JOURNAL OF VIROLOGY, Nov. 1990, p. 5270–5276
0022-538X/90/115270-07$02.00/0

Vol. 64, No. 11

Construction and Use of a Human Immunodeficiency Virus Vector for Analysis of Virus Infectivity

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Received 25 April 1990/Accepted 27 July 1990

We constructed a recombinant human immunodeficiency virus (HIV) vector to facilitate studies of virus infectivity. A drug resistance gene was inserted into a gp160$^-$ HIV proviral genome such that it could be packaged into HIV virions. The HIV genome was rendered replication defective by deletion of sequences encoding gp160 and insertion of a *gpt* gene with a simian virus 40 promoter at the deletion site. Cotransfection of the envelope-deficient genome with a gp160 expression vector resulted in packaging of the defective HIV-gpt genome into infectious virions. The drug resistance gene was transmitted and expressed upon infection of susceptible cells, enabling their selection in mycophenolic acid. This system provides a quantitative measure of HIV infection, since each successful infection event leads to the growth of a drug-resistant colony. The HIV-gpt virus produced was tropic for CD4$^+$ human cells and was blocked by soluble CD4. In the absence of gp160, noninfectious HIV particles were efficiently produced by cells transfected with the HIV-gpt genome. These particles packaged HIV genomic RNA and migrated to the same density as gp160-containing virions in a sucrose gradient. This demonstrates that HIV virion formation is not dependent on the presence of a viral envelope glycoprotein. Expression of a murine leukemia virus amphotropic envelope gene in cells transfected with HIV-gpt resulted in the production of virus capable of infecting both human and murine cells. These results indicate that HIV can incorporate envelope glycoproteins other than gp160 onto particles and that this can lead to altered host range. Like HIV type 1 and vesicular stomatitis virus(HIV) pseudotypes, gp-160$^+$ HIV-gpt did not infect murine NIH 3T3 cells that bear human CD4, confirming that these cells are blocked at an early stage of HIV infection.

There are few quantitative and sensitive assays for human immunodeficiency virus (HIV) infection in current use. As with many other retroviruses, direct, accurate bioassay of HIV is difficult because of the limited cytopathicity of the virus for susceptible adherent cell lines. A focal immunoassay for HIV that is highly sensitive for infectious HIV but requires antibody staining and microscopic observation has been previously described (4). In this report, we describe a self-packaging HIV vector that carries a drug resistance gene. Infection of cells with this vector is quantitated by enumerating colonies that form in the presence of drug-containing medium. This system is relatively safe and simple and provides a quantitative measure of HIV infection which can be applied to antiviral drug testing. The vector, referred to as HIV-gpt, permits dominant selection of cells permissive for the early events of HIV infection. Thus, rare infectable cells within a population can be identified and isolated. Since selection against expression of the *gpt* gene is also possible (2), HIV-gpt can also be used to eliminate HIV-infectable cells within a population, thereby selecting for HIV-resistant variants. Also, studies of the role of the *env* gene product are facilitated by the use of HIV-gpt, since *env* is supplied on a separate plasmid.

A replication-defective HIV vector that carries a chloramphenicol acetyltransferase gene in the *nef* region of HIV has previously been described (10). HIV-gpt differs from this vector in that it carries *rev* and a selectable marker gene. Genes other than *gpt* may be substituted in HIV-gpt to permit their packaging and delivery to infectable cells. HIV-gpt can be used in conjuction with a variety of *env* genes such that the host range of the viral vector can be manipulated. It is likely that HIV-gpt will prove to be a useful vector for a variety of purposes.

We have used HIV-gpt to study a block to HIV infection in murine NIH 3T3 cells. It has previously been shown that NIH 3T3 fibroblasts that express human CD4 (NIH 3T3-T4 cells) bind HIV but are resistant to infection with HIV and vesicular stomatitis virus(HIV) pseudotypes (16). These results suggested that the block to HIV infection was at the level of gp160-mediated entry and/or uncoating. In the experiments described here, NIH 3T3-T4 cells were found to be resistant to infection with HIV-gpt which carried gp160 *env* but could be infected with HIV-gpt carrying a murine leukemia virus (MLV) amphotropic *env*. This result confirms that gp160 is directly involved in a block to infection in NIH 3T3-T4 cells and indicates that postentry events of HIV DNA synthesis and integration are not blocked. The use of HIV-gpt as a tool for unraveling the virus-cell interactions that occur during HIV infection may lead to identification of cellular factors that control virus infection.

MATERIALS AND METHODS

Cells and virus. HeLa, COS-7, and NIH 3T3 cells and transfected derivatives were maintained in Dulbecco modified Eagle medium H21 supplemented with 10% fetal calf serum (GIBCO, Long Island, N.Y.) and antibiotics. HeLa T4 cells were obtained through the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, from R. Axel. NIH 3T3-T4 cells were obtained by cotransfection of NIH 3T3 cells with a CD4 expression plasmid, pCDMCD4 (from B. Seed), and pSV2neo (21). Hut78 cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. HIV

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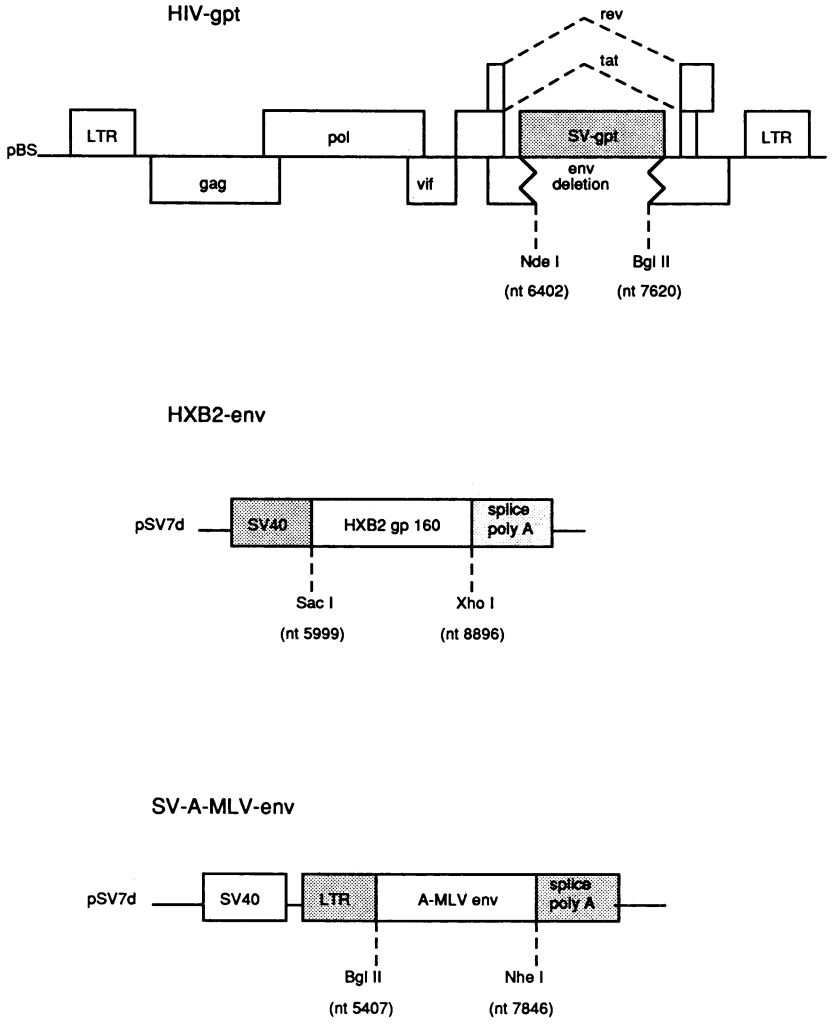

FIG. 1. Plasmid constructs. The restriction enzyme sites in *env* genes that were used for plasmid construction are indicated. Nucleotide (nt) numbers refer to the position of the restriction sites in a complete provirus. LTR, Long terminal repeat.

type 1 (HIV-1) strain LAV-1 (1) was propagated in Hut78 cells.

Vector construction. HIV-1 proviral and flanking cellular sequences were excised from pHXB2gpt (6) with *Xba*I and *Hpa*I and cloned into *Hinc*II- and *Xba*I-cut pBS (Bluescript; Stratagene, San Diego, Calif.). A 1.2-kb deletion in the envelope gene was made from the *Nde*I site at HIV nucleotide 6402 to the *Bgl*II site at HIV nucleotide 7620. Inserted at this site was a 1.1-kb fragment from pSV2gpt (18) from *Pvu*II to *Dra*I that includes the simian virus 40 (SV40) origin of replication and promoter and coding sequences for the *gpt* gene. The *gpt* gene was inserted in the same orientation as the HIV sequences such that the *gpt* gene could use the polyadenylation signal in the HIV 3′ long terminal repeat. This construct was named HIV-gpt. The gp160 expression plasmid HXB2-env was constructed by inserting HIV HXB2 gp160 sequences from *Sac*I (nucleotide 5999) to *Xho*I (nucleotide 8896) into the *Sma*I site of the SV40 expression vector pSV7d (23). An MLV long terminal repeat (17) and MLV amphotropic *env* coding sequences (9) were subcloned into pSV7d to generate plasmid SV-A-MLV-env. Schematic diagrams for all of the constructs used in this study are shown in Fig. 1.

Transfection. Virus was generated by transfection of plasmid DNA into COS-7 cells by the calcium-phosphate procedure (7) with the following modifications. Fifteen micrograms of each plasmid per 10-cm (diameter) dish of COS-7 cells was used, and 50 μM chloroquine was added to enhance transfection efficiency. At 14 to 20 h after transfection, the medium was replaced with fresh Dulbecco modified Eagle medium containing 10% fetal calf serum and antibiotics. Optimal results were obtained when the COS-7 cells were approximately 50% confluent at the time of transfection. COS-7 culture supernatants were harvested from 48 to 65 h after transfection, filtered through a 0.45-μm membrane, and stored at −70°C.

Infection and drug selection. Monolayer cells to be infected with viruses produced by transfection were seeded into 6-well culture plates such that they were 10 to 30% confluent at the time of infection. Transfected COS-7 cell serially diluted supernatant (200 μl) was added to the wells. Virus was allowed to absorb for 1 h at 37°C before an additional 1

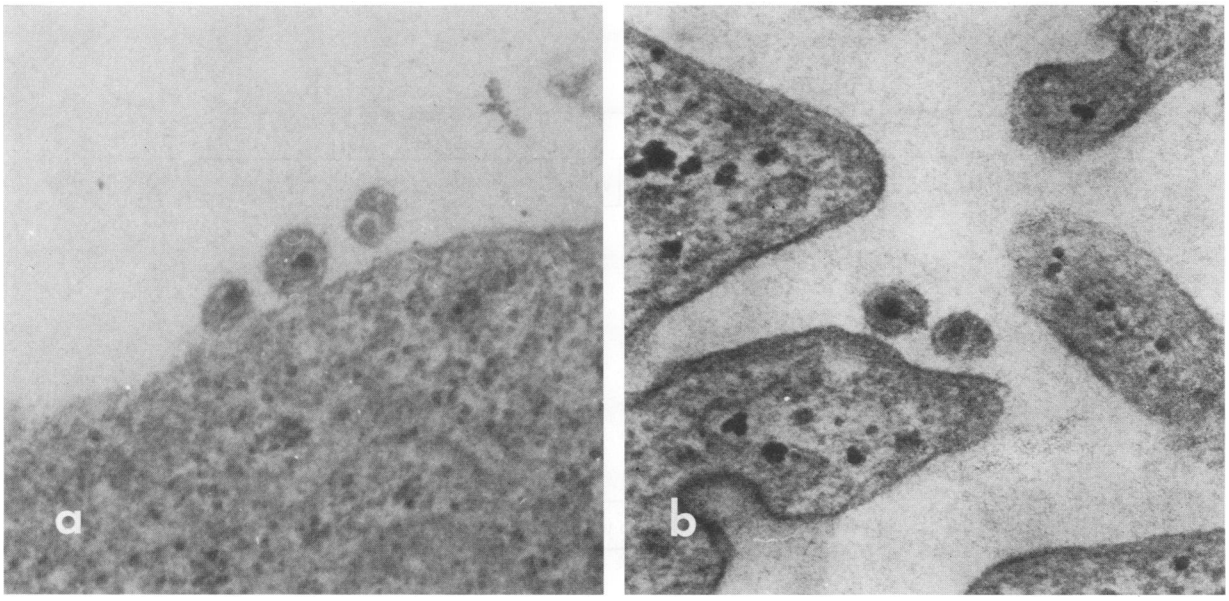

FIG. 2. Electron micrographs of viral particles produced by transfected COS-7 cells and infected Hut78 cells. (a) Hut78 cells were infected with HIV-1 strain LAV-1, harvested 7 days later, and fixed with 3% glutaraldehyde. (b) COS-7 cells were transfected with HIV-gpt and HXB2-env, harvested 65 h later, and fixed with 3% gluaraldehyde. Sectioned cells were stained with uranyl acetate and lead citrate and viewed at ×25,000 magnification.

ml of culture medium was added per well. At 14 to 28 h later, the medium was replaced with Dulbecco modified Eagle medium containing 10% fetal calf serum, 250 μg of xanthine per ml, 14 μg of hypoxanthine per ml, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.5), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 25 μg (NIH 3T3 and NIH 3T3-T4 cells) or 50 μg (HeLa and HeLa T4 cells) of mycophenolic acid (Calbiochem, La Jolla, Calif.) per ml. The medium was changed every 3 to 4 days until colonies of drug-resistant cells formed (10 to 15 days). Colonies were fixed and stained with a combination of 1% Formalin, 50% ethanol, and 0.5% crystal violet. For each cell line, a concentration of mycophenolic acid that killed 80 to 100% of the cells in 3 to 6 days was chosen. To infect Hut78 cells, 2×10^5 cells per ml were added to COS-7 cells that had been transfected 14 to 20 h previously. After 2 days of cocultivation, Hut78 cells were recovered and cultured in the presence of mycophenolic acid until drug-resistant cells grew out (2 to 3 weeks). Hut78 cells were selected in RPMI 1640 containing the same supplements described for the adherent cells and with 10 μg of mycophenolic acid per ml.

Sucrose density gradient analysis of HIV-gpt. Particles were concentrated by pelleting 9 ml of culture supernatant onto a 0.15-ml 45% sucrose cushion at 160,000 × *g* for 1.5 h at 4°C. The sucrose cushion was diluted threefold with phosphate-buffered saline and layered over an 11-ml continuous gradient of 20 to 45% (wt/vol) sucrose in phosphate-buffered saline. Virions were banded by centrifugation of the gradients at 160,000 × *g* for 2 h at 4°C. Gradient fractions were analyzed for p24 concentration by enzyme-linked immunoassay (HIVAG, Abbott), for density by measurement of the refractive index, and for HIV RNA content by hybridization. The hybridization was carried out by diluting a portion of each fraction into denaturation solution (0.2% sodium dodecyl sulfate, 2 M formaldehyde, 50% formamide, 40 mM MOPS [morpholinepropanesulfonic acid; pH 7.2]) and heating it to 65°C for 10 min. The fractions were then bound to Genescreen hybridization filters (NEN, Dupont, Boston, Mass.) by using a slot blot apparatus and hybridized to a radiolabeled HIV-specific probe. After autoradiography of the hybridization filters, the slot blot band intensities were quantitated by densitometry.

RESULTS

Construct design. A 1.2-kb deletion in the *env* gene of HIV HXB2 was made, leaving the *rev* responsive element and *tat* and *rev* exons intact. SV40 and *gpt* sequences were inserted into the *env* deletion site. This construct, named HIV-gpt, is shown in Fig. 1. The *env* region of the HIV HXB2 provirus was cloned into the SV40 expression vector pSV7d. This plasmid, named HXB2-env, contains the complete coding region for the *env* gene. Translation initiation codons for *rev*, *tat*, and *vpu* are not present in HXB2-env. Thus, gp160 is the only HIV gene product that is supplied by HXB2-env; all other viral gene products are derived from HIV-gpt. In order to minimize the chances for recombination between HIV-gpt and HXB2-env, the amount of HIV proviral sequence in HXB2-env was limited to 224 bp 5′ and 102 bp 3′ flanking the *env* coding sequence. Two homologous recombination events would be required to regenerate a replication-competent virus from HIV-gpt and HXB2-env.

Physical properties of virus produced by transfection. When HIV-gpt and HXB2-env were cotransfected into COS-7 cells, p24 antigen was released into the culture supernatant and viral particles with typical HIV morphology could be identified by electron microscopy (Fig. 2). Virus produced in this manner is refered to as HIV(HXB2) to indicate that the envelope glycoprotein of HIV strain HXB2 is present on HIV core particles. Transfection of HIV-gpt alone into COS-7 cells also resulted in p24 antigen release at approximately the same level (typically, 10 to 100 ng/ml) as that of transfections including the gp160 construct. Virus produced

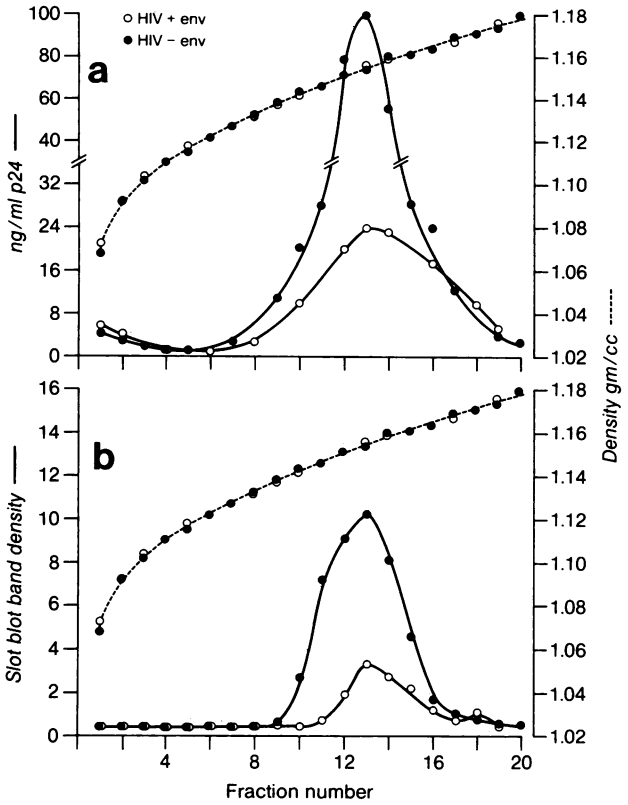

FIG. 3. Density gradient fractionation of gp160$^+$ and gp160$^-$ HIV-gpt. COS-7 cells were transfected with HIV-gpt alone (●) or HIV-gpt and HXB2-env (○). At 65 h, the culture supernatants were filtered through a 0.45-μm-pore-size filter and inactivated by the addition of Formalin to 0.5%. The methods used for density gradient analysis of the culture supernatants are described in Materials and Methods. (a) p24 content of the gradient fractions (——) and the sucrose densities of the fractions (– – –); (b) intensity of nucleic acid hybridization to an HIV probe of each gradient fraction after RNA slot blotting (——) and the sucrose densities (– – –).

in the absence of a viral glycoprotein is refered to as HIV$^-$ to indicate the lack of an envelope glycoprotein. The amount of p24 associated with particles was determined by pelleting virus in the culture supernatant through a layer of 20% sucrose and then quantitating the amount of p24 in the pellet and supernatant by enzyme immunoassay. The amount of p24 that was recovered in the pellet was 74% for HIV (HXB2), 88% for HIV$^-$, and 75% from a culture supernatant of lymphadenopathy-associated virus type 1-infected Hut78 cells. This indicates that p24 released by COS-7 cells transfected with HIV-gpt is stably associated with particles that pellet as efficiently as lymphadenopathy-associated virus type 1 derived from infected T cells. In order to compare the properties of virions made in the presence and absence of gp160, filtered culture supernatants containing HIV(HXB2) or HIV$^-$ were analyzed by density gradient centrifugation to determine their buoyant densities. The p24 released by the COS-7 cells banded at a density of 1.145 to 1.170 g/ml (Fig. 3a), as would be predicted for retroviral particles. In the experiment shown in Fig. 3, particles were produced somewhat more efficiently by cells transfected with HIV-gpt alone than by cells cotransfected with the gp160 expression plasmid. HIV$^-$ particles banded at the same density as HIV (HXB2) particles and contained a similar amount of HIV

TABLE 1. Infectivity of virus produced by COS-7 cells

Expt no. and virus prepn	p24 (ng/ml)	Infectious units/ml on:	
		HeLa T4 cells	HeLa cells
Expt 1			
HIV(HXB2)	13	1,920	6
HIV$^-$	41	0	1
No virus		0	0
Expt 2			
HIV(HXB2)	18	3,000	0
HIV$^-$	39	0	0
No virus		0	0
Expt 3			
HIV(HXB2)	18	825	
HIV(HXB2) + sCD4[a]	18	0	
Expt 4			
HIV(HXB2)	88	2,550	
HIV(HXB2) + sCD4	88	0	

[a] sCD4, Soluble CD4 (40 μg/ml).

RNA as detected by hybridization of disrupted, density-banded particles to an HIV-specific probe (Fig. 3b). These results demonstrate that gp160 is not required for efficient HIV assembly and release from cells.

CD4-dependent and independent infection. To test the infectivity of virus produced by transfected COS-7 cells, filtered culture supernatants were titrated on subconfluent monolayers of HeLa and HeLa T4 cells. The cells were then selected in medium containing mycophenolic acid such that only the cells which successfully integrated proviral DNA containing the HIV-gpt gene survived to form colonies. HeLa T4 cells were highly susceptible to infection with HIV (HXB2) but not with HIV$^-$ (Table 1). As would be predicted for retroviral infection, the numbers of colonies formed titrated linearly with the amount of viral supernatant added, indicating that infection with HIV-gpt followed single-hit kinetics (data not shown). Interestingly, HeLa cells were infected at a low efficiency with HIV(HXB2), and a single drug-resistant HeLa colony was obtained after infection with HIV$^-$. Drug-resistant HeLa colonies were obtained in several independent experiments after infection with HIV (HXB2) but always at a very low frequency relative to colonies formed on HeLa T4 cells (data not shown). Infection with HIV$^-$ was observed in a single experiment (Table 1). To verify that the drug-resistant HeLa colonies were derived from HIV(HXB2) infection events and not from either breakthrough of selection or spurious transfection with plasmid DNA carried over from the COS-7 cell transfection, DNA from two of the HeLa colonies was isolated and analyzed. HIV sequences but not plasmid vector sequences were detected by Southern blot hybridization of the isolated DNA, indicating that the viral DNA was inserted by retrovirus-mediated integration (data not shown). The apparent HIV$^-$ infection event has not been verified in this manner. To confirm that infection of HeLa T4 cells with HIV (HXB2) was mediated by CD4 on the surface of those cells, HIV(HXB2) was incubated for 1 h at 4°C with or without 40 μg of soluble recombinant CD4 per ml before addition of the virus to cells. Soluble recombinant CD4 completely prevented HIV(HXB2) infection of HeLa T4 cells (Table 1). These results indicate that HIV(HXB2) infection is largely CD4 and gp160 dependent but that non-CD4-mediated infection may occur at a low frequency. HIV(HXB2) infection

TABLE 2. Virus infectivity

Virus prepn	p24 (ng/ml)	Infectious units/ml on:		
		HeLa T4 cells	HeLa cells	Hut78 cells[a]
HIV(HXB2)	22	1,100	0	+
HIV⁻	10	0	0	−

[a] +, Infection appeared; −, no infection appeared.

was not restricted to adherent cell lines, since a human T-cell line, Hut78, was readily infected with HIV(HXB2) after cocultivation of Hut78 cells with transfected COS-7 cells (Table 2) or with filtered COS-7 cell supernatants (data not shown).

Assay for generation of replication-competent virus. The two constructs used to generate HIV(HXB2) contain homologous sequences that have the potential to recombine to form replication-competent HIV. To test for this, Hut78 T cells were cocultivated with COS-7 cells that had been transfected with HIV-gpt and HXB2-env. After 2 days of coculture, the T cells were recovered and further cultured for 45 days. A low level (<1 ng/ml) of p24 was detected throughout the culture period, as would be expected from infection of some of the cells with the replication-defective HIV(HXB2). No consistent increase in p24 levels and no cytopathology were observed during the culture period. Therefore, there was no obvious indication of replication-competent virus spread in the culture. We conclude that the level of recombination yielding replication-competent virus in this system was below our limit of detection. This experiment does not rule out generation of low levels of replication-competent virus that might spread very slowly or with limited cytopathic effect in Hut78 cells but does demonstrate that HIV strongly virulent for Hut78 cells was not produced.

Infection of murine fibroblasts. Mouse NIH 3T3 fibroblasts that bear human CD4 (NIH 3T3-T4 cells) can bind HIV but cannot be infected. Since vesicular stomatitis virus(HIV) pseudotypes also cannot infect NIH 3T3-T4 cells, it is likely that these cells are resistant to gp160-mediated entry or uncoating (15). In order to confirm these results, infection of NIH 3T3-T4 cells was attempted with HIV(HXB2). As expected, these cells were resistant to HIV(HXB2) infection (Table 3). The NIH 3T3-T4 cells used in this study expressed slightly higher CD4 levels than the HeLa T4 cells (Fig. 4), so the lack of infection cannot be ascribed to insufficient receptor levels. In order to determine whether postentry blocks to HIV DNA synthesis and integration exist in NIH 3T3-T4 cells, a virus with HIV core proteins and a MLV amphotropic envelope glycoprotein was made. This virus was produced by cotransfection of HIV-gpt with a plasmid (SV-A-MLV-env) that expresses the envelope glycoprotein of MLV strain 4070 A (Fig. 1). HIV readily formed pseudotypes with the MLV amphotropic envelope glycoprotein, yielding virus HIV(A-MLV) that infected human and murine

TABLE 3. Virus infectivity

Virus prepn	p24 (ng/ml)	Infectious units/ml on:			
		HeLa T4 cells	HeLa cells	NIH 3T3-T4 cells	NIH 3T3 cells
HIV(HXB2)	88	950	0	0	0
HIV(A-MLV)	96	1,825	300	250	115
HIV⁻	57	0	0	0	0
No virus		0	0	0	0

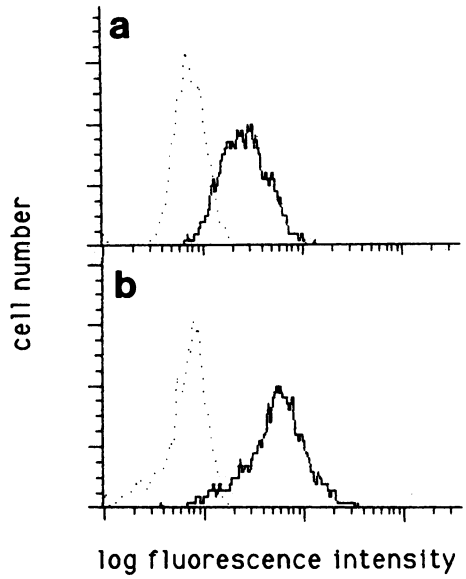

FIG. 4. Surface expression of CD4. Tissue culture cells were stained with fluoresceinated anti-CD4 monoclonal antibody Leu3A (1 μg/10^6 cells), washed, and analyzed by fluorescence-activated cell scanning (FACSCAN, Becton Dickinson). (a) HeLa (. . . .) and HeLa T4 (——) cells; (b) NIH 3T3 (. . . . .) and NIH 3T3-T4 (——) cells.

cells (Table 3). These results indicate that murine fibroblasts are permissive for HIV DNA synthesis and integration when the block to viral entry is circumvented by pseudotyping HIV with an amphotropic envelope glycoprotein. It should be noted that up to 50-fold variations in titer were obtained when several different lines of NIH 3T3 fibroblasts were tested for infection with the same preparation of HIV(A-MLV). The reason for this variation is unclear and may be dependent on the passage level and precise culture conditions of individual lines of NIH 3T3 cells.

DISCUSSION

Use of HIV-gpt to study HIV infectivity. HIV-gpt is a self-packaging retroviral vector that can be used to quantitate viral infection events. Cells permissive for the early events of virus infection up to and including provirus integration can be infected with HIV-gpt, since stable *gpt* expression from an SV40 promoter is used as the marker for infection. Thus, HIV-gpt allows analysis of a unique window of virus infection events from binding to integration. In this study, the vector has been used to compare the permissiveness of a variety of cells for the early events of HIV infection. HIV-gpt also provides a means to assess the antiviral activity of agents that block HIV infection. For example, inhibition of HIV-gpt infection by soluble recombinant CD4 was readily demonstrated.

Studies of the role of *env* in HIV host range are facilitated by HIV-gpt, since *env* is supplied from a separate expression vector. HIV isolates vary in their ability to infect T cells, monocytes, and CD4⁻ cells (3, 8, 12, 14). It is possible that variations in *env* among different HIV isolates account for their variation in tropism. It has previously been shown that certain mutations in gp160 lead to altered tropism of HIV-1 for T-cell and monocyte lines (5). The effect of *env* on tropism can be studied by complementing HIV-gpt with *env* genes derived from various HIV isolates and examining

changes in the host range. In addition, by providing envelope glycoproteins from viruses other than HIV, well-defined HIV pseudotype viruses with altered viral host ranges can be formed.

We observed a low level of CD4-independent infection with HIV(HXB2). HIV may infect cells in the absence of CD4 via a different cellular receptor or by nonspecific binding of virus to cells, followed by a conformational change in gp160 which exposes a fusogenic domain. If an alternate receptor for HIV is present on certain cells, HIV-gpt may be used to identify cells that bear that receptor. Different isolates of HIV vary considerably in their rates of replication in $CD4^-$ cells (14). Thus, it is possible that variations in gp160 affect the ability of HIV to infect $CD4^-$ cells. The design of HIV-gpt will enable further studies on the role of gp160 in determining the tropism of HIV for $CD4^-$ cells.

It is unclear why NIH 3T3-T4 cells are resistant to HIV entry. Conceivably, there are cellular factors other than CD4 that are required for initiation of HIV infection. Alternatively, NIH 3T3-T4 cells may have an inhibitor of the viral entry process. Expression of specific human genes in NIH 3T3-T4 cells may permit their infection with HIV-gpt. Such genes may be identified by selecting for HIV-gpt-infectable cells after transfer of human genes to NIH 3T3-T4 cells. In this manner, cellular factors involved in HIV infection may be isolated. CD4-positive murine cells other than NIH 3T3 fibroblasts are also resistant to HIV infection. Mice transgenic for human CD4 did not support any detectable viral replication when inoculated with HIV (M. McCune, personal communication), and mitogen-stimulated lymphocytes from these animals could not be productively infected in vitro (K. Page, unpublished data). These studies highlight the difficulties involved in developing mouse models to study HIV infection. It is likely that multiple blocks to productive HIV infection exist in many mouse cells. Since mice transgenic for the HIV provirus produce a low but detectable level of virus, it appears that blocks to HIV expression in mice significantly impair virus production but are not absolute (13).

Role of gp160 in virion assembly. HIV-gpt forms virions with the same approximate buoyant density and RNA content with or without gp160. This observation confirms and extends the results of Karacostas et al. which revealed that immature retrovirus-like particles appeared in cultures of cells infected with a recombinant vaccinia virus that expressed HIV *gag-pol* polypeptides (11). The present study demonstrates that packaging of HIV RNA and release of viral particles from cells are just as efficient in the absence as in the presence of gp160. Studies with defective avian and murine retroviruses have also shown that the *env* protein is not required for particle assembly (19, 20). It is apparent that gp160 does not control virus assembly, since the budding process is not slowed by the lack of a viral envelope glycoprotein. The incorporation of gp160 onto particles may proceed in a passive manner because of the presence of gp160 on the plasma membrane. If this is so, then it is possible that cellular plasma membrane proteins are also incorporated onto HIV virions. It will be interesting to determine the structural requirements for loading of a given protein onto the HIV envelope.

Viral pseudotypes. An MLV amphotropic *env* was able to substitute for gp160 in the formation of infectious virus after COS-7 cell transfection. Predictably, the presence of the amphotropic glycoprotein on HIV core particles enabled infection of both human and murine cells. It has recently been shown that dual infection of human cells with HIV and amphotropic MLV resulted in the formation of mixed-phenotype viruses that could transmit the HIV provirus to cells bearing the amphotropic MLV receptor (22). Our results confirm these findings and demonstrate that *env* is the only MLV gene product required to alter the host range of HIV. The pseudotyping of MLV *env* with HIV was an efficient process; titers of HIV(A-MLV) were generally higher than those of HIV(HXB2) generated in parallel transfections (data not shown). It should be noted that HIV and MLV *env* proteins do not share significant sequence homology. Thus, the ability of HIV to incorporate viral glycoproteins other than gp160 onto particles reveals a flexibility in HIV assembly. It is tempting to speculate that the structural features(s) present in HIV *env* and MLV *env* that permits their incorporation onto virions may be present in a variety of other proteins. It was recently reported that HIV forms phenotypically mixed particles with xenotropic MLV (15) and herpes simplex virus and vesicular stomatitis virus (24). In addition, we have shown that pseudotypes between HIV and human T-cell lymphotropic virus type I *env* can be formed (N. R. Landau, K. A. Page, and D. R. Littman, submitted for publication). The host range of HIV is extended to $CD4^-$ cells when such mixing occurs. It is possible that the permissiveness of HIV for inclusion of various glycoproteins in its envelope provides the virus with a mechanism to extend the host range in vivo. The potential to form mixed-phenotype viruses is clearly present in acquired immunodeficiency syndrome patients, since they are often infected with many different viruses. Studies on the ability of HIV to utilize non-HIV proteins to expand viral host range may lead to a better understanding of disease progression in acquired immunodeficiency syndrome.

ACKNOWLEDGMENTS

We gratefully acknowledge Mei Lie Wong for performing electron microscopy, and we thank Robert von Borstel III, Paul Skiles, and Kathlelyn Steimer for help and advice with HIV cultures. We also thank Brian Seed and Alejandro Aruffo for the plasmid clone pCDMCD4 and Richard Locksley for use of his biocontainment laboratory.

This work was supported by Public Health Service grants 1F32-AI 0890 to K.A.P. and AI 24286 to D.R.L. from the National Institutes of Health and a Cancer Research Institute/Carolyn Spitzer memorial fellowship to N.R.L.

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