JOURNAL OF VIROLOGY, Nov. 1991, p. 5782–5789
0022-538X/91/115782-08$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 65, No. 11

Identification of Human Immunodeficiency Virus Envelope Gene Sequences Influencing Viral Entry into CD4-Positive HeLa Cells, T-Leukemia Cells, and Macrophages

BRUCE CHESEBRO,[1]* JANE NISHIO,[1] SYLVIA PERRYMAN,[1] ALAN CANN,[2]† WILLIAM O'BRIEN,[2] IRVIN S. Y. CHEN,[2] AND KATHY WEHRLY[1]

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840,[1] and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024-1678[2]

Received 8 April 1991/Accepted 1 August 1991

Infectious recombinant viruses were constructed from three molecularly cloned human immunodeficiency virus (HIV) strains varying in cell tropism. All recombinants showed a high infectivity titer on phytohemagglutinin-stimulated normal T lymphocytes. However, a 120-bp region of the envelope gene including the area of the V3 hypervariable loop was found to influence infectivity titer on both clone 1022 CD4-positive HeLa cells and CD4-positive CEM leukemia cells. Infectivity for macrophages was more complex. All viruses replicated in macrophages to a low level, but viral sequences both inside and outside the V3 loop region influenced the efficiency of replication. Two experiments showed that the mechanism of restriction of infection of 1022 cells by HIV strain JR-CSF was related to lack of virus entry. First, productive virus infection occurred after transfection of 1022 cells with viral plasmid DNA. Second, the nonpermissive HIV strain JR-CSF could infect 1022 cells when pseudotyped with the envelope of other retroviruses, including human T-cell leukemia virus type I (HTLV-I), HTLV-II, and amphotropic murine leukemia virus. These results demonstrate the possibility that unexpected cell types might be infected with HIV in human patients coinfected with HIV and HTLV-I or HTLV-II.

Human immunodeficiency virus (HIV) is known for its ability to infect CD4-positive T lymphocytes. However, HIV is also capable of infecting other cell types, including macrophages of blood and various organs (22, 39, 45), intestinal epithelial cells (2, 41), brain capillary endothelial cells (53), placental cells (38), and various cells derived from neural and connective tissues (8, 9, 13, 14, 17, 49, 51). HIV-infected macrophages have been observed in human brain tissues (28, 32, 50), and such cells may be involved in the AIDS dementia syndrome. HIV infection of macrophages and T lymphocytes has also been studied in vitro. HIV can be isolated from peripheral blood mononuclear cells (PBMCs) of most seropositive individuals by cocultivating with differentiated macrophages (23, 24) or phytohemagglutinin (PHA)-stimulated CD4-positive lymphoblasts from normal donors (30). These results suggest that the HIV in most patients is either dual tropic for both macrophages and T cells or that mixed macrophage-tropic and T-cell-tropic virus populations coexist in these individuals. Some HIV stocks may show a preference for infecting macrophages rather than T lymphoblasts (22), but other cloned macrophage-tropic HIV strains infect both macrophages and T lymphoblasts (34). However, none of these macrophage-tropic HIV strains infects T-leukemia cell lines. Conversely, many HIV strains have been adapted to continuous laboratory passage in T-cell leukemia lines. These viruses can also infect PHA-stimulated T lymphoblasts but usually fail to infect macrophages (16, 24). Recent data have identified sequences in the HIV envelope gene which appear to be important for macrophage and T-leukemia cell line tropism (42, 47). However, the molecular details of the virus-cell interactions responsible for these differences are currently not well understood and may be important in altering disease pathogenesis.

We have recently generated CD4-positive HeLa cells (clone 1022) capable of being infected by HIV produced by PBMCs of AIDS patients within 0 to 4 days of blood sampling. HIV from 53 of 56 patients readily infected 1022 cells; however, HIV from 3 patients failed to infect these cells (12). Thus, 1022 cells appeared to detect distinguishable patterns of cell tropism among these AIDS patient HIV isolates. In the present study we used several molecularly cloned HIV strains which differed in their abilities to infect 1022 cells. The region of the HIV genome influencing tropism for 1022 cells was identified by using infectious recombinant HIV constructions, and the mechanism of resistance to infection was shown to be related to viral entry.

MATERIALS AND METHODS

Cells. Clone 1022 CD4-positive HeLa cells (12); uninfected A3.01 and CEM human CD4-positive leukemia cells (18); amphotropic murine leukemia virus (MuLV)-infected A3.01 cells (9); 729, an Epstein-Barr virus-transformed human B-lymphocyte cell line (7); human T-cell leukemia virus type II (HTLV-II)-transfected 729 cells (729-neo) (7, 46); and HTLV-I-transformed human lymphoid cells (SLB) (31) were described previously.

Viruses. HIV strains NL4-3 (1), JR-CSF (34), JR-FL (34), and BA-L (22) were propagated on PHA-stimulated normal human PBMC cultures as previously described (12). JR-CSF was previously found to infect macrophages less efficiently than JR-FL (34), and in most of the present experiments

* Corresponding author.
† Present address: Department of Microbiology, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester, LE1 9HN, England.

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

macrophage infection by JR-CSF was detected later than infection by JR-FL and BA-L.

Construction of infectious recombinant HIV clones. Construction of viruses NF-Xho and NFN-SX was described previously (42). Viruses CN-Sal, CN-Dra, NC-Dra, CNC-DX, CNC-MX, and NCN-AX were made at UCLA by using similar methods. Viruses NCN-SB, SN, SM, MN, and NB were made at the Rocky Mountain Laboratories by using a modified version of pNL4-3 obtained from Malcolm Martin, National Institute of Allergy and Infectious Diseases, Bethesda, Md. This clone contained the pNL4-3 insert ligated into the *Pvu*II site of a pUC18 vector lacking the polylinker segment. Subsequently, we removed an extra *Stu*I site in the 5′ flanking region by excision of two *Fsp*I fragments of 614 and 457 bp. This vector, pNL4-3-10-17, contained unique restriction sites at 6822 (*Stu*I), 7250 (*Nhe*I), and 8465 (*Bam*HI) and produced infectious HIV after transfection into HeLa 1022 cells or PHA-stimulated PBMCs. Recombinant plasmids were created by using polymerase chain reaction with oligonucleotides containing the *Stu*I, *Nhe*I, or *Bam*HI sites in the NL4-3 sequence to amplify DNA from pBRN-BJRCSF (5). Conditions were 30 cycles of 94°C for 1 min, 40 or 50°C for 1 min and 72°C for 2 min, 200 μM deoxynucleoside triphosphate, 0.1 μM each oligonucleotide, 1 U of *Taq* polymerase (ProMega) per 50 μl in 50 μl of 1× commercial buffer from ProMega. Sense strand oligonucleotides were 6813 (*Stu*), 5′-TTA CAC AGG CCT GTC CAA AGG-3′; 7241 (*Nhe*), 5′-CAG ATA GCT AGC AAA TTA AGA G-3′; and 7097 (*Mlu*), 5′-TGT ACA AGA CCC AAC AAC AAT ACG CGT AAA AG-3′. Antisense strand oligonucleotides were 7129C (*Mlu*), 5′-CTT TTA CGC GTA TTG TTG TTG GGT CTT GTA CA-3′; 7262C (*Nhe*), 5′-C TCT TAA TTT GCT AGC TAT CTG-3′; and 8472C (*Bam*), 5′-GCT AAG GAT CCG TTC ACT AAT-3′. After phenol-chloroform extraction and ethanol precipitation, amplified DNA was digested with the appropriate restriction enzymes, reextracted and precipitated and then ligated into clones of pNL4-3-10-17 which had the appropriate NL4-3 fragment previously excised and substituted by an irrelevant "stuffer" DNA fragment or synthetic oligonucleotides.

A unique *Mlu*I site was introduced into pNL4-3 by first inserting a pair of synthetic oligonucleotides containing *Stu*I, *Mlu*I, *Xba*I, *Sac*II, and *Nhe*I sites between the unique *Stu*I and *Nhe*I sites of pNL4-3-10-17. The upper strand was 5′-CCT ACG CGT CTA GAC CGC GG-3′, and the lower strand was 5′-CTA GCC GCG GTC TAG ACG CGT AGG-3′. Then sequences from NL4-3 and JR-CSF were amplified by polymerase chain reaction with oligonucleotides 6813 (*Stu*) plus 7129C (*Mlu*) or 7097 (*Mlu*) plus 7262C (*Nhe*), and these fragments were inserted sequentially into the appropriate *Stu*I, *Mlu*I, and *Nhe*I sites of the vector described above to create viruses NCN-SM and MN as well as NL4-3 and JR-CSF, all containing a unique *Mlu*I site. The creation of this new *Mlu*I site did not alter the amino acid sequence of NL4-3, and the live HIV derived from this clone was indistinguishable from the original NL4-3 in the infectivity experiments described in this paper.

To produce infectious HIV stocks, recombinant viral plasmids were transfected into 3-day PHA-stimulated PBMCs. Cells were washed once in serum-free RPMI 1640 and resuspended at 5×10^6 cells per ml with DNA at 1 μg/ml in hypotonic DEAE-dextran transfection medium using 67% RPMI 1640 (27). Cells were incubated at 37°C for 5 to 10 min, washed once by centrifugation with serum-free RPMI 1640, resuspended at 2×10^6 cells per ml in RPMI 1640–15% fetal bovine serum–5% interleukin-2 (Electronucleonics), and cultured with an equal volume of untransfected cells at the same concentration in the same medium. Stocks were made from culture fluid taken 1 to 2 days prior to maximum visible cytopathic effect. In some experiments plasmids were transfected into 3×10^5 1022 cells in 35-mm wells by using the calcium phosphate technique. On the following day cells were trypsinized and split into two separate wells, and 24 h later 0.5×10^6 to 1×10^6 3-day PHA-stimulated lymphoblasts were added to wells. One day later suspended lymphocytes were removed and cultured in 24-well plates with medium containing 5% interleukin-2. Supernatant fluid was followed for reverse transcriptase, and virus stocks were made from positive wells as usual.

HIV infectivity. Viruses were titered on HeLa clone 1022 cells by focal immunoassay or on PHA-stimulated PBMC cultures by endpoint dilution (10, 12). Human macrophage cultures were prepared from monocytes purified from peripheral blood of normal donors by adherence and EDTA elution on dishes coated with gelatin plus fibronectin (20). Cells were cultured at 37°C in 24-well plates at 1.5×10^6 cells per well in 1.5 ml of RPMI 1640 with 10% heat-inactivated human serum. Medium was changed on day 4, and cells were infected with 0.2 ml of undiluted HIV stock on day 6 or 7. Cells were washed three or four times with medium 2 days later to remove input virus, and medium was changed every 4 days thereafter. HIV infection was detected by using supernatant fluid to transfer HIV infection to PHA-stimulated PBMC cultures or by following reverse transcriptase activity in supernatant fluid by a dot blot assay (54). In some experiments positive spots were excised and radioactivity was analyzed in a liquid scintillation counter.

Infection of CEM cells was done by incubating 0.2 ml of undiluted virus stock with 10^6 cells in 1.5 ml of medium with polybrene at 8 μg/ml. Two days later cells were split 1:4 as usual. This was repeated every 3 to 4 days to keep the cell concentration between 5×10^5 and 2×10^6/ml. Supernatant fluid was assayed every 3 to 4 days for reverse transcriptase (54).

Transfection. 1022 cells and normal HeLa cells were transfected with infectious molecular clones of HIV strain NL4-3 and JR-CSF by using the calcium phosphate precipitate method as previously described (11). Plasmid DNA of infectious molecular clones, pBRNBJRCSF, and pNL4-3 was transfected into 729, 729-neo, SLB, A3.01, and amphotropic MuLV-infected A3.01 cells by using electroporation at 960 μF and 250 V. Two days later, dilutions of electroporated cells were plated in culture wells previously seeded with normal HeLa or CD4-positive HeLa clone 1022 cells. Suspended cells were removed after 24 h, and 2 days later wells were fixed and scored by focal immunoassay for HIV (10, 12).

RESULTS

Initially four HIV strains were tested for infectivity in 1022 cells, CEM CD4-positive leukemia cells, macrophages, and PHA-stimulated PBMCs. All four viruses readily infected the PHA-stimulated PBMCs. However, three HIV strains, JR-CSF, JR-FL, and BA-L, infected macrophages at different levels but failed to infect 1022 cells or CEM cells (Table 1). In contrast, the T-cell-tropic HIV, strain NL4-3, infected 1022 cells, and CEM cells but failed to produce significant levels of reverse transcriptase after infection of macrophages (Table 1). Thus, among these HIV strains there was an inverse correlation between ability to infect macrophages versus 1022 or CEM cells.

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

TABLE 1. Comparative infectivity of four HIV strains in various cells

HIV strain	Infectivity titer[a]		Reverse transcriptase[b]	
	PHA blasts	HeLa 1022	CEM	Macrophages
NL4-3	1×10^4	4.4×10^3	6,500	40
JR-CSF	2×10^4	<3	50	240
JR-FL	1×10^4	<3	40	400
BA-L	1×10^4	<3	55	5,500

[a] Titer in HeLa 1022 cells represents focus-forming units per 0.2 ml, as detected by focal immunoassay; standard errors were within 30% of values shown. Titer in PHA-stimulated PBMC blasts represents 50% tissue culture infectious doses per 0.2 ml. Standard error is within 10-fold of the values shown.

[b] Macrophage infectivity was determined by culturing monocytes (20) in RPMI 1640 with 10% human serum. Cells were infected with 0.2 ml of undiluted HIV stock at 6 to 7 days after initiating cultures. Infection was monitored by measuring reverse transcriptase in the supernatant fluid at 3- to 4-day intervals from day 3 to day 24 by using a ^{32}P dot blot assay (54). CEM cells were infected and monitored in a similar fashion. Values shown are cpm/5 μl of supernatant fluid and were determined by liquid scintillation counting of positive spots detected by autoradiography. Background was 40 to 50 cpm/5 μl. The earliest day of reverse transcriptase-positive supernatant was day 8 for JR-FL, day 14 for BA-L, and day 17 for JR-CSF. NL4-3 remained negative through day 24. Values shown are mean peak counts per minute observed in three experiments. Macrophage culture supernatant fluid from day 8 or 10 was also used to infect PHA-stimulated PBMCs, and positive HIV infectivity was detected from all macrophage cultures including those with only background reverse transcriptase levels.

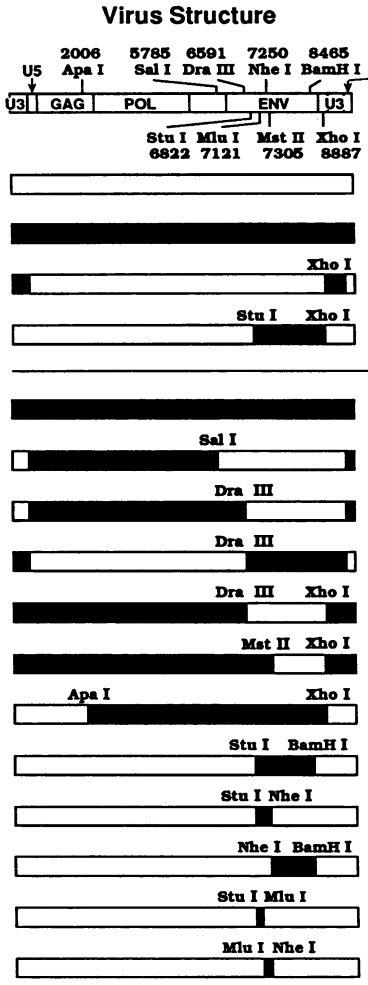
Infectivity of recombinant HIV constructs. Infectious molecular clones were available for strains JR-CSF, JR-FL, and NL4-3. Therefore, recombinant infectious HIV constructs were made by exchanging different portions of these HIV genomes. Constructs were used to determine which region(s) of the viral genome influenced infectivity of various cell types. All constructs had a high infectivity titer on PHA-stimulated PBMCs (Fig. 1). Titers were not altered by removal of macrophages and monocytes by plastic adherence prior to infection (data not shown). Thus, the majority of infected cells in these cultures were CD4-positive lymphocytes, and no differences in tropism were observed among the constructs tested. In contrast, on clone 1022 CD4-positive HeLa cells recombinant constructs segregated into two groups according to infectivity titers. One group gave no detectable titers, and the other group gave titers similar to those observed on PHA blasts (Fig. 1). The smallest fragment which could abolish NL4-3 infectivity in 1022 cells was a 120-bp region of JR-CSF between an *Mlu*I site at position 7121 and a *Nhe*I site at position 7250 in construct NCN-MN. All other constructs containing this portion of JR-CSF or JR-FL also failed to infect 1022 cells. Furthermore, in the background of the JR-CSF genome, the *Dra*III-to-the *Xho*I insert of NL4-3 in construct CNC-DX was sufficient to permit 1022 infectivity, whereas presence of the NL4-3 portion from *Mst*II to *Xho*I in construct CNC-MX lacked 1022 infectivity. These two constructs differed only for the region between *Dra*III and *Mst*II, and thus these results supported the conclusion that the region of envelope from *Dra*III to *Mst*II around the V3 loop was critical for cell tropism. All recombinant constructs were also tested on CEM leukemia cells, and results were identical to those observed with 1022 cells.

Macrophage infectivity was tested by infection with undiluted virus stocks because in preliminary experiments neither JR-CSF nor JR-FL strains could infect macrophages beyond the 10^{-1} dilution (data not shown). Replication efficiency in macrophages was tested by analysis of reverse transcriptase activity in supernatant fluid between 7 and 21 days after infection. Macrophage tropism appeared to be more complex than tropism for 1022 and CEM cells. All constructs produced HIV infectivity detectable by transfer of supernatant to PHA blast cultures (data not shown). However, the constructs differed significantly in peak levels of reverse transcriptase produced. All viruses which infected 1022 cells or CEM cells showed only background levels of reverse transcriptase (Fig. 1). In contrast viruses NFN-SX and NCN-SB with large envelope gene inserts from JR-FL and JR-CSF respectively showed a 10- to 20-fold increase in reverse transcriptase levels. However, cultures of recombinants with smaller inserts from this envelope region consistently had lower reverse transcriptase levels, although viruses NLN-SN and -MN with the V3 region of JR-CSF were always three- to fourfold above the background levels seen with other constructs (Fig. 1). Thus, high HIV replication efficiency in macrophages appeared to require JR-CSF sequences within the V3 loop but also was significantly augmented by the presence of other envelope gene sequences further downstream from the V3 loop in JR-CSF and JR-FL. However, two viruses (CNC-MX and NCN-AX) replicated poorly in macrophages as well as in CEM and 1022 cells, even though both contained the V3 loop region of JR-CSF. Thus, complex interactions between different viral gene sequences appeared to be responsible for these phenotypes.

Transfection of infectious HIV DNA. In some areas of the HIV envelope gene overlapping open reading frames encoding *tat*, *rev*, and *vpu* occur, but in the *Mlu*I-to-*Nhe*I region found to influence cell tropism, the envelope protein itself is the only known open reading frame (42). Thus, it appeared likely that structural differences in envelope protein were responsible for the differences in cell tropism detected in our experiments. Since envelope proteins are involved in interactions with cellular receptors and virus-cell fusion during the viral entry phase of infection, it is possible that the infectivity differences observed might represent different abilities of HIV strains to enter the various target cells used. To test this possibility, the usual HIV entry into 1022 cells was bypassed with two different approaches: (i) direct transfection of infectious viral DNA clones into cells and (ii) phenotypic mixing to form HIV pseudotypes with envelope proteins of other retroviruses capable of entering cells through different receptors. 1022 cells were highly susceptible to transfection by cloned viral DNA, and expression of HIV structural proteins was detected in 10 to 50% of the cells 48 h after transfection with either NL4-3 or JR-CSF (Fig. 2). However, whereas NL4-3 induced formation of massive syncytia in the 1022 cells, JR-CSF caused only rare syncytia, usually with less than 5 nuclei, and the majority of cells expressing viral protein in these cultures remained mononuclear (Fig. 2). Despite this difference in virus-induced cell fusion, 1022 cells transfected with either virus made infectious HIV which could be transferred to PHA-stimulated PBMCs by cocultivation (data not shown). Thus, introduction of JR-CSF genomes into 1022 cells by transfection led to productive infection, and this suggested that the main block to successful infection of 1022 cells by JR-CSF was at the level of viral entry.

Pseudotyping of HIV by HTLV-I and HTLV-II. We have previously shown that HIV genomes pseudotyped by mixed infection with murine leukemia viruses could successfully enter and productively infect various CD4-negative human and mouse cell lines (11). We recently observed that both 1022 cells and HeLa cells could be infected by both HTLV-I and HTLV-II. Therefore, it was also of interest to test

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest



Virus Strain	Infectivity Titer: PHA-blasts	Infectivity Titer: HeLa 1022	Reverse Transcriptase: CEM	Reverse Transcriptase: Macrophages
NL4-3	1×10^4	7.4×10^3	6500	50
JR-FL	3×10^4	< 3	40	220
NF-Xho	3×10^4	1.1×10^4	6000	40
NFN-SX	3×10^4	< 3	45	850
JR-CSF	3×10^4	< 3	50	240
CN-Sal	5×10^3	8.7×10^3	5500	45
CN-Dra	3×10^4	1.3×10^4	5850	50
NC-Dra	3×10^5	< 3	40	860
CNC-DX	3×10^3	3.5×10^2	5950	40
CNC-MX	3×10^4	< 3	48	55
NCN-AX	3×10^3	< 3	50	50
NCN-SB	1×10^4	< 3	45	430
NCN-SN	1×10^5	< 3	43	150
NCN-NB	1×10^4	3×10^3	6100	40
NCN-SM	2×10^4	4×10^3	6350	40
NCN-MN	1×10^4	< 3	45	200

FIG. 1. Comparison of HIV infectivity in HeLa 1022 cells, CEM leukemia cells, macrophages, and normal PHA-stimulated PBMCs by using infectious HIV molecular clones NL4-3, JR-FL, JR-CSF and molecular recombinant constructs of these clones. Restriction enzyme sites refer to positions in HIV strain NL4-3: *Apa*I, 2006; *Sal*I, 5785; *Dra*III, 6591; *Stu*I, 6822; *Nhe*I, 7250; *Mst*II, 7305; *Bam*HI, 8465; and *Xho*I, 8887. An *Mlu*I site was introduced at position 7121 in NL4-3 as described in Materials and Methods. Titers in HeLa 1022 cells are focus-forming units/0.2 ml, as detected by focal immunoassay. Titers in PHA blasts are 50% tissue culture infectious doses/0.2 ml. CEM and macrophage infectivity was determined by monitoring reverse transcriptase activity as described in Table 1. Values shown are mean peak cpm/5-μl supernatant and are taken from two to four experiments.

whether JR-CSF infection of 1022 cells could be achieved by using pseudotyped retrovirus particles containing HIV RNA genomes and non-HIV envelope proteins from HTLV-I or -II. This approach used the usual virus-cell interactions and fusion processes which occur during retrovirus infection, rather than the artifactual situation of transfection by high amounts of calcium phosphate-precipitated DNA. Furthermore, since pseudotyped virus particles might form in vivo in human patients infected by HIV and HTLV-I or -II (6, 36, 43), this experiment would test the possible effects on cell tropism of pseudotyping by these human retroviruses. To obtain mixed retrovirus infections and pseudotype production, uninfected clone 729 human B-lymphocyte leukemia cells or 729-neo cells chronically infected by HTLV-II or SLB cells infected with HTLV-I were transfected with cloned infectious DNA of HIV strains NL4-3 or JR-CSF by using the electroporation method. Dilutions of these cells were used to infect both 1022 cells and normal CD4-negative HeLa cells, and cultures were followed for foci of HIV infection. As expected, uninfected 729 cells transfected with NL4-3 could infect 1022 cells but not normal HeLa cells, whereas uninfected 729 cells transfected with JR-CSF could infect neither 1022 nor normal HeLa cells (Table 2). In contrast, HTLV-II-infected 729 cells (729-neo) and HTLV-I-infected SLB cells transfected with JR-CSF could transfer HIV infection to both 1022 cells and normal HeLa cells. Similar results were seen after transfection of NL4-3. Thus, coinfection of human leukemia cells by HTLV-I or -II and the JR-CSF strain of HIV produced viruses capable of infecting both CD4-positive and CD4-negative HeLa cells, presumably by using the receptors for HTLV-I or -II. Once viral entry was achieved, these infected cells produced HIV proteins detected by the focal immunoassay as well as infectious HIV virions (data not shown). Similar results were also obtained with 1022 cells and HeLa cells by using pseudotyped HIV produced when human A3.01 leukemia cells were preinfected with amphotropic MuLV before transfection with JR-CSF DNA (Table 2). Infection of 1022 cells or HeLa cells by these amphotropic MuLV pseudotypes could be blocked by preinfection of the target cells with

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

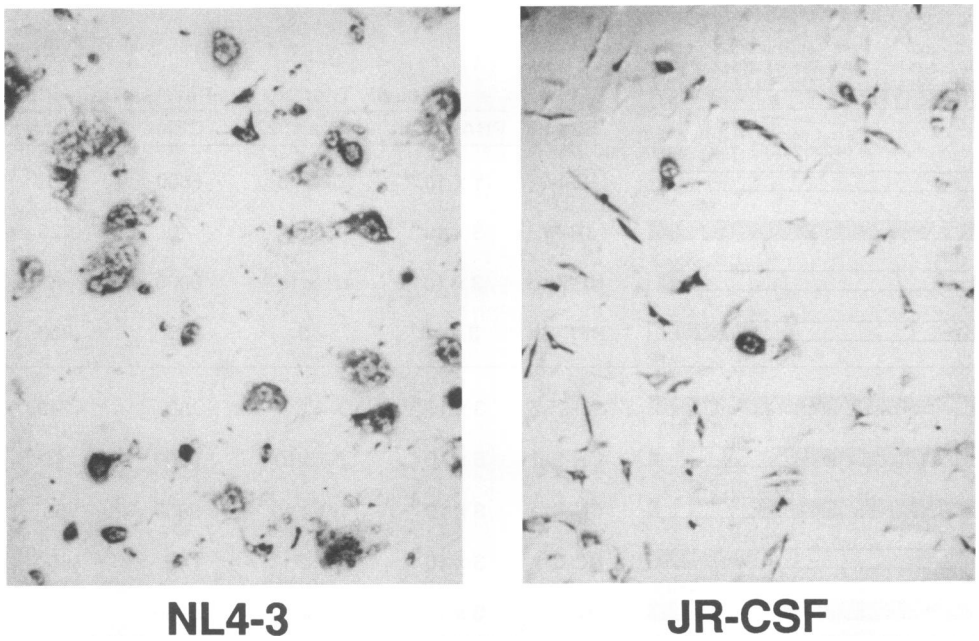


FIG. 2. Expression of HIV proteins after transfection of 1022 cells with infectious molecular clones of strain NL4-3 and JR-CSF. Forty-eight hours after transfection, cells were fixed and stained with anti-HIV antibodies by using indirect immunoperoxidase for detection (10). Both cultures show many cells positive for viral antigen expression. With NL4-3 most positive cells are multinucleated, but with JR-CSF most positive cells are mononuclear.

amphotropic MuLV, as was shown previously with NL4-3 (11). This confirmed that the mechanism of infection by JR-CSF involved viral pseudotype formation and utilization of the cellular receptor for amphotropic MuLV.

DISCUSSION

Among the recombinant HIV strains used in this study tropism for macrophages and 1022 cells was mutually exclusive. No dual-tropic strains were detected. The present results were similar to those of recent studies using different HIV clones which found no dual-tropic recombinants capable of infecting both macrophages and T-leukemia cell lines (29, 47). In our studies the recombinant virus strains detected by 1022 cells could also infect CEM T-leukemia cells. Therefore, 1022 cells appeared to be selective for T-leukemia cell line-tropic HIV clones. However, 1022 cells are able to detect HIV directly from PBMCs of most AIDS patients (12), whereas CEM and other leukemia cells cannot. Thus, 1022 cells appear to be less restrictive than T-leukemia cell lines but more restrictive than PHA-stimulated lymphoblasts, which can propagate most HIV isolates efficiently.

TABLE 2. Pseudotyping of HIV strain JR-CSF by human retroviruses HTLV-I and HTLV-II or amphotropic MuLV results in successful infection of HeLa and HeLa 1022 cells[a]

Leukemia cell line	Pseudotyping virus	Transfected HIV	HIV foci/10^6 cells	
			HeLa 1022	HeLa
729	None	NL4-3	3,300	0
729-neo	HTLV-II	NL4-3	5,600	2,900
729	None	JR-CSF	0	0
729-neo	HTLV-II	JR-CSF	1,900	1,450
729-neo	HTLV-II	None	0	0
SLB	HTLV-I	NL4-3	375	210
SLB	HTLV-I	JR-CSF	370	400
SLB	HTLV-I	None	0	0
A3.01	None	NL4-3	300	0
A3.01	Ampho	NL4-3	15	90
A3.01	None	JR-CSF	0	0
A3.01	Ampho	JR-CSF	280	610
A3.01	Ampho	None	0	0

[a] Titers are expressed as HIV foci per 10^6 leukemia cells plated. Results are geometric means for two or three replicate experiments. Ampho, amphotropic MuLV. SLB cells not infected with HTLV-I were unavailable because this cell line was derived by using HTLV-I as the transforming virus (31).

The present results indicate that the main mechanism of resistance of 1022 cells to infection by macrophage-tropic clones is lack of viral entry. This conclusion is in agreement with previous results showing that JR-CSF could not enter or infect HUT 78 or A3.01 T-cell leukemia lines (5). It was interesting that HIV pseudotyping by other retroviruses could overcome this resistance to infection. Since a significant number of HIV-infected drug addicts are also infected with HTLV-I or -II (6, 36, 43), it is possible that similar pseudotyping could occur in these individuals. If so, this might change the pattern of infection and/or manifestations of disease in these patients.

Recent studies have shown that JR-FL and JR-CSF envelope protein show high binding affinity for CD4 similar to NL4-3 (4). Thus, it may not be surprising that cell tropism differences among these viruses did not map to the CD4-binding region of the envelope. In contrast, the *Mlu*I-to-*Nhe*I region of the envelope involved in macrophage and 1022 cell tropism includes the V3 hypervariable loop structure (25) rather than the CD4-binding region or gp41 fusion domain (21, 33, 35) (Fig. 3). Type-specific antibodies have been found to recognize the V3 loop, block cell fusion, and neutralize virus infectivity (25, 37, 44), so the loop appears to be exposed at the outside of the virion and should be available for interactions with cell surface receptors. In addition, recent data indicate that there is a constant se-

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

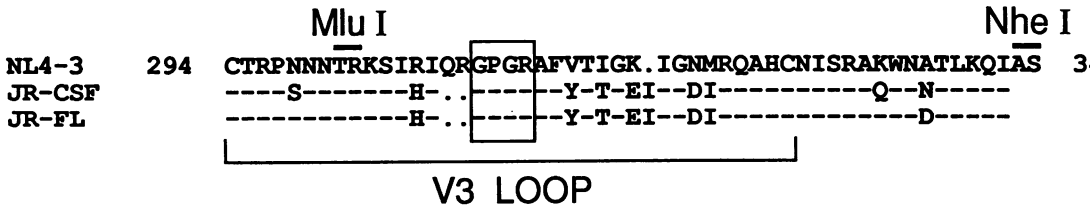


FIG. 3. Comparison of amino acid sequences of HIV clones NL4-3 (1), JR-CSF (40), and JR-FL (42) between *Mlu*I and *Nhe*I sites near the V3 region of the envelope gene. Mutations in the boxed GPGR sequence have been shown to block fusion of CD4-positive HeLa cells (19).

quence of four amino acids (Gly-Pro-Gly-Arg) at the tip of the V3 loop in many HIV clones and that mutations altering these amino acids inhibit fusion of CD4-positive HeLa cells by HIV envelope (19). Interestingly, HIV strains NL4-3 and JR-CSF both contain this sequence, and therefore both should be competent to induce cell fusion. Since JR-CSF does not fuse or infect 1022 cells efficiently, this virus must lack other sequences in the *Mlu*I-to-*Nhe*I region which are also required for fusion and entry of 1022 cells. NL4-3 and JR-CSF differ by 11 amino acids between the *Mlu*I and *Nhe*I sites (Fig. 3). Nine of these changes are within the V3 loop. However, it is unclear at present how the extreme sequence variability in the V3 loop could give rise to the general subgroups of HIV strains with tropism for T cells or macrophages. It seems likely that some common structural features would be required to maintain these consistent patterns of infectivity in spite of the high variability of the loop itself. Recent data suggest that portions of the V3 loop can be hydrolyzed by various proteases, and such proteolysis might be important in activation of the fusing activity of the envelope protein (15, 26). If in different cell types such proteolysis were carried out by cellular enzymes with different target sequences, the variability of the amino acid sequence around the V3 loop might determine susceptibility of the HIV envelope to proteolysis and this might in turn influence entry of a particular cell type by a particular HIV strain. Alternatively, proteolysis of the V3 loop of different HIV strains by the same protease would still produce protein structures with different sequences which might differ in their abilities to carry out the fusion step with different cell types.

The present results showing the importance of the *Mlu*I-to-*Nhe*I region in cell tropism differences among HIV strains NL4-3, JR-CSF, and JR-FL are in agreement with studies using HIV strains SF2 and SF162 (47) and with recent studies using BA-L and IIIB strains (29). However, it is unclear at present whether these findings can be generalized to all HIV strains. Furthermore, none of these results exclude the possibility that other regions of the HIV genome also play an important role in cell tropism. Two previous studies suggested that adjacent envelope gene sequences could also influence HIV productivity or infectivity in macrophages (42) or T-leukemia cell lines (47). In addition, on the basis of results with murine and avian retroviruses, it should be expected that tissue-specific enhancer elements in the long terminal repeat or other regions might also influence cell tropism of HIV (3, 48, 52). Since tropism effects mapping to the long terminal repeat were not observed in our studies, it is likely that the virus strains we compared do not differ functionally in long terminal repeat or other regions which also might influence cell tropism. A wider variety of HIV strains will have to be studied to search for additional viral factors involved in this phenomenon.

REFERENCES

1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59:**284–291.
2. **Adachi, A., S. Koenig, H. E. Gendelman, D. Daugherty, S. Gattoni-Celli, A. S. Fauci, and M. A. Martin.** 1987. Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. J. Virol. **61:**209–213.
3. **Arrigo, S., M. Yun, and K. Beemon.** 1987. *cis*-acting regulatory elements within *gag* genes of avian retroviruses. Mol. Cell. Biol. **7:**388–397.
4. **Brighty, D. W., M. Rosenberg, I. S. Y. Chen, and M. Ivey-Hoyle.** Envelope proteins from clinical isolates of HIV-1 which are refractory to neutralization by soluble CD4 possess high affinity for the CD4 receptor. Proc. Natl. Acad. Sci. USA, in press.
5. **Cann, A. J., J. A. Zack, A. S. Go, S. J. Arrigo, Yoshio Koyanagi, P. L. Green, Yasuko Koyanagi, S. Pang, and I. S. Y. Chen.** 1990. Human immunodeficiency virus type 1 T-cell tropism is determined by events prior to provirus formation. J. Virol. **64:**4735–4742.
6. **Chang, K. S., L. C. Wang, C. L. Gao, S. Alexander, R. C. Ting, A. Bodner, T. Log, A. F. Kuo, and P. Strickland.** 1988. Concomitant infection of HTLV-I and HIV-1: prevalence of IgG and IgM antibodies in Washington, D.C. area. Eur. J. Epidemiol. **4:**426–434.
7. **Chen, I. S. Y., D. J. Slamon, J. D. Rosenblatt, N. P. Shah, S. G. Quan, and W. Wachsman.** 1985. The *x* gene is essential for HTLV replication. Science **229:**54–58.
8. **Cheng-Mayer, C., J. T. Rutka, M. L. Rosenblum, T. McHugh, D. P. Stites, and J. A. Levy.** 1987. Human immunodeficiency virus can productively infect cultured human glial cells. Proc. Natl. Acad. Sci. USA **84:**3526–3530.
9. **Chesebro, B., R. Buller, J. Portis, and K. Wehrly.** 1990. Failure of human immunodeficiency virus entry and infection in CD4-positive human brain and skin cells. J. Virol. **64:**215–221.
10. **Chesebro, B., and K. Wehrly.** 1988. Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. J. Virol. **62:**3779–3788.
11. **Chesebro, B., K. Wehrly, and W. Maury.** 1990. Differential expression in human and mouse cells of human immunodeficiency virus psedotyped by murine retroviruses. J. Virol. **64:**4553–4557.
12. **Chesebro, B., K. Wehrly, J. Metcalf, and D. E. Griffin.** 1991. Use of a new CD4-positive HeLa cell clone for direct quantitation of infectious human immunodeficiency virus from blood cells of AIDS patients. J. Infect. Dis. **163:**64–70.
13. **Chiodi, F., S. Fuerstenberg, M. Gidlund, B. Asjo, and E. M. Fenyo.** 1987. Infection of brain-derived cells with the human immunodeficiency virus. J. Virol. **61:**1244–1247.
14. **Clapham, P. R., J. N. Weber, D. Whitby, K. McIntosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, and R. A. Weiss.** 1989. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. Nature (London) **337:**368–370.
15. **Clements, G. J., M. J. Price-Jones, P. E. Stephens, C. Sutton, T. F. Schulz, P. R. Clapham, J. A. McKeating, M. O. McClure,**

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

S. Thomson, M. Marsh, J. Kay, R. A. Weiss, and J. P. Moore. 1991. The V3 loops of the HIV-1 and HIV-2 surface glycoproteins contain proteolytic cleavage sites: a possible function in viral fusion? AIDS Res. Hum. Retroviruses 7:3–16.

16. Collman, R., N. F. Hassan, R. Walker, B. Godfrey, J. Cutilli, J. C. Hastings, H. Friedman, S. D. Douglas, and N. Nathanson. 1989. Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). Monocyte-tropic and lymphocyte-tropic strains of HIV-1 show distinctive patterns of replication in a panel of cell types. J. Exp. Med. 170:1149–1163.
17. Dewhurst, S., J. Bresser, M. Stevenson, K. Sakai, M. J. Evinger-Hodges, and D. J. Volsky. 1987. Susceptibility of human glial cells to infection with human immunodeficiency virus (HIV). FEBS Lett. 213:138–143.
18. Folks, T., S. Benn, A. Rabson, T. Theodore, M. D. Hoggan, M. Martin, M. Lightfoote, and K. Sell. 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA 82:4539–4543.
19. Freed, E. O., D. J. Myers, and R. Risser. 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. J. Virol. 65:190–194.
20. Freundlich, B., and N. Avdalovic. 1983. Use of gelatin/plasma coated flasks for isolating human peripheral blood monocytes. J. Immunol. Methods 62:31–37.
21. Gallaher, W. R. 1987. Detection of a fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. Cell 50:327–328.
22. 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.
23. Gendelman, H. E., L. M. Baca, H. Husayni, J. A. Turpin, D. Skillman, D. C. Kalter, J. M. Orenstein, D. L. Hoover, and M. S. Meltzer. 1990. Macrophage-HIV interaction: viral isolation and target cell tropism. AIDS 4:221–228.
24. Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. S. Burke, D. Skillman, and M. S. Meltzer. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. 167:1428–1441.
25. Goudsmit, J., C. Debouck, R. H. Meloen, L. Smit, M. Bakker, D. M. Asher, A. V. Wolff, C. J. Gibbs, Jr., and D. C. Gajdusek. 1988. Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc. Natl. Acad. Sci. USA 85:4478–4482.
26. Hattori, T., A. Koito, K. Takatsuki, H. Kido, and N. Katunuma. 1989. Involvement of tryptase-related cellular protease(s) in human immunodeficiency virus type 1 infection. FEBS Lett. 248:48–52.
27. Hesse, J. E., J. M. Nickol, M. R. Lieber, and G. Felsenfeld. 1986. Regulated gene expression in transfected primary chicken erythrocytes. Proc. Natl. Acad. Sci. USA 83:4312–4316.
28. Ho, D. D., T. R. Rota, R. T. Schooley, J. C. Kaplan, J. D. Allan, J. E. Groopman, L. Resnick, D. Felsenstein, C. A. Andrews, and M. S. Hirsch. 1985. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. N. Engl. J. Med. 313:1493–1497.
29. Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen. 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science 253:71–74.
30. Jackson, J. B., R. W. Coombs, K. Sannerud, F. S. Rhame, and H. H. Balfour, Jr. 1988. Rapid and sensitive viral culture method for human immunodeficiency virus type 1. J. Clin. Microbiol. 26:1416–1418.
31. Koeffler, H. P., I. S. Y. Chen, and D. W. Golde. 1984. Characterization of a novel HTLV-infected cell line. Blood 64:482–490.
32. Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. M. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 233:1089–1093.
33. 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.
34. Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen. 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science 236:819–822.
35. 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.
36. Lee, H., P. Swanson, V. S. Shorty, J. A. Zack, J. D. Rosenblatt, and I. S. Y. Chen. 1989. High rate of HTLV-II infection in seropositive IV drug abusers from New Orleans. Science 244: 471–475.
37. Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takatsuki, and S. Putney. 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J. Virol. 62:2107–2114.
38. Maury, W. J., B. J. Potts, and A. B. Rabson. 1989. Infection of human placental tissue with HIV-1: a possible mode of maternal-fetal transmission. J. Infect. Dis. 160:583–588.
39. Meltzer, M. S., D. R. Skillman, P. J. Gomatos, D. C. Kalter, and H. E. Gendelman. 1990. Role of mononuclear phagocytes in the pathogenesis of human immunodeficiency virus infection. Annu. Rev. Immunol. 8:169–194.
40. Myers, G., A. B. Rabson, J. A. Berzofsky, and T. F. Smith. 1990. Human retroviruses and AIDS (1990 supplement). Theoretical Biology and Biophysics Group T-10, Los Alamos, N.M.
41. Nelson, J. A., C. A. Wiley, C. Reynolds-Kohler, C. E. Reese, W. Margaretten, and J. A. Levy. 1988. Human immunodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet i:259–262.
42. O'Brien, W. A., Y. Koyanagi, A. Namazie, J.-Q. Zhao, A. Diagne, K. Idler, J. A. Zack, and I. S. Y. Chen. 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. Nature (London) 348:69–73.
43. Robert-Guroff, M., S. H. Weiss, J. A. Giron, A. M. Jennings, H. M. Ginzburg, I. B. Margolis, W. A. Blattner, and R. C. Gallo. 1986. Prevalence of antibodies to HTLV-I, -II, and -III in intravenous drug abusers from an AIDS endemic region. JAMA 255:3133–3137.
44. 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.
45. Schrier, R. D., J. A. McCutchan, J. C. Venable, J. A. Nelson, and C. A. Wiley. 1990. T-cell-induced expression of human immunodeficiency virus in macrophages. J. Virol. 64:3280–3288.
46. Shimotohno, K., W. Wachsman, Y. Takahashi, D. W. Golde, M. Miwa, T. Sugimura, and I. S. Y. Chen. 1984. Nucleotide sequence of the 3′ region of an infectious human T-cell leukemia virus type II genome. Proc. Natl. Acad. Sci. USA 81:6657–6661.
47. Shioda, T., J. A. Levy, and C. Cheng-Mayer. 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature (London) 349:167–169.
48. Short, M. K., S. A. Okenquist, and J. Lenz. 1987. Correlation of leukemogenic potential of murine retroviruses with transcriptional tissue preference of the viral long terminal repeats. J. Virol. 61:1067–1072.
49. Srinivasan, A., D. Dorsett, D. York, C. Bohan, and R. Anand. 1988. Human immunodeficiency virus replication in human brain cells. Arch. Virol. 99:135–141.
50. Stoler, M. H., T. A. Eskin, S. Benn, R. C. Angerer, and L. M.

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest

Angerer. 1986. Human T-cell lymphotropic virus type III infection of the central nervous system. A preliminary in situ analysis. JAMA **256**:2360–2364.

51. **Tateno, M., F. Gonzalez-Scarano, and J. A. Levy.** 1989. Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. Proc. Natl. Acad. Sci. USA **86**:4287–4290.
52. **Thiesen, H.-J., Z. Bosze, L. Henry, and P. Charnay.** 1988. A DNA element responsible for the different tissue specificities of Friend and Moloney retroviral enhancers. J. Virol. **62**:614–618.
53. **Wiley, C. A., R. D. Schrier, J. A. Nelson, P. W. Lampert, and M. B. A. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83**:7089–7093.
54. **Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. L. Earl, B. Moss, D. J. Capon, and M. A. Martin.** 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J. Virol. **62**:139–147.

Downloaded from http://jvi.asm.org/ on November 18, 2020 by guest