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Derivation of a biologically contained replication system for human immunodeficiency virus type 1

(Tat/Rev/mutant human immunodeficiency virus type 1/trans complementation)

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ABSTRACT **Human immunodeficiency virus type 1 (HIV-1) proviral mutants that lack viral regulatory genes are unable to replicate unless rescued by complementation in trans. Structurally intact virus can be produced by infecting recombinant cell lines expressing the deficient genes. A HIV-1 mutant functionally defective in *tat* and *rev* (vIIIB$^{\Delta Tat/Rev}$), which replicates only in a recombinant T-cell line expressing *tat* and *rev* (CEM^{TART}), is described in this report. Infection of the CEM^{TART} cell line with vIIIB$^{\Delta Tat/Rev}$ permits the complete HIV-1 life cycle, including cytopathology, decreased expression of CD4, and production of viral structural proteins, to be biologically contained. Culture supernatants from infected CEM^{TART} contain virus that is able to replicate only in uninfected CEM^{TART}. No reversion of vIIIB$^{\Delta Tat/Rev}$ to wild-type HIV-1 was observed as measured either by sequencing proviral vIIIB$^{\Delta Tat/Rev}$ or by detecting the ability of vIIIB$^{\Delta Tat/Rev}$ to replicate in CEM or activated CD4-bearing T lymphocytes. Defective HIV-1 mutants produced by trans complementation of essential genes permit infection and analysis of defined genotypes on cellular function and phenotype. Authentic HIV-1 structural proteins and infected cells can be prepared in mass, and agents that interfere with the HIV-1 life cycle can be studied on a large scale with minimum risk of exposing workers to virulent HIV-1.**

Human immunodeficiency virus type 1 (HIV-1) proviral mutants are important to help determine the function of HIV-1 gene products. The effect of HIV-1 genes on host cell function can be examined by deletion of genes such as *nef*. Mutants that lack essential viral genes are unable to replicate and aspects of their function on the host cell cannot be determined. A recombinant cell line expressing essential viral regulatory genes in trans was used to complement defective molecular clones of HIV-1 to generate high-titer structurally intact virus. The defective HIV-1 generated is capable of binding and integrating in CD4-bearing T cells but of replicating only in the recombinant cells.

The initial events of the HIV-1 life cycle, including binding, fusion, entry, reverse transcription, and integration, are followed by the expression of a class of fully spliced mRNA that encodes the viral regulatory gene products Tat, Rev, and Nef (1–3). Tat and Rev are essential trans-acting proteins, each encoded by two exons, that act to increase transcription of viral mRNA and to allow transport of unspliced mRNA to the cytoplasm, respectively. It is only following the generation of the second class of viral mRNA, which consists of unspliced and singly spliced mRNA encoding the viral proteins Gag, Pol, and Env, that virion assembly, maturation, and budding can occur (1). The critical control of viral gene expression exerted by *tat* and *rev* made them attractive candidates for deletion to create replication-incompetent HIV-1 because each is independently required for *gag*, *pol*, and *env* expression.

To create a HIV-1 provirus functionally deficient in *tat* and *rev*, physically separate mutations in the first exon of *tat* and both exons of *rev* were made. The complementary Tat and Rev gene products were both expressed from a single bicistronic mRNA derived from a spliced cDNA. These reagents therefore provided a biologically contained system to study the replication of HIV-1 in culture.

MATERIALS AND METHODS

Viruses. HIV-1 was generated as described by transfection of the proviral plasmid IIIB, a derivative of HXB-3 (4). Oligonucleotide-directed mutagenesis with a bacteriophage M13 system (Amersham) was used to introduce five point mutations into the coding region of Tat and Rev in pIIIB$^{\Delta Tat/Rev}$. Since Tat and Rev are encoded in different frames, mutations in the first exon of *tat* and *rev* result in a premature stop codon in *tat* while deleting the *rev* start codon. In the second exon of *rev*, mutations result in a premature stop codon. All mutations are shown in Table 1.

The Tat-responsive chloramphenicol acetyltransferase (CAT) expression vector pLTR-CAT contains the HIV-1 long terminal repeat (LTR) fused to the CAT gene (4). The Rev-responsive CAT expression vector pDM128/CMV contains the simian virus 40 (SV40) origin of replication, the cytomegalovirus (CMV) immediate-early promoter, and the bacterial CAT gene inserted into an intron regulated by Rev. pBC12/CMV/CAT contains the CMV immediate-early promoter driving expression of the CAT gene (5).

The Moloney murine leukemia virus-based retroviral vector LXSN (a generous gift of A. Dusty Miller, Fred Hutchinson Cancer Center, Seattle, WA) contains the neomycin phosphotransferase gene (neomycin-resistance gene) transcribed from an internal SV40 early promoter (6). The L^{TART}SN vector is a modification of LXSN containing the *Sal* I–*Xho* I fragment from pcTART which is inserted into the *Xho* I site of LXSN and transcribed from the 5′ retroviral LTR. L^{TART}SN virus (vL^{TART}SN) was produced by calcium phosphate transfection of the amphotropic packaging cell line PA317 with 2.5 mg of pL^{TART}SN. Cell-free supernatant from transfected PA317 was isolated after 24 hr, and vL^{TART}SN titer was determined on NIH 3T3 cells by detecting G418-resistant colony-forming units per ml as described (7).

Cells. COS cells were grown in Iscove's modified Dulbecco's medium/10% fetal bovine serum (FBS). CEM cells were grown in RPMI 1640 medium/20% FBS. Activated peripheral

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Abbreviations: HIV-1, human immunodeficiency virus type 1; CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat; CMV, cytomegalovirus; SV40, simian virus 40; PBL, peripheral blood mononuclear cell; AZT, 3′-azido-3′-deoxythymidine; FBS, fetal bovine serum.

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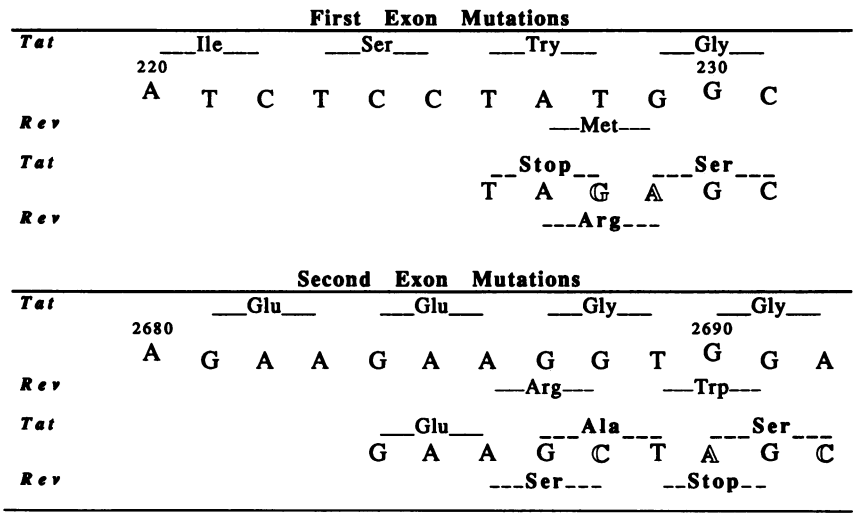
Table 1. Mutations in Tat and Rev of vIIIB$^{\Delta Tat/Rev}$



blood mononuclear cells (PBLs) were isolated from normal donor HIV-seronegative blood, centrifuged over Ficoll/Hypaque, washed and suspended in RPMI 1640 medium/20% FBS with 5 mg of phytohemagglutinin per ml (GIBCO) for 72 hr, washed, and resuspended in medium with 20 units of recombinant interleukin 2 per ml (DuPont).

To introduce cTART into CEM cells, CEM cells were cultured with the vL^{TART}SN at a virus-to-cell ratio of 0.2 in the presence of protamine sulfate (5 mg/ml; Lilly) for 2 hr, washed, and, after 48 hr in culture, placed under selection with the neomycin analogue G418 (Geneticin; GIBCO), 0.3 mg/ml (active drug) for 14 days. After selection, limiting dilution cloning was performed, and genomic DNA extraction of CEM^{TART} and PCR amplification were performed as described (8) (CEM^{TART} 5′ primer, ATCGTGACCTGGGAAGCCTTGGCTTT; CEM^{TART} 3′ primer, CTGGGGACTTTCCACACCTGGTTGC).

CAT Assay. Tat and Rev indicator elements linked to CAT were used as described (5). COS cell cultures (60 mm) were cotransfected by calcium phosphate with 2.5 mg of either pcTAT, pcREV, pcTART, pL^{TART}SN, or pLXSN and 2.5 mg of either pLTR-CAT, pDM128/CMV, or pBC/CMV or with 5.0 mg of pBC12/CMV/CAT (9). Protein extracts were prepared from cells 48 hr after transfection and levels of CAT activity were determined as described (10). Relative protein concentrations were used to normalize CAT activity.

pIIIB$^{\Delta Tat/Rev}$ Transfection. COS cells were cotransfected by calcium phosphate with 2.5 mg of either pIIIB or pIIIB$^{\Delta Tat/Rev}$ and 2.5 mg of either pcTAT, pcREV, pcTART, pL^{TART}SN, or pLXSN. Soluble p24 Gag expression was monitored using an ELISA and was quantitated in pg of p24 expression per ml by reference to standards supplied by the manufacturer (DuPont).

vIIIB$^{\Delta Tat/Rev}$ Infection. Following cotransfection of COS cells with 2.5 mg of either pIIIB or pIIIB$^{\Delta Tat/Rev}$ and 2.5 mg of pL^{TART}SN, COS cells were maintained in culture for 72 hr and then cocultivated by the addition of 2.5 × 10^6 CEM or CEM^{TART} cells and fresh medium to maintain cells at 10^6 cells per ml. After an additional 72 hr, nonadherent cells were aspirated, washed, and suspended in fresh medium at 2 × 10^5 cells per ml at 37°C in 5% CO_2 containing humidified air. Every 48 hr, cells were washed and counted, viability was determined by trypan blue dye exclusion, and cells were resuspended in fresh medium. Cells were maintained in culture for 12 weeks and 48-hr supernatants were assayed for p24 every 8 days.

Culture supernatants of vIIIB$^{\Delta Tat/Rev}$-infected CEM^{TART} cells (CEM^{TART}/IIIB$^{\Delta Tat/Rev}$) were assayed for soluble p24 concentration and used to infect permissive cells. Infection of fresh CEM^{TART} was performed by collecting supernatant from CEM^{TART}/IIIB$^{\Delta Tat/Rev}$ after 48 hr, passing the supernatant through a 22-μm filter, and incubating 1 ml with 10^5 CEM^{TART} for 1 hr at 37°C. Infected CEM^{TART} cells were then washed twice, counted, and resuspended in fresh medium at 2 × 10^5 cells per ml. Every 48 hr supernatants were aspirated and assayed for p24. Cells were washed and counted, and viability was determined by trypan blue dye exclusion. CEM^{TART} cells were then resuspended in fresh medium at 2 × 10^5 cells per ml. Every 7 days, culture supernatants were used to infect fresh CEM^{TART} or either CEM or PBLs. Culture supernatants from CEM^{TART} infected with progeny virus were serially passed every 7 days on fresh CEM^{TART}, and the progeny vIIIB$^{\Delta Tat/Rev}$ produced after passage no. 4 were assayed for infectivity on CEM^{TART}, CEM, and PBLs as described.

DNA Analysis. Oligonucleotides corresponding to the 5′ and 3′ sequences flanking the mutations in vIIIB$^{\Delta Tat/Rev}$ were designed to allow PCR amplification of the mutated *tat* and *rev* genes in the integrated IIIB$^{\Delta Tat/Rev}$ provirus (*tat* and *rev* exon 1 mutation 5′ primer, CAAACAACTGCTGTTTATCCA; *tat* and *rev* exon 1 mutation 3′ primer, CATGGATCCACTGCTTTGATAGAGAAACT; *tat* and *rev* exon 2 mutation 5′ primer, TGGCTGTGGTATATAAAATTA; *tat* and *rev* exon 2 mutation 3′ primer, CATAAGCTTGCTGAAGAGGCACAGGCT). Following genomic DNA extraction of CEM^{TART} infected with vIIIB$^{\Delta Tat/Rev}$ and maintained in culture for 9 weeks or infected with vIIIB$^{\Delta Tat/Rev}$ recovered after four serial passages in CEM^{TART}, PCR amplification and dideoxynucleotide sequencing were performed as described (11). In addition, genomic DNA was extracted from CEM exposed to vIIIB$^{\Delta Tat/Rev}$ and maintained in culture for 10 days. PCR amplification (Amplitaq; Cetus) and dideoxynucleotide sequencing (Sequenase; United States Biochemical) were performed as described (12).

Inhibition with 3′-Azido-3′-deoxythymidine (AZT). Infection of CEM^{TART} with vIIIB$^{\Delta Tat/Rev}$ was performed in the presence of various concentrations of AZT (13). On day 0, 0.5 × 10^6 CEM or CEM^{TART} cells were placed in six-well plates (Falcon) and incubated with AZT at final concentrations of 0, 50, 5, and 0.5 mM for 1 hr in 1 ml of RPMI 1640 medium/20% FBS at 37°C and 5% CO_2 containing humidified air. One milliliter of a filtered (22-μm), 72-hr culture supernatant from COS cells cotransfected with either 2.5 μg of pIIIB or pIIIB$^{\Delta Tat/Rev}$ and 2.5 μg of pL^{TART}SN, and diluted with RPMI 1640 medium/20% FBS to contain 3 ng of p24 per ml, was added to each well. After a 1-hr incubation, 1 ml of medium

containing AZT was added to bring the final concentration of AZT to either 0, 50, 5, or 0.5 mM, respectively. After 48 hr the cells in culture were aspirated, washed twice, and resuspended at 0.25×10^6 cells per ml in fresh medium containing AZT. On day 5, cells were washed twice and resuspended at 0.25×10^6 cells per ml in fresh medium containing AZT. Ninety-six-hour culture supernatants were collected at day 9 and assayed for soluble p24.

RESULTS

Construction of pIIIB$^{\Delta Tat/Rev}$, pL^{TART}SN, and CEM^{TART}. The full genome of HIV-1 (IIIB) is depicted in Fig. 1*A*. The expanded view depicts the coding region between the unique *Sal* I and *Xho* I restriction sites in pIIIB. The lower, upward pointing arrows depict the locations of the missense mutations in pIIIB$^{\Delta Tat/Rev}$ that functionally delete *tat* and *rev*.

pcTART is a cDNA expression vector that encodes both exons of *tat* and *rev* (Fig. 1*B*) (31). The retroviral vector LXSN is also depicted in Fig. 1*B*. The construction of L^{TART}SN is depicted by brackets surrounding cTART and the arrow pointing at the *Xho* site of LXSN. The L^{TART}SN vector contains the *Sal* I–*Xho* I fragment from pcTART inserted into the *Xho* I site of LXSN and transcribed from the 5′ retroviral LTR.

Functional assays of Tat and Rev performed by transfecting COS cells with Tat or Rev indicator elements linked to CAT independently confirmed the presence of functional Tat and Rev in pcTART- and pL^{TART}SN-transfected COS cells (data not shown).

After CEM cells were cultured 48 hr with vL^{TART}SN (virus-to-cell ratio, 0.2) and placed under selection with G418 for 14 days (CEM^{TART}), limiting dilution cloning was performed. Genomic DNA extraction and PCR amplification using 5′ and 3′ oligonucleotides flanking cTART confirmed the presence of cTART in CEM^{TART} clones (data not shown).

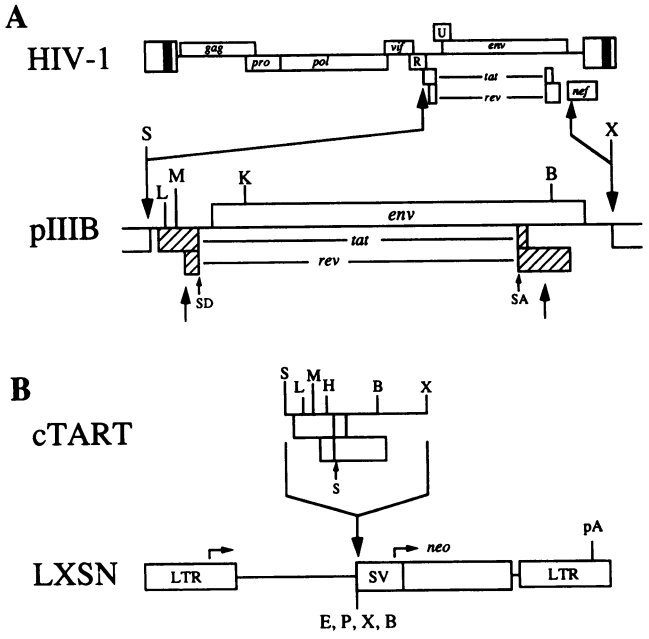


FIG. 1. (*A*) HIV-1 genome shown at top with enlarged view of *tat* and *rev* coding regions below. Lower arrows depict the locations of the mutations that functionally delete *tat* and *rev*. (*B*) pcTART is a cDNA expression vector that encodes both exons of *tat* and *rev*. pcTART includes the overlapping sequences of pcTAT and pcREV (*Mst* II–*Bam*HI, containing the splice site of *tat* and *rev*, the *rev* initiation codon, and the *tat* stop signal) and unique sequences from pcTAT (*Sal* I–*Mst* II, containing the *tat* initiation signal) and pcREV (*Bam*HI–*Xho* I, containing the *rev* stop signal). Ltart SN contains the *Sal* I–*Xho* I fragment from pcTART inserted into the *Xho* I site of LXSN. L, *Xho* II; K, *Kpn* I; E, *Eco*RI; P, *Hpa* I; LTR, LTR sequences; SV, SV40 promoter; *neo*, neomycin-resistance gene (neomycin phosphotransferase); pA, poly(A) signals; SD, splice donor; SA, splice acceptor.

vIIIB$^{\Delta Tat/Rev}$ Infection. Supernatants of COS cells transfected with pIIIB for 72 hr contained >200 pg of p24 per ml. When pIIIB was cotransfected with pcTART, pcTAT, pcREV, or pL^{TART}SN, similar levels (200–800 pg/ml) of p24 were produced. In contrast, supernatants of COS cells transfected with pIIIB$^{\Delta Tat/Rev}$ for 72 hr contained no detectable p24. When pIIIB$^{\Delta Tat/Rev}$ was cotransfected with pcTART or pL^{TART}SN, p24 was detected in COS cell supernatants after 72 hr at 30 pg/ml and 200 pg/ml, respectively (Fig. 2). Cotransfection of pIIIB or pIIIB$^{\Delta Tat/Rev}$ with pL^{TART}SN leads to higher p24 levels than cotransfection with pcTART. This is partially due to transcription from a single SV40 origin of replication in COS cells transfected with pIIIB or pIIIB$^{\Delta Tat/Rev}$ with pL^{TART}SN, whereas COS cells containing pIIIB or pIIIB$^{\Delta Tat/Rev}$ with pTART contain competing SV40 origins of replication, leading to a decrease in transcription. COS cell supernatants 72 hr after cotransfection of pIIIB$^{\Delta Tat/Rev}$ with pcTAT alone or pcREV alone were shown to contain no detectable p24, confirming that pIIIB$^{\Delta Tat/Rev}$ rescue required functional Tat and Rev.

CEM and CEM^{TART} were then infected with vIIIB$^{\Delta Tat/Rev}$ produced by cotransfection of COS cells with pL^{TART}SN and pIIIB$^{\Delta Tat/Rev}$. Infection was performed by incubating 10^6 cells with 3 ml of the supernatants of transfected COS cells containing 200 pg of p24 per ml. CEM^{TART} infected with vIIIB$^{\Delta Tat/Rev}$ typically have a decrease in cell viability (<50%) after 72 hr, with a return to >90% viability after 7 days. CEM cells exposed to vIIIB$^{\Delta Tat/Rev}$ did not demonstrate any cytopathology. Culture supernatants of CEM^{TART} cells infected with vIIIB$^{\Delta Tat/Rev}$ demonstrate rising concentrations of p24 until they are $>10^5$ pg/ml by day 10 and remain at high levels over 45 days in culture (Fig. 3). No p24 was detected from the supernatants of CEM cells infected with vIIIB$^{\Delta Tat/Rev}$.

Flow cytometric analysis of CEM^{TART} infected with vIIIB$^{\Delta Tat/Rev}$ using monoclonal antibodies against CD4 revealed a marked decrease in CD4 expression, in a fashion similar to CEM cells infected with vIIIB. CEM exposed to vIIIB$^{\Delta Tat/Rev}$ did not demonstrate any decrease in expression of CD4 (data not shown). Western blot analysis of CEM^{TART} infected with vIIIB$^{\Delta Tat/Rev}$ reveals patterns of HIV-1 protein expression identical to CEM cells infected with vIIIB (data not shown).

Inhibition by AZT. AZT inhibited replication of vIIIB in CEM cells and vIIIB$^{\Delta Tat/Rev}$ in CEM^{TART} cells at concentrations of 50 and 5 μg/ml and partially inhibited replication at 0.5 μg/ml (Fig. 4). Other anti-HIV drugs, including dextran

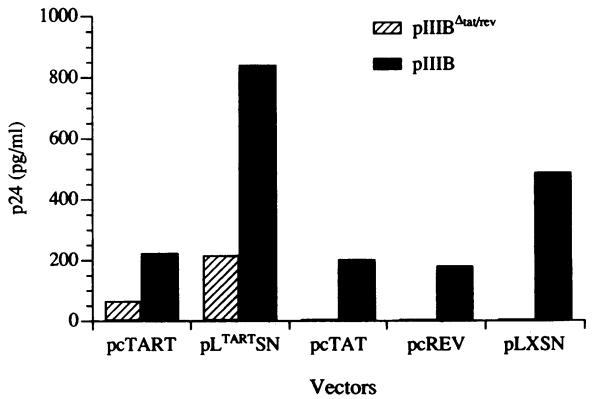


FIG. 2. HIV-1 p24 ELISA of 72-hr COS cell supernatants after cotransfection by calcium phosphate with listed vectors.

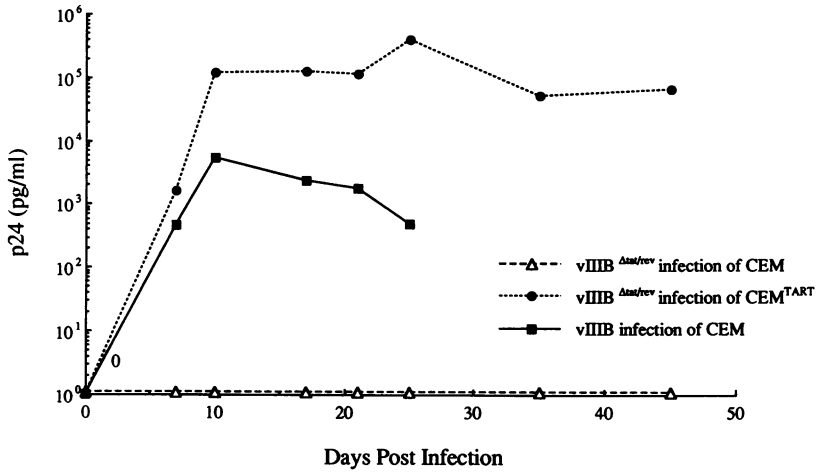


FIG. 3. HIV-1 p24 ELISA of long-term cell culture supernatants after infection with the listed viruses.

sulfate and dideoxycytidine, inhibited vIIIB$^{\Delta Tat/Rev}$ infection of CEM^{TART} at levels similar to their inhibition of vIIIB infection of CEM (data not shown).

Because CEM^{TART} constitutively express Tat and Rev and are not subject to the down-regulation of Tat and Rev expression that normally occurs in the HIV-1 life cycle, it was felt that CEM^{TART} would support higher levels of HIV-1 virion production than CEM. To ensure that identical amounts of infectious vIIIB are used to infect CEM or CEM^{TART}, equal amounts of a 72-hr supernatant from a single COS cell culture transfected with pIIIB was used (vIIIB, Fig. 4). Following infection of CEM (denoted as triangles) or CEM^{TART} (denoted as circles) with vIIIB, the concentration of p24 in culture supernatants of infected CEM^{TART} after 9 days was ≈1000-fold higher than p24 concentrations in infected CEM.

Detection of Replication-Competent vIIIB$^{\Delta Tat/Rev}$. CEM^{TART} infected with vIIIB$^{\Delta Tat/Rev}$ for 9 weeks continued to produce large amounts of soluble p24 and vIIIB$^{\Delta Tat/Rev}$ capable of replicating in CEM^{TART}, but the vIIIB$^{\Delta Tat/Rev}$ produced maintained its phenotype by remaining replication incompetent in CEM or PBLs (Table 2). Similarly, after four serial passages of vIIIB$^{\Delta Tat/Rev}$ on CEM^{TART}, no virus capable of replication in CEM or PBLs was detected in culture supernatants, although the vIIIB$^{\Delta Tat/Rev}$ remained capable of replicating in CEM^{TART}.

No reversions in either of the two separate mutations were detected in the IIIB$^{\Delta Tat/Rev}$ provirus present in the genomic DNA of five clones of CEM^{TART} after 12 weeks of infection with vIIIB$^{\Delta Tat/Rev}$. Similarly, no reversions were detected in five clones of the IIIB$^{\Delta Tat/Rev}$ provirus serially passaged in CEM^{TART}.

To determine if vIIIB$^{\Delta Tat/Rev}$ infection and proviral integration occurred in CEM cells, genomic DNA from CEM cells 10 days after exposure to vIIIB$^{\Delta Tat/Rev}$ was extracted. PCR amplification and DNA sequencing of the IIIB$^{\Delta Tat/Rev}$ provirus present in the genomic DNA demonstrated the presence of proviral IIIB$^{\Delta Tat/Rev}$ in CEM cells. Culture supernatants from these CEM cells did not contain detectable p24. In addition, no cytopathology or CD4 down-regulation occurred.

DISCUSSION

The possibility of mutations or recombinations to replication-competent virus was carefully considered in the design of this system. The possibility of mutations or recombination to revert to wild-type HIV-1 was minimized by creating two physically separate mutations in the two exons of *tat* and *rev* in vIIIB$^{\Delta Tat/Rev}$. Recombination in the T-cell line containing the complementary Tat and Rev gene products expressed from a single bicistronic mRNA derived from a spliced cDNA would have to occur over two short, physically separated sequences. Furthermore, such a recombination would have a high probability of disrupting *env* expression, because the *env* coding region physically separates the exons of *tat* and *rev*. As predicted by these considerations, reversion of the defective HIV-1 to wild type was not observed.

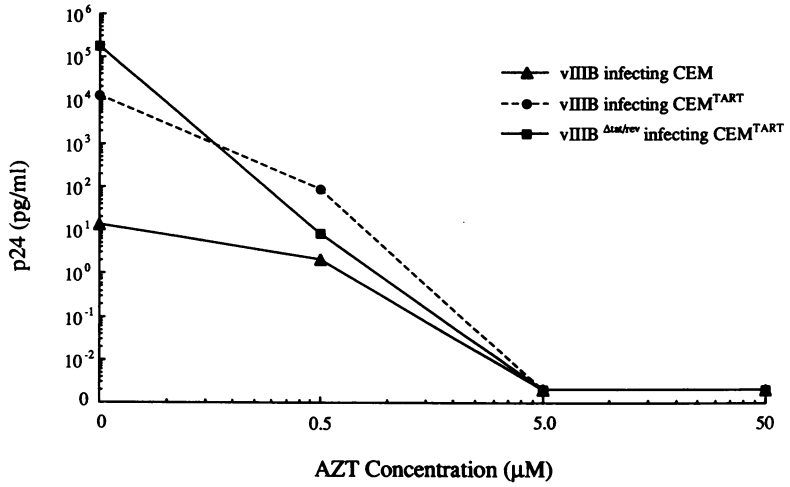


FIG. 4. HIV-1 p24 ELISA of cell culture supernatants 9 days after infection with the listed viruses in the presence of various concentrations of AZT.

Table 2. Detection of replication-competent vIIIB$^{\Delta Tat/Rev}$

IIIB$^{\Delta Tat/Rev}$ source	Target cells	Mean p24 after infection, pg/ml	
		vIIIB$^{\Delta Tat/Rev}$*	vIIIB
CEM^{TART} infected with IIIB$^{\Delta Tat/Rev}$ for 9 weeks	CEM	0	>1000
	PBLs†	0	>1000
	CEM^{TART}	112,000 ± 1000	>1000
CEM^{TART} infected with IIIB$^{\Delta Tat/Rev}$ after four passages	CEM	0	>1000
	PBLs†	0	>1000
	CEM^{TART}	150,000 ± 1500	>1000

HIV-1 p24 ELISA of cell culture supernatants 2 weeks after infection with the listed virus.
*Mean ± SEM.
†Activated PBLs.

A number of questions regarding the biology of HIV-1 infection may be addressed by the strategies described. First, the kinetics of a single cycle of infection of cells may be determined using replication-incompetent virus. Although direct detection of proviral HIV-1 requires relatively large numbers of infected cells, amplification of full-length proviral DNA by quantitative PCR would allow a semiquantitative analysis of the cells infected in the absence of cell-to-cell spread of virus in culture.

Using a similar strategy, the cells initially infected in experimental animal models of HIV-1 following intravenous, intravaginal, or intraanal infection may be determined. The cells initially infected after viral challenge of animals could be determined using high-titered replication-incompetent virus because secondary infection from progeny virus would not occur. Strategies to increase the ability to detect cells containing vIIIB$^{\Delta Tat/Rev}$ include replacing nonessential genes such as *nef* with an indicator gene such as CAT. Although only chimpanzees can be reliably infected with HIV-1, similar strategies to create high-titer replication-incompetent simian immunodeficiency virus (SIV) may allow similar studies to be performed in other primates. Since HIV-1 Tat and Rev can functionally replace SIV Tat and Rev, CEM^{TART} may be permissive for SIV mutants defective in Tat and/or Rev (14–16).

Currently, only transfection techniques allow the introduction of defined genotypes of HIV-1 with mutations in essential genes into cells. In contrast to the inefficient methods of transfection, replication-incompetent HIV-1 would allow the efficient introduction of genetically defined HIV-1 mutants into T cells and allow the phenotypic analysis of mutations in essential genes.

Native viral proteins, complete viral particles, and virus-infected cells can all be generated in mass using the described techniques. By exploiting the constitutive expression of *tat* and *rev* in recombinant cell lines large levels of viral structural proteins are produced. Inactivated, whole killed virus preparations that may be used as vaccines against HIV-1 could be prepared with these techniques with enhanced safety over wild-type, inactivated HIV-1.

Finally, attenuated or replication-defective HIV-1 may be used in research laboratories for the safety of laboratory workers (17–21). At least two cases of accidental, laboratory-acquired HIV-1 infection have been documented (21). Biological as well as physical containment has been used to limit the risk of infection to laboratory workers (20, 22–27). In eukaryotic host systems, risks to laboratory workers are reduced if either no virus vector particles are produced or, if virus particles are produced, essential genes are deleted, making them replication-incompetent (28).

Strategies to develop attenuated HIV-1 include deletion of nonessential genes such as *nef*, which may play a role in pathogenesis of disease (29). This approach is based on the assumption that the pathogenesis of HIV-1 infection is related to expression of *nef*. Use of *nef*-defective mutants, though not eliminating the risk of HIV-1 infection, would theoretically reduce the risk of developing AIDS (30). Other approaches include the use of viral mutants incapable of replication; the disadvantages of this latter approach are the inability to express all HIV-1 genes and the inability to detect standardized end points of replication.

The biologically contained HIV-1 replication system described here will likely yield comparable results in a variety of HIV-1-based investigative studies. These include tests of virus neutralization and tests for antiviral drugs. Studies with AZT demonstrate that results using vIIIB$^{\Delta Tat/Rev}$ infecting CEM^{TART} parallel results obtained using wild-type HIV-1 infecting CEM. Other antiviral agents have been similarly tested, yielding comparable results.

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