−	87	≤1:10
R6	2,465.9	22.8	1,452.1	3.6	2.9	+	50	≥1:1,000

[a] Recombinant viruses recovered from transfection of RD-4 cells were assayed for biologic and serologic properties according to the procedures described in Table 1 and in Materials and Methods. For replication in PMC, RT values (RT, $\times 10^3$ cpm/ml) at day 10 postinfection are presented. For replication in the T-cell line (HUT-78) and primary macrophages, RT values ($\times 10^3$ cpm/ml) at day 7 and day 30 postinfection are presented. Serum neutralization was conducted by using one HIV-1-positive serum sample. All data are representative of at least two independent experiments. CPE, Cytopathic effect in purified CD4+ lymphocytes.

sequence of RP135 implicated to participate in viral fusion processes (46–48). Whether these changes are responsible for macrophage tropism and other biologic properties of HIV-1_{SF162} requires further study.

Finally, the variations present in the immunodominant RP135 loop region (Fig. 2, amino acids 298 to 412) (46, 48) are likely to be responsible for the difference in neutralization patterns of the two isolates. Fine structure mapping of the envelope region of HIV-1 with additional recombinant viruses, together with site-directed mutagenesis studies, should allow the identification of the specific domains within the *env* region that control these host range and cytopathic and antigenic properties of HIV-1.

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LITERATURE CITED

1. **Ahmad, N., and S. Venkatesan.** 1988. *nef* protein of HIV-1 is a transcriptional repressor of HIV-1 LTR. Science **241:** 1481–1485.
2. **Alizon, M. S., S. Wain-Hobson, L. Montagnier, and P. Sonigo.** 1986. Genetic variability of the AIDS virus. Cell **46:**63–74.
3. **Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal.** 1985. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science **229:**69–73.
4. **Asjo, B., J. Albert, A. Karlsson, L. Morfeldt-Mamson, G. Biberfeld, K. Lidman, and E. M. Fenyo.** 1986. Replicative properties of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet **ii:**660–662.
5. **Benn, S., R. Rutledge, T. Folks, J. Gold, L. Baker, J. McCormick, P. Feorino, P. Piot, T. Quinn, and M. Martin.** 1985. Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. Science **230:**1548–1553.
6. **Berman, P. W., W. M. Nunes, and O. K. Haffar.** 1988. Expression of membrane-associated and secreted variants of gp160 of HIV-1 in vitro and in continuous cell lines. J. Virol. **62:** 3135–3142.
7. **Bosch, M. L., P. L. Earl, K. Fargnoli, S. Picciafuoco, F. Giombini, F. Wong-Staal, and G. Franchini.** 1989. Identification of the fusion peptide of primate immunodeficiency virus. Science **244:**694–697.
8. **Castro, B. A., C. Cheng-Mayer, L. A. Evans, and J. A. Levy.** 1988. HIV heterogeneity and viral pathogenesis. AIDS **2:** s17–s28.
9. **Cheng-Mayer, C., J. Homsy, L. Evans, and J. A. Levy.** 1988. Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization. Proc. Natl. Acad. Sci. USA **85:**2815–2819.
10. **Cheng-Mayer, C., J. Rutka, M. Rosenblum, T. McHugh, D. Stites, and J. A. Levy.** 1987. The human immunodeficiency virus (HIV) can productively infect cultured human glial cells. Proc. Natl. Acad. Sci. USA **84:**3526–3530.
11. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV that correlate with virulence in the host. Science **240:**80–82.
12. **Cheng-Mayer, C., C. Weiss, D. Seto, and J. A. Levy.** 1989. Isolates of human immunodeficiency virus type 1 from the brain may constitute a special group of the AIDS virus. Proc. Natl. Acad. Sci. USA **86:**8575–8579.
13. **Cohen, E. A., E. F. Terwilliger, J. G. Sodroski, and W. A. Haseltine.** 1988. Identification of a protein encoded by the *vpu* gene of HIV-1. Nature (London) **334:**532–534.
14. **Cordonnier, A., L. Montagnier, and M. Emerman.** 1989. Single amino-acid changes in HIV envelope affect viral tropism and receptor binding. Nature (London) **340:**571–574.
15. **Dowbenko, D., G. Nakamura, C. Fennie, C. Shimasaki, L. Riddle, R. Harris, T. Gregory, and L. Lasky.** 1988. Epitope mapping of the human immunodeficiency virus type 1 gp120 with monoclonal antibodies. J. Virol. **62:**4703–4711.
16. **Evans, L. A., T. M. McHugh, D. P. Stites, and J. A. Levy.** 1987. Differential ability of HIV isolates to productively infect human cells. J. Immunol. **138:**3415–3418.
17. **Evans, L. A., J. Moreau, K. Odehouri, H. Legg, A. Barboza, C. Cheng-Mayer, and J. A. Levy.** 1988. Characterization of a noncytopathic HIV-2 strain with unusual effects on CD4 expression. Science **240:**1522–1525.
18. **Feinberg, M. B., R. F. Jarrett, A. Aldovini, R. C. Gallo, and F. Wong-Staal.** 1986. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell **46:**807–817.
19. **Fisher, A. G., G. Ensoli, D. Looney, A. Rose, R. C. Gallo, M. S. Saag, G. M. Shaw, B. H. Hahn, and F. Wong-Staal.** 1988. Biologically diverse molecular variants within a single HIV-1 isolate. Nature (London) **334:**444–448.
20. **Gallaher, W. R.** 1986. Detection of a fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. Cell **50:**327–328.
21. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV III/LAV infection. Science **233:**215–219.
22. **Gonzalez-Scarano, F., M. N. Waxham, A. M. Ross, and J. A. Hoxie.** 1987. Sequence similarities between human immunodeficiency virus gp41 and paramyxovirus fusion proteins. AIDS Res. Hum. Retroviruses **3:**245–252.
23. **Haffar, O. K., D. J. Dowbenko, and P. W. Berman.** 1988. Topogenic analysis of the HIV-1 envelope glycoprotein gp160 in microsomal membranes. J. Cell Biol. **107:**1677–1687.
24. **Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D.**

Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks, and W. P. Parks. 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science **232**:1548–1553.

25. **Harada, S., Y. Koyanagi, and N. Yamamoto.** 1985. Infection of HTLV-III/LAV in HTLV-I carrying cells MT-2 and MT-4 and application in a plaque assay. Science **229**:563–566.
26. **Ho, D. D., M. G. Sarngadharan, M. S. Hirsch, R. T. Schooley, T. R. Rota, R. C. Kennedy, T. C. Chanh, and V. L. Sato.** 1987. Human immunodeficiency virus neutralizing antibodies recognize several conserved domains on the envelope glycoproteins. J. Virol. **61**:2024–2028.
27. **Hoffman, A. D., B. Banapour, and J. A. Levy.** 1985. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology **147**:326–335.
28. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
29. **Lasky, L. A., J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz, and P. W. Berman.** 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **233**:209–212.
30. **Lasky, L. A., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
31. **Levy, J. A., C. Cheng-Mayer, D. Dina, and P. A. Luciw.** 1986. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. Science **232**:998–1001.
32. **Levy, J. A., L. Evans, C. Cheng-Mayer, L.-Z. Pan, A. Lane, D. Dina, C. Staben, C. Wiley, and J. Nelson.** 1986. Biologic and molecular properties of the AIDS-associated retrovirus that affect antiviral therapy. Ann. Inst. Pasteur (Paris) **138**:101–111.
33. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
34. **Levy, J. A., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro.** 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. Virology **147**:441–448.
35. **Levy, J. A., L. H. Tobler, T. M. McHugh, C. H. Casavant, and D. P. Stites.** 1985. Long-term cultivation of T cell subsets from patients with acquired immune deficiency syndrome. Clin. Immunol. Immunopathol. **35**:328–336.
36. **Luciw, P. A., C. Cheng-Mayer, and J. A. Levy.** 1987. Mutational analysis of the human immunodeficiency virus: the orf-B region downregulates virus replication. Proc. Natl. Acad. Sci. USA **84**:1434–1438.
37. **Luciw, P. A., H. Oppermann, J. M. Bishop, and H. E. Varmus.** 1984. Integration and expression of several molecular forms of Rous sarcoma virus DNA used for transfection of mouse cells. Mol. Cell. Biol. **4**:1260–1264.
38. **Luciw, P. A., S. J. Potter, K. Steimer, D. Dina, and J. A. Levy.** 1984. Molecular cloning of AIDS-associated retrovirus. Nature (London) **312**:760–763.
39. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Martinez, H.** 1988. A flexible multiple sequence alignment program. Nucleic Acids Res. **16**:1683–1691.
41. **Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takasuki, and S. Putney.** 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of a neutralizing epitope. J. Virol. **62**:2107–2114.
42. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double digest restriction fragments. Gene **19**:269–276.
43. **Modrow, S., B. H. Hahn, G. M. Shaw, R. C. Gallo, F. Wong-Staal, and H. Wolf.** 1987. Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable regions. J. Virol. **61**:570–578.
44. **Niederman, M. J., B. J. Thielan, and L. Ratner.** 1989. Human immunodeficiency virus type 1 negative factor is a transcriptional silencer. Proc. Natl. Acad. Sci. USA **86**:1128–1132.
45. **Pan, L.-Z., C. Cheng-Mayer, and J. A. Levy.** 1987. Patterns of antibody response to the human immunodeficiency virus (HIV). J. Infect. Dis. **155**:626–632.
46. **Putney, S. D., T. J. Matthews, W. G. Robey, D. L. Lynn, M. Robert-Guroff, W. T. Mueller, A. J. Langlois, J. Ghrayeb, S. R. Petteway, Jr., K. J. Weinhold, P. J. Fischinger, F. Wong-Staal, R. C. Gallo, and D. P. Bolognesi.** 1986. HTLV-III/LAV-neutralizing antibodies to an *E. coli*-produced fragment of the virus envelope. Science **234**:1392–1395.
47. **Robey, W. G., L. O. Arthur, T. J. Matthews, A. Langlois, T. D. Copeland, N. W. Lerche, S. Oroszlan, D. P. Bolognesi, R. V. Gilden, and P. J. Fischinger.** 1986. Prospect for prevention of human immunodeficiency virus infection: purified 120-kDa envelope glycoprotein induces neutralizing antibody. Proc. Natl. Acad. Sci. USA **83**:7023–7027.
48. **Rusche, J. R., K. Javaherian, C. McDanal, J. Petro, D. L. Lynn, R. Grimaila, A. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino acid sequence of the viral envelope, gp120. Proc. Natl. Acad. Sci. USA **85**:3198–3202.
49. **Sagg, M. S., B. H. Hahn, J. Gibbons, Y. Li, E. S. Parks, W. P. Parks, and G. M. Shaw.** 1988. Extensive variation of human immunodeficiency virus type-1 in vivo. Nature (London) **334**: 440–444.
50. **Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. W. Gee, A. Ranard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw.** 1985. Nucleotide sequence and expression of the AIDS-associated retrovirus (ARV-2). Science **227**:484–492.
51. **Sanchez-Pescador, R., and M. S. Urdea.** 1984. Use of purified synthetic deoxynucleotide primers for rapid dideoxynucleotide chain termination sequencing. DNA **3**:339–343.
52. **Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature (London) **322**: 470–474.
53. **Sodroski, J., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. A. Haseltine.** 1986. A second post-transcriptional transactivator gene required for HTLV-III replication. Nature (London) **321**:412–417.
54. **Sodroski, J., R. Patarca, C. Rosen, F. Wong-Staal, and W. A. Haseltine.** 1985. Location of the transactivating region of the genome of human T-cell lymphotropic virus type III. Science **229**:74–77.
55. **Starich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeeley, S. Modrow, H. Wolf, E. S. Parks, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45**:637–648.
56. **Stein, B. S., S. D. Gowda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engleman.** 1987. pH-independent HIV-entry into CD4-positive T cells via virus envelope fusion to plasma membrane. Cell **49**:659–668.
57. **Strebel, K., T. Klimkait, and M. A. Martin.** 1988. A novel gene of HIV-1, *vpu*, and its 16-kilodalton product. Science **241**: 1221–1223.
58. **Tateno, M., and J. A. Levy.** 1988. MT-4 plaque formation can distinguish cytopathic subtypes of the human immunodeficiency virus (HIV). Virology **167**:299–301.
59. **Tersmette, M., R. E. Y. de Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency

syndrome (AIDS) and AIDS-related complex. J. Virol. **62:** 2026–2032.

60. **Tersmette, M., R. A. Gruters, F. de Wolf, R. E. Y. de Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J. Virol. **63:**2118–2125.
61. **Terwilliger, E. F., E. A. Cohen, Y. Lu, J. G. Sodroski, and W. A. Haseltine.** 1986. Functional role of human immunodeficiency virus type 1 *vpu*. Proc. Natl. Acad. Sci. USA **86:**5163–5167.
62. **Terwilliger, E., J. G. Sodroski, C. A. Rosen, and W. A. Haseltine.** 1986. Effects of mutations within the 3′ *orf* open reading frame region of human T-cell lymphotropic virus type III (HTLV-III/LAV) on replication and cytopathogenicity. J. Virol. **60:**754–760.
63. **Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo.** 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science **229:**759–762.
64. **Wright, C. M., B. K. Felber, H. Paskalis, and G. N. Pavlakis.** 1986. Expression and characterization of the trans-activator of HTLV-III/LAV virus. Science **234:**988–992.
65. **Wysocki, L. J., and V. L. Sato.** 1978. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. USA **75:**2884–2888.
66. **York-Higgins, D., C. Cheng-Mayer, D. Bauer, J. A. Levy, and D. Dina.** 1990. Human immunodeficiency virus type 1 cellular host range, replication, and cytopathicity are linked to the envelope region of the viral genome. J. Virol. **64:**4016–4020.