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Human Immunodeficiency Virus Type 1 T-Cell Tropism Is Determined by Events Prior to Provirus Formation

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Different strains of human immunodeficiency virus type 1 (HIV-1) vary in the ability to replicate in cells that bear the HIV-1 receptor, CD4. The mechanism responsible for these cell tropism differences is unknown. We examined different isolates of HIV-1 with regard to replication in specific tumor-derived CD4-positive T-cell lines and normal peripheral blood lymphocytes. To investigate early events in the virus life cycle at low multiplicities of infection, we used a modification of the polymerase chain reaction method. Use of a molecularly cloned primary HIV-1 isolate, HIV-1_{JR-CSF}, restricted for replication in T-cell lines, demonstrated that little or no viral DNA or RNA was synthesized in nonpermissive cells after infection. However, transfection of proviral DNA resulted in efficient transient virus production from these cells. Therefore, we conclude that at least one block to infection for HIV-1 strains in nonpermissive T cells occurs at a point in entry or uncoating before provirus formation.

The replication cycle of retroviruses proceeds through a number of stages which include binding of virions to specific receptors, penetration into the cell, synthesis of viral DNA, formation of the provirus by integration of the viral DNA into the host cell genome, transcription of mRNA, and assembly and release of mature virions (reviewed in reference 41). For most retroviruses, these processes occur within a single cell generation (7, 40). However, human immunodeficiency virus type 1 (HIV-1) differs from other retroviruses in that for a given isolate, the kinetics of replication vary widely among different cell types (9, 22, 28). Although all HIV-1 strains infect primary T lymphocytes, virus isolates with differing tropisms for macrophages (12, 15, 22) and immortalized T-cell lines (5, 8, 11, 34, 39) have been identified. The specific stage of the HIV-1 replication cycle responsible for these differences in cell tropism is not known. Cell tropism differences have been reported to correlate with the disease states of individuals from whom viruses were isolated (5, 8, 11, 34, 39). Thus, it is of critical importance to determine the mechanisms that control HIV-1 cell tropism.

We investigated variations in HIV-1 replication by studying primary isolates of HIV-1 that replicate efficiently in primary peripheral blood lymphocytes (PBL) but are incapable of establishing a productive infection in CD4-bearing T-cell lines. We find, similar to previous studies, that this restricted cell tropism is a general property of most strains of HIV-1 isolated from patients with acquired immunodeficiency syndrome (AIDS) by short-term culture in PBL. In contrast, and as expected, viruses that have been passaged and selected for growth in immortalized cell lines (including most common laboratory strains of HIV-1, such as HIV-1_{LAV} [3], HIV-$1_{HTLV-IIIB}$ [31], HIV-1_{ARV-2} [25], and HIV-1_{NL4-3} [1]) will replicate in immortalized cell lines. The differences between these two classes of HIV-1 strains could be explained by differences in the viral envelope glycoprotein, gp120, and its interactions with CD4, or could relate to differences in subsequent processes in HIV-1 infection, such as fusion, entry, reverse transcription, integration, or transcription. Given the high genetic variation of HIV-1, previous studies of HIV-1 T-cell tropism have been complicated by the use of patient-derived viral isolates that are neither biologically nor molecularly cloned. In this study, we used a molecularly cloned isolate of HIV-1, termed HIV-1_{JR-CSF}, which was molecularly cloned after short-term culture in PBL without adaptation in cell lines. Thus, we are able to use a homogeneous virus derived from this molecular clone for comparative studies in different cell types and among different viral strains. To investigate the molecular basis for the restriction of replication of some HIV-1 strains, we performed a detailed molecular analysis of events following HIV-1_{JR-CSF} infection.

MATERIALS AND METHODS

Cells and virus strains. The immortalized CD4-positive T-cell lines Jurkat (36), HUT 78 (16), MOLT-4 (29), and A3.01 (13) were maintained in Iscove medium containing 10% (vol/vol) fetal calf serum. Primary human PBL were prepared as follows. Mononuclear cells from whole blood were separated by centrifugation over Ficoll-Hypaque (Pharmacia) and cultured for 3 days in RPMI 1640 medium containing 20% fetal calf serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM glutamine, 1% phytohemagglutinin (PHA; HA15; Wellcome Diagnostics), and recombinant interleukin-2 (30 U/ml). Monocytes/macrophages were removed by adherence to the tissue culture flasks. After 3 days, the stimulated PBL were maintained in the same medium without PHA. HIV-1_{JR-CSF} was isolated from the cerebrospinal fluid of an AIDS patient (22). The virus was cultured in PHA-stimulated PBL for 11 days, at which time an infectious molecular clone of an integrated

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HIV-1 provirus was obtained from the DNA of these cells. Other primary clinical isolates of HIV-1, strains HIV-1_{LA1} to HIV-1_{LA4}, were isolated similarly by culture of infected patient cells for 7 to 14 days with PHA-stimulated PBL but were not molecularly cloned. All virus infections were performed as follows. Cells to be infected were mixed with virus suspension in 1- to 2-ml volume. The amounts of input virus used in each experiment were normalized to equivalent amounts of *gag* p24 antigen. Polybrene was added to a concentration of 10 μg/ml. The mixtures were incubated for 2 h at 37°C, with occasional shaking. After incubation, the cells were washed once with medium to remove nonadsorbed virus and then placed in the appropriate growth medium at a density of 10^6 cells per ml. For subsequent polymerase chain reaction (PCR) analysis, virus stocks were pretreated with DNase I before infection (see Fig. 3).

Molecular clones of HIV-1. Cloned DNA of HIV-1_{NL4-3} was generously provided by M. Martin, National Institute of Allergy and Infectious Diseases, and has been previously described (1). The isolation of HIV-1_{JR-CSF} from an AIDS patient has been previously described (22). A full-length HIV-1 provirus was isolated into a lambda vector (14) as a 14.2-kilobase *Eco*RI fragment that also includes 4.5 kilobases of flanking cellular DNA (unpublished data). This fragment was subcloned into the *Eco*RI site of a plasmid vector, pBRN/B (18), to form plasmid pBRNBJRCSF. Virus was rescued from the molecular clones by electroporation of PBL with plasmid DNA. Detailed description of the electroporation conditions and kinetics of virus rescue after electroporation (6) have been previously published. Virus stocks prepared by electroporation of PBL were titrated by p24 enzyme-linked immunosorbent assay (ELISA), and these stocks were used for subsequent experiments.

Preparation of nucleic acids and PCR analysis. DNA for PCR amplification experiments was prepared as follows. Cells were washed once in phosphate-buffered saline and lysed in Hirt buffer (10 mM Tris hydrochloride [pH 7.4], 20 mM EDTA [pH 7.4], 0.5% [wt/vol] sodium dodecyl sulfate). Proteins were digested with proteinase K (100 μg/ml) at 37°C overnight, followed by phenol-chloroform extraction and ethanol precipitation. Total nucleic acids (DNA plus RNA) resulting from this extraction procedure were used for PCR amplification. Because of the extreme sensitivity of the PCR assay, the following steps were taken to eliminate false-positive results. All biochemical and tissue culture reagents used were specifically prepared for PCR DNA isolation and proven to be totally free of contamination with HIV-1 DNA. DNA from uninfected cells was always extracted in parallel when DNA isolations were performed, to serve as negative controls in the PCR and eliminate the possibility that PCR signals seen were due to contamination with HIV-1 DNA. A specific biosafety level 3 facility is designated for DNA extractions and preparation of PCR reagents. Analysis of PCR products is performed in a physically separate facility. PCR amplification was performed as follows. Each reaction mixture was 25 μl in volume and contained 1 μg of total cellular DNA plus RNA (see above); a total of 100 ng of each oligonucleotide primer, representing 10^5 to 5×10^6 cpm (see below); 0.25 mM each of the four deoxynucleoside triphosphates; 25 mM Tris hydrochloride (pH 8.0); 5 mM $MgCl_2$; 100 μg of bovine serum albumin per ml; and 1.25 U of *Taq* DNA polymerase (New England BioLabs, Inc.). This reaction mixture was covered with 25 μl of mineral oil to prevent evaporation and subjected to denaturation (1 min at 91 to 94°C) and extension (2 min at 65°C) for 30 cycles. Products from the PCR amplifications were analyzed by electrophoresis in nondenaturing 6% polyacrylamide gels and visualized by direct autoradiography of the dried gels. Oligonucleotide primers used for amplification of HIV-1 sequences are shown in Fig. 3A. In addition, a pair of oligonucleotides complementary to the first exon of the human β-globin gene (LA1, positions 14 to 33 [5′-ACACAACTGTGTTCACTAGC-3′], and LA2, positions 123 to 104 [5′-CAACTTCATCCACGTTCACC-3′] relative to the translation initiation site [23, 33]) were used to normalize each reaction mixture for total amounts of cellular DNA present. One oligonucleotide of each complementary pair was 5′ end labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase to a specific activity of 10^8 to 10^9 cpm/μg. The specific activity of labeled oligonucleotides used in the PCR was adjusted to 10^6 cpm/μg for β-globin and 5×10^7 cpm/μg for HIV-1 oligonucleotides by the addition of unlabeled oligonucleotides to the reaction mixture. Quantitation of HIV-1 DNA in the PCR was performed by using dilutions of cloned HIV-1_{JR-CSF} DNA. Plasmid pBRNBJRCSF (17.5 kilobase pairs [kbp]) was digested with *Eco*RI to release the 14.2-kbp HIV-1-containing insert. Approximately 2×10^{-17} g of this digested plasmid represents one copy of the HIV-1_{JR-CSF} provirus (9.7 kbp). The *Eco*RI-digested DNA was diluted in carrier DNA or RNA and subjected to PCR amplification as described above.

RNA for PCR amplification was prepared by lysis of cells in urea lysis buffer (4.7 M urea, 1.3% [wt/vol] sodium dodecyl sulfate, 0.23 M NaCl, 0.67 mM EDTA [pH 8.0], 6.7 mM Tris hydrochloride [pH 8.0]), followed by phenol-chloroform extraction and ethanol precipitation. DNA was removed by digestion with RNase-free DNase (200 μg/ml; Worthington Diagnostics) for 1 h at 37°C, followed by phenol-chloroform extraction and ethanol precipitation. RNA prepared in this way was completely free of HIV-1 DNA and was used for reverse transcription. PCR analysis of DNA-free RNA (2) was performed as follows. Oligonucleotide primer M668 (see Fig. 3A) was annealed to the RNA in a 10-μl mixture containing 1 μg of RNA, 25 to 30 ng of M668 (unlabeled), 20 mM Tris hydrochloride (pH 8.3), 0.2 mM EDTA, and 50 mM KCl, incubated at 65°C for 1 h, and then cooled to room temperature. The annealing mixture was then split into two 5-μl samples. One sample (designated +RT) was used for primer extension of the M668 oligonucleotide in a 15-μl reaction mixture containing 5 μl of the annealed RNA-oligonucleotide mixture, 100 mM Tris hydrochloride (pH 8.3), 10 mM $MgCl_2$, 100 mM KCl, 0.5 mM each of the four deoxnucleoside triphosphates, 35 mM β-mercaptoethanol, and 2.5 U of avian myeloblastosis virus reverse transcriptase. Primer extension reactions were incubated for 1 h at 37°C, followed by denaturation of the enzyme by heating to 65°C for 10 min. The other 5-μl sample of the annealing mixture (designated −RT) was incubated under the same conditions without the addition of reverse transcriptase. Paired samples of the +RT and −RT primer extension reactions were then subjected to PCR amplification. A 2.5-μl amount of each primer extension reaction (equivalent to 83 ng of the input RNA) was used directly in a PCR amplification as described above. The RNA specificity of the PCR-amplified products was ensured by the absence of a signal in the −RT primer extension samples. To determine the sensitivity of this RNA PCR method, HIV-1 RNA synthesized in vitro was used. A 714-bp fragment of HIV-1_{JR-CSF} (nucleotides 493 to 1206) was subcloned into the SP6 expression vector pGEM-2 (Promega Biotec). This insert was linearized at nucleotide position 961, and a 469-nucleotide RNA runoff product synthesized in vitro

from the SP6 promoter as directed by the supplier (Promega Biotec). Dilutions of this RNA were used to estimate the sensitivity of the reaction.

RESULTS

HIV-1 phenotypic variation. One general observation has been that HIV-1 is more readily isolated from infected individuals by using normal human PBL as targets rather than using T-cell lines. In initial experiments, we tested primary isolates of HIV-1, derived by short-term culture of PBL from AIDS patients, for their ability to infect mitogen-stimulated normal human PBL and the CD4-positive Jurkat T-cell line (Fig. 1). As expected, all replicated efficiently in normal PBL. With one exception, the primary isolates tested were not able to replicate in the Jurkat tumor T-cell line (Fig. 1). The one isolate that was able to infect Jurkat cells, HIV-1_{LA4}, did not produce detectable levels of virus until 2 to 3 weeks after the initial infection. However, when the virus recovered from HIV-1_{LA4}-infected Jurkat cells 21 days after infection was used to reinfect fresh Jurkat cells with amounts equivalent to that of the parental HIV-1_{LA4} stock, the rescued virus was found to infect Jurkat cells more efficiently than did the parent virus (Figure 1C). This may be the result of the accumulation of mutations followed by selection of a Jurkat-adapted virus during the initial 21-day culture period in the cell line, or it may be the result of the selection of a preexisting variant in the original stock (17, 28, 32). These initial observations confirm the tropism differences of HIV-1 strains and demonstrate that under in vitro selection pressures, isolates with altered cell tropism can be selected.

To investigate the mechanism of the growth restriction of HIV-1 isolates in cell lines, we used infectious molecular clones of two strains of HIV-1 that differ in the ability to productively infect $CD4^+$ T-cell lines. Use of virus rescued from a molecular clone ensures that every experiment is performed with a homogeneous virus stock and not a population of viruses that may contain many minor variants (17, 28, 32). HIV-1_{JR-CSF}, a primary isolate, is described in detail elsewhere (22). An infectious molecular clone of this isolate was obtained from infected PBL cocultivated for 11 days with cerebrospinal fluid from patient JR (see Materials and Methods). Repeated attempts to propagate virus rescued from this infectious clone in the Jurkat, HUT 78, and MOLT-4 cell lines by continued cocultivation with infected PBL for as long as 4 months were unsuccessful (data not shown). Therefore, the restricted cell tropism is a stable property of this molecularly cloned virus. We compared this primary isolate with an infectious clone of a laboratory strain of virus, HIV-1_{NL4-3} (1). In contrast to HIV-1_{JR-CSF}, HIV-1_{NL4-3} is adapted for growth in many established T-cell lines (see below).

Virus was generated from the molecular clones by electroporation of 729-6 B cells as previously described (5, 6), and virus stocks were prepared by short-term coculture (7 days) with PBL. These virus stocks were tested for the ability to replicate in PBL and cell lines. As expected, HIV-1_{NL4-3} productively infected both PBL and the CD4-positive cell lines HUT 78 and A3.01, whereas HIV-1_{JR-CSF} productively infected only PBL (Fig. 2). Neither of these virus isolates productively infected the Jurkat cell line under these experimental conditions (Fig. 2). Therefore, subsequent experiments were performed in the HUT 78 cell line.

We also investigated whether HUT 78 cells infected with HIV-1_{JR-CSF} might release low levels of virus or contain intracellular virus. HUT 78 cells were infected with HIV-1_{JR-CSF} or HIV-1_{NL4-3}. Seventeen days after infection, half of the cells were cocultivated with PHA-stimulated PBL to amplify any virus production. The other half were disrupted

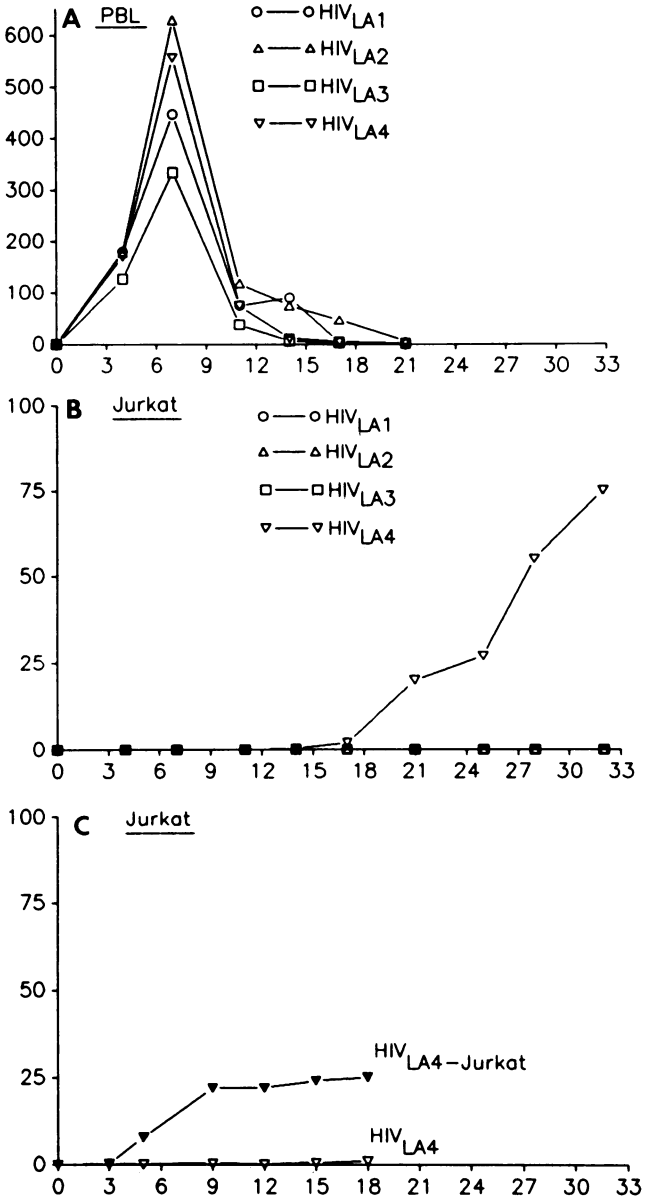

FIG. 1. Cell tropism of primary HIV-1 isolates. PHA-stimulated primary human PBL and Jurkat cells were infected with the isolates of HIV-1 indicated (see Materials and Methods); 50 ng of p24 antigen (approximately 5×10^3 tissue culture infectious doses) was used to infect 5×10^6 cells. In each graph, days in culture are shown on the *x* axis and nanograms of p24 in culture supernatants are shown on the y axis. (A) Virus production from PBL; (B) virus production from Jurkat cells; (C) infection of Jurkat cells with virus recovered from HIV-1_{LA4}-infected Jurkat cells at day 21. A total of 5×10^6 cells were infected with 34 ng of either the original HIV-1_{LA4} isolate or a Jurkat-adapted isolate. Virus production from infected cells was measured by ELISA against HIV-1 *gag* p24 antigen (Abbott Laboratories). Serial dilution experiments showed that 10 pg of p24 antigen was equivalent to approximately 1 infectious unit for PBL (data not shown), and this factor was used to estimate multiplicities of infection for PBL.

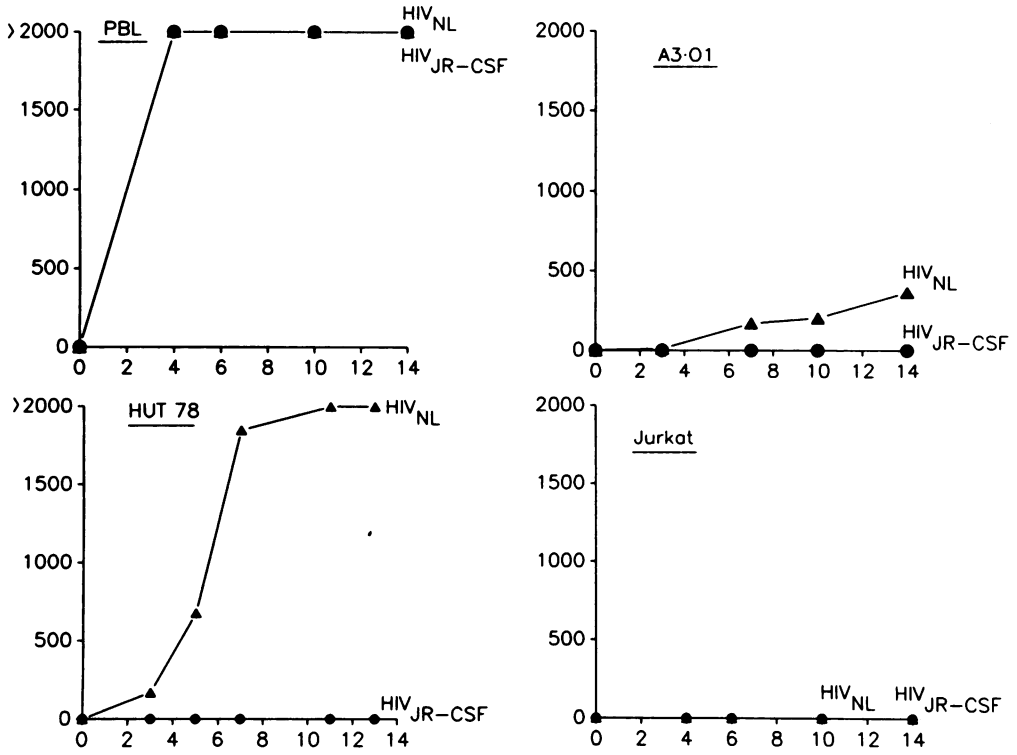

FIG. 2. Cell tropism of HIV-1_{JR-CSF} and HIV-1_{NL4-3} recovered from infectious molecular clones. Virus stocks of HIV-1_{JR-CSF} and HIV-1_{NL4-3} prepared from infectious molecular clones were used to infect the cells indicated as described in the legend to Fig. 1 (see also Materials and Methods); 10^6 of each cell type was infected with 10 ng of each virus.

by freeze-thaw lysis to test for intracellular virus, and the resulting suspension was added to a PHA-stimulated PBL culture. As expected, high titers of virus were recovered from the HIV-1_{NL4-3}-infected HUT 78 cells, but neither the intact nor the disrupted HIV-1_{JR-CSF}-infected cultures gave rise to any virus production (data not shown).

PCR assay for early events following HIV-1_{JR-CSF} and HIV-1_{NL4-3} infection. Molecular analysis of the early events in infection of PBL and HUT 78 cells was performed in cells infected with either HIV-1_{JR-CSF} or HIV-1_{NL4-3}. Because of the relatively low multiplicities of infection achievable (usually 0.01 to 0.05), it was necessary to use highly sensitive molecular detection methods for analysis of virus nucleic acids in the infected cells. Therefore, the PCR method was used, which allows the analysis of low-abundance viral DNA species (33). To achieve maximum sensitivity and to perform quantitative analysis of low-abundance species of nucleic acid, we adapted the PCR method by using synthetic oligonucleotide primers for amplification which were 5′ end labeled with γ-^{32}P, followed by direct autoradiography of gel-separated products (2, 24, 30) (Materials and Methods). The oligonucleotide primers used for these experiments are shown in Fig. 3A.

Figure 3B demonstrates the detection of amounts of cloned HIV-1_{JR-CSF} DNA ranging from 10 to 5,000 copies of cloned HIV-1 DNA. The β-globin gene was detected in 0.3 to 2 μg of uninfected PBL DNA and used as an internal PCR standard for the amount of cellular DNA present in the PCR amplifications. This analysis shows the capacity of this modified PCR method to quantitatively detect and discriminate between the different amounts of input nucleic acids.

We next used this modified PCR method to quantitatively analyze the levels of viral DNA synthesized after infection of HUT 78 cells by HIV-1_{JR-CSF} and HIV-1_{NL4-3}. Total cellular DNA was isolated from infected cells 16 to 24 h after infection and analyzed by PCR (Fig. 3B). This short period of time was chosen to avoid possible selection processes that may occur with longer times in culture and to examine the events that occur during the initial round of infection. HIV-1_{NL4-3} DNA was detected in these cells, whereas HIV-1_{JR-CSF} did not snythesize levels of DNA above those seen in a control infection using heat-inactivated HIV-1_{JR-CSF}. In contrast, infection of cells permissive for HIV-1_{JR-CSF} production (PBL) resulted in easily measurable levels of HIV-1_{JR-CSF} DNA.

We also tested another cell line, termed A2.01, into which the CD4 gene had been transfected and in which CD4 is expressed on the cell surface (4). This cell line is also permissive for HIV-1_{NL4-3} infection and resistant to productive infection by HIV-1_{JR-CSF} (data not shown). We investigated the early events following infection (Fig. 3C) in a manner similar to that described above. Again, HIV-1_{NL4-3} synthesized high levels of viral DNA within a short time period. Unlike HUT 78 cells, the signal detected after HIV-1_{JR-CSF} infection was significantly above that of an HIV-1_{JR-CSF} heat-inactivated control, although much less viral DNA was detected than in stimulated PBL infected with the same virus or A2.01 infected with equal amounts of HIV-1_{NL4-3}. Infection of A2.01 cells expressing CD8 rather than CD4 was negative for both HIV-1_{JR-CSF} and HIV-1_{NL4-3} (data not shown). Thus, in the case of this cell line, although some HIV-1_{JR-CSF} DNA was synthesized, the levels of infection were apparently insufficient to sustain a productive infection. Possible reasons for failure of HIV-1_{JR-CSF} infection to spread in these cells are considered below.

Infected HUT 78 cells were similarly examined for expres-

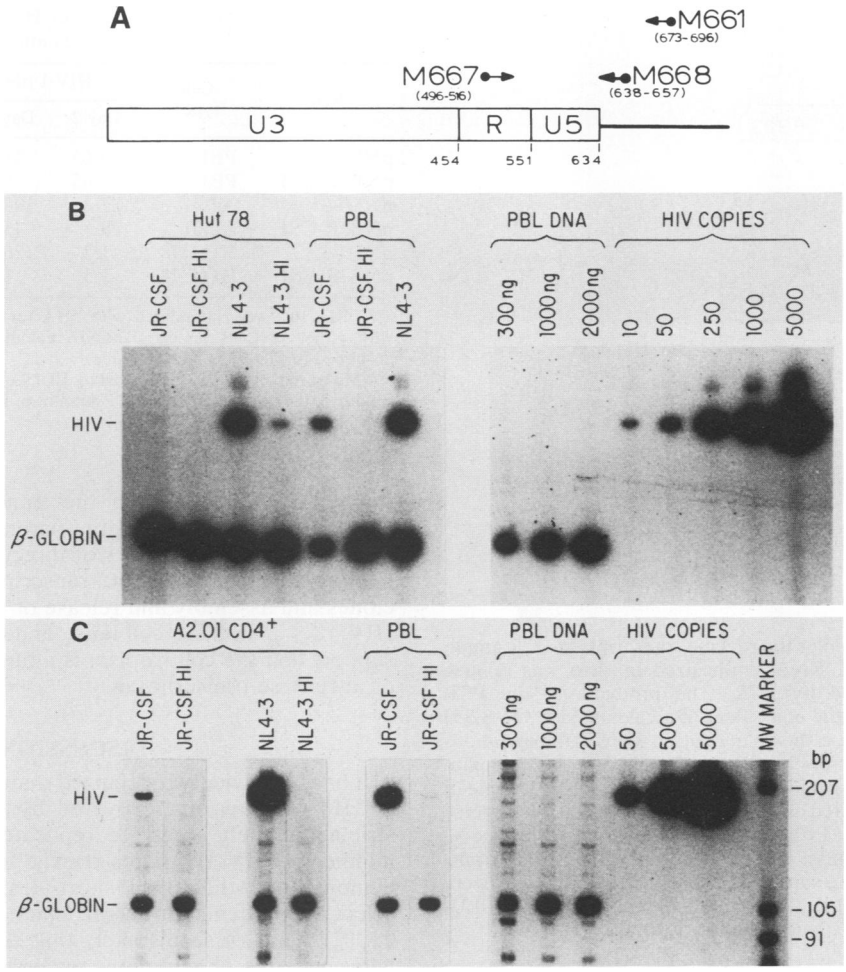

FIG. 3. (A) Oligonucleotide primers used for PCR amplification. Positions of synthetic oligonucleotides directed against conserved regions of the HIV-1 genome are shown relative to the 5′ long terminal repeat of HIV-1_{ARV-2} (35). Sequences of oligonucleotides used (5′ to 3′) are as follows: M661, CCTGCGTCGAGAGAGCTCCTCTGG; M667, GGCTAACTAGGGAACCCACTG; and M668, CGCGTCCCTGT TCGGGCGCC. (B) HIV-1 DNA in infected HUT 78 cells and PBL. The indicated cells (10^6) were infected in parallel with each virus (150 ng of p24), and total nucleic acids were prepared as described in the text. All virus stocks were filtered through 0.22-μm-pore-size filters and treated with DNase I (200 ng/ml; Worthington) in the presence of 10 mM $MgCl_2$ before use for infection to eliminate viral DNA in the viral supernatants. Heat inactivation controls (HI) were performed identically except that virus preparations were incubated at 56°C for 30 min before infection. A total of 2×10^5 cell equivalents of nucleic acid from HIV-1_{JR-CSF}- or HIV-1_{NL4-3}-infected cells were analyzed by PCR (Materials and Methods) except for HIV-1_{JR-CSF} infection of PBL, in which case less sample was analyzed as a result of sample loss during processing of the cells (note reduced β-globin signal intensity). (C) Detection of viral DNA in CD4-positive A2.01 cells infected by HIV-1_{JR-CSF} and HIV-1_{NL4-3}. Each cell type (10^6 cells) was infected with 400 ng of p24 of HIV-1_{JR-CSF} and 350 ng of p24 of HIV-1_{NL4-3}. PCR amplifications were done as for Fig. 3B. Parallel infections of stimulated PBL by HIV-1_{JR-CSF} and CD4-positive A2.01 by HIV-1_{NL4-3} are shown as controls. bp, Base pairs.

sion of viral RNA. To detect and analyze very low levels of HIV-1 RNA, we used a further modification of our PCR amplification technique (2) (Materials and Methods). RNA from HIV-1-infected cells was converted to cDNA, using Avian myeloblastosis virus reverse transcriptase and the antisense synthetic oligonucleotide primer used for PCR amplification (M668, Fig. 3). After conversion of RNA molecules to cDNA, PCR amplification was performed as before. In vitro-synthesized HIV-1 RNA was used to determine the sensitivity of this technique (Fig. 4A). The control RNA was diluted in carrier tRNA, and between 8.3 and 8.3 $\times 10^3$ calculated copies of viral RNA were subjected to reverse transcription, followed by PCR amplification (Fig. 4). A PCR-amplified signal was seen only in the samples that were subjected to reverse transcription, and no signal is seen in the samples that underwent a mock reverse transcriptase reaction, demonstrating that there was no viral DNA in the DNase I-treated RNA samples. An amplified signal could be seen in the PCR sample containing as few as 83 copies of input RNA. This amplified signal intensified with increasing amounts of HIV-1 RNA added.

Figure 4B shows the use of this technique to detect HIV-1 RNA in infected HUT 78 cells. Consistent with the failure to detect HIV-1_{JR-CSF} DNA in infected HUT 78 cells, little viral RNA was detected in HIV-1_{JR-CSF}-infected cells, whereas increasing levels of RNA were seen in HIV-1_{NL4-3}-infected cells, consistent with the increasing levels of HIV-1 p24 produced in culture supernatants (Fig. 4C, legend). The low signal in HIV-1_{JR-CSF}-infected cells detected at the earliest time point (day 1) probably represents detection of

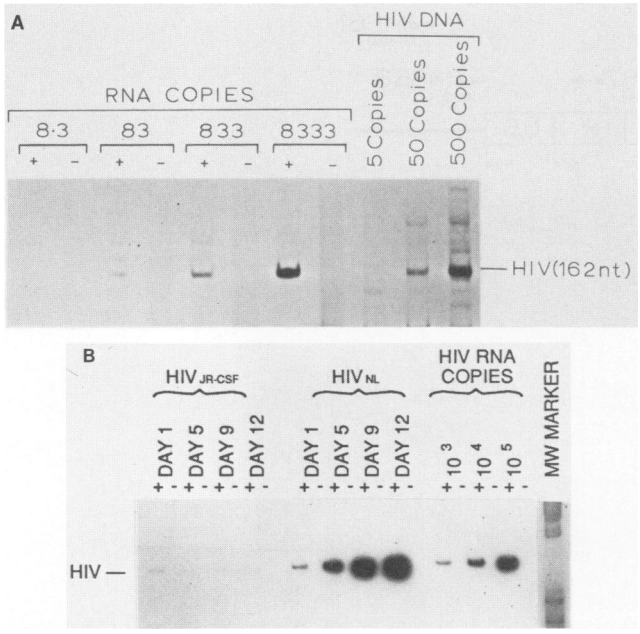

FIG. 4. (A) Quantitation of the reverse transcriptase-PCR amplification technique. HIV-1 RNA synthesized in vitro was reverse transcribed and amplified by PCR. The products of the PCR amplifications containing the equivalent of a calculated 8.3 to 8,333 copies of synthetic RNA are shown in relation to PCR amplifications containing 5 to 500 copies of cloned HIV-1 DNA. (B) PCR amplification of RNA from HIV-1-infected HUT 78 cells. A 1-μg sample of total RNA from cells infected as for Fig. 3C was subjected to reverse transcription, followed by PCR. +, With reverse transcriptase; −, without reverse transcriptase. The levels of HIV-1 p24 (picograms per milliliter) present in the supernatant at days 1, 5, 9, and 12 were as follows: HIV-1_{JR-CSF}; 103, 170, <50, and <50; HIV-1_{NL4-3}; 127, 231, 6,000, and 43,000. nt, Nucleotides.

genomic RNA from input virion particles either bound to the surface of the cell or internalized.

Transfection of molecular clones into PBL and cell lines. The apparent abortive infection of HIV-1_{JR-CSF} in A2.01 cells (Fig. 3C), in which a low level of DNA was synthesized after HIV-1_{JR-CSF} infection, suggests that events subsequent to DNA formation may also be involved in controlling HIV-1 cell tropism. To directly test whether events such as transcription, packaging, or release of virus are involved, we tested molecular clones of HIV-1_{JR-CSF} and HIV-1_{NL4-3} for virus production after direct DNA transfection of T cells. The levels of virus produced for each cell type were comparable for HIV-1_{JR-CSF} and HIV-1_{NL4-3} at early times (day 2) after transfection (Table 1). The levels of virus produced early after transfection were a result of transient transfection and expression from the cloned DNA introduced into the cells. The virus produced from transient transfection of many cell types, including COS fibroblasts and T-, B-, and monocytoid cell lines, maintained the characteristics of the original virus (data not shown). After the transient burst of virus, production increased for HIV-1_{NL4-3} transfection of all cell types as the virus spread through the culture. In the case of HIV-1_{JR-CSF}, virus production increased in PBL, indicating continued spread; however, the amounts of virus produced gradually decreased in the A2.01 and HUT 78 T-cell line. HIV-1_{JR-CSF} harvested 3 days after transfection of HUT 78 cells was retested for replication in HUT 78 and PBL cells (Table 2). HIV-1_{JR-CSF} replicated only in PBL;

TABLE 1. Transfection of HIV-1 proviral clones into cell lines and PBL

Clone	Cell type[a]	HIV-1 p24 antigen produced (ng/ml)[b]			
		Day 2	Day 5	Day 8	Day 14
pNL4-3	PBL	0.65	21	95	84
pNBJR-CSF	PBL	0.65	6.9	28	38
pNL4-3	A2.01	27	105	100	21,000
pNBJR-CSF	A2.01	29	45	6.6	0.77
pNL4-3	HUT 78	1.2	26	45	2,000
pNBJR-CSF	HUT 78	1.3	1.4	0.077	0.06

[a] PBL (10^7) were stimulated with PHA for 2 days before electroporation with either pNL4-3 or pNBJR-CSF. Conditions for electroporation are described in Materials and Methods.

[b] Measured on the days indicated by ELISA (Abbott Laboratories). Except for day 2, the numbers indicate the amount of HIV-1 p24 produced within the preceding 24-h period.

thus, virus rescued from the transfection had the same tropism phenotype as did the parental virus. These results are consistent with results of direct virus infection experiments. We conclude that transcription from the proviral clones and assembly and release of virions is unaffected for HIV-1_{JR-CSF} in these cell lines, and the data provide further support that the cell tropism is a function of events prior to or at reverse transcription.

DISCUSSION

It has been widely recognized that primary clinical isolates of HIV-1 are easily recovered by using PBL and are restricted in their ability to replicate in cell lines. A large number of HIV-1 variants coexist in vivo, and it has been demonstrated that even short-term passage in PBL will select for particular strains from among that population (17, 28, 32). Thus, it seems likely that either selection of preexisting variants or mutations occurring in vitro followed by selection result in the outgrowth of viruses that are able to infect certain T-cell lines. Viruses such as HIV-1_{JR-CSF}, which have been molecularly cloned after only short-term culture in PBL without selection in cell lines, are unable to replicate in the cell lines.

These studies show that isogenic molecularly cloned HIV-1 strains differ in their ability to productively infect the same cell, implicating different interactions between HIV-1 and a given cell type. It is noteworthy that HIV-1_{JR-CSF} differs in its ability to infect the two different cell lines, HUT 78 and A2.01, although neither leads to a productive infection. HUT 78 appears to be completely resistant to HIV-1_{JR-CSF} infection, whereas some HIV-1_{JR-CSF} DNA is detected after infection of A2.01, although considerably less than after infection of the same cells with HIV-1_{NL4-3} or infection

TABLE 2. Phenotype of virus rescued after DNA transfection[a]

Cell type[b]	HIV-1 p24 antigen produced (ng/ml)[c]		
	Day 4	Day 7	Day 10
HUT 78	<0.02	<0.02	<0.02
PBL	0.19	1.9	20.1

[a] HUT 78 cells (10^7) were electroporated with pNBJR-CSF as described in Materials and Methods.

[b] One milliliter of virus (1.8 ng/ml) harvested on day 2 after electroporation of HUT 78 cells was used to infect fresh HUT 78 or PBL.

[c] Measured as in Table 1; 0.02 ng was considered the lower limit of sensitivity in this assay.

of PBL with HIV-1_{JR-CSF}. Thus, it is possible that quantitative differences in the levels of infection could explain the rapid or slow kinetics of virus spread through a culture, as has been observed (5, 8, 11, 34, 39). It is noteworthy that the low level of HIV-1_{JR-CSF} DNA seen after infection of A2.01 does not lead to spread of virus throughout the culture and eventual productive infection. This result seems particularly curious since there does not seem to be any subsequent block to transcription, assembly, or release of virus in A2.01 cells. One possibility is that those cells productively infected by HIV-1_{JR-CSF} die rapidly, or their growth is slowed such that the uninfected cells overtake the culture.

The restriction to productive infection occurs at an early phase in the HIV-1 replication cycle, which could include binding to CD4 and other events up to and including reverse transcription of viral DNA. Our results exclude later events in the virus life cycle, such as differing efficiencies of transcription, integration, assembly, or packaging, as being events responsible for differences in T-cell line tropism. Reverse transcription does not appear to be responsible, since we have recently constructed recombinants between HIV-1_{NL4-3} and HIV-1_{JR-CSF} and find that regions which include the *pol* gene do not influence cell tropism (A. J. Cann, unpublished data). There are several possibilities for the early block to productive infection. Differences in cell tropism may be due to affinities of binding to CD4. However, the equivalent infectivity per unit amount of p24 of HIV-1_{JR-CSF} and HIV-1_{NL4-3} for PBL suggests that HIV-1_{JR-CSF} can efficiently bind its receptor. In addition, monoclonal antibody Leu 3a efficiently blocks HIV-1_{JR-CSF} infection of PBL (D. Ho, unpublished observations), indicating that CD4 is an essential component of the HIV-1_{JR-CSF} receptor and excluding the formal possibility that a different receptor is used by HIV-1_{JR-CSF}. Little is understood about the events of HIV-1 infection subsequent to binding to CD4. HIV-1 has been shown to enter cells after CD4 binding via direct fusion with the cell membrane (26, 38); thus, differing efficiencies of fusion to cell lines but not to PBL may also explain the differing cell tropism. Mutants of HIV-1 *env* and neutralizing antibodies directed to gp120 have been reported which prevent a productive infection but do not prevent binding to CD4 (9, 19, 20, 37). Such studies implicate gp120 as playing a role not only in HIV-1 binding to its receptor but also in as yet undefined subsequent early events. The role of viral proteins, including gp120 and gp41, in the early steps of internalization and uncoating will likely relate to the mechanism of the restriction for infection of T-cell lines by certain HIV-1 strains.

One implication of our results is that other cell proteins in addition to CD4 may be required for efficient infection and that these factors interact differently with different strains of HIV-1. Cellular factors may also be involved in the subsequent internalization or uncoating of HIV-1 in the cells. This experimental system may provide the opportunity to investigate the role of possible accessory molecules in HIV-1 infection. Further understanding of HIV-1 T-cell tropism will be informative about early events in HIV-1 infection and HIV-1 pathogenesis in general.

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ADDENDUM IN PROOF

Our recent results indicate that infection of quiescent primary T cells results in incomplete HIV-1 DNA transcripts (J. A. Zack, S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen, Cell **61**:213–222, 1990). We tested oligonucleotide PCR probes designed to detect incomplete reverse transcripts in HIV-1_{JR-CSF}-infected HUT 78 or A2.01 cells and found identical results to those shown in Fig. 3. Thus, nonproductive infection of T-cell lines differs from that of quiescent primary T cells.

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