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

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

HERBERT CHEN*, TERENCE J. BOYLE*, MICHAEL H. MALIM†, BRYAN R. CULLEN†, AND H. KIM LYERLY*‡§

*Department of Surgery, †Howard Hughes Medical Institute, Center for AIDS Research, Departments of Medicine, Microbiology and Immunology, and ‡Pathology, Duke University Medical Center, Durham, NC 27710

Communicated by Gertrude B. Elion, April 13, 1992 (received for review February 24, 1992)

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 recombinaant cell lines expressing the deficient genes. A HIV-1 mutant functionally defective in tat and rev (vIIIBATat/Rev), which replicates only in a recombiant T-cell line expressing tat and rev (CEM'TART), is described in this report. Infection of the CEM'TART cell line with vIIIIBATat/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 vIIIIBATat/Rev to wild-type HIV-1 was observed as measured either by sequencing proviral vIIIIBATat/Rev or by detecting the ability of vIIIIBATat/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 recombiant 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 recombiant 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 bisctronic 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 pIIIBATat/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 viral regulatory genes are 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 LTRTSN vector is a modification of LXSN containing the SalI-XhoI fragment from pCTART which is inserted into the XhoI site of LXSN and transcribed from the 5' retroviral LTR. LTRTSN virus (vLTRTSN) was produced by calcium phosphate transfection of the amphotropic packaging cell line PA317 with 2.5 mg of pLTRTSN. Cell-free supernatant from transfection PA317 was isolated after 24 hr, and vLTRTSN 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

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.

‡To whom reprint requests should be addressed.
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 vIIIBATSN 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 (Genetecic; GIBCO), 0.3 mg/ml (active drug) for 14 days. After selection, limiting dilution cloning was performed, and genomic DNA extraction of CEMTART and PCR amplification were performed as described (8) (CEM'TART 5' primer, ATCGTGACCTGGGACTT; primer, ATCGTGACCTGGGACTT). 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, plTARTSN, 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.

p218BAT/Rev Transfection. COS cells were cotransfected by calcium phosphate with 2.5 mg of either plIIB or pIIIBAT/Rev and 2.5 mg of either pcTAT, pcREV, pcTART, plTARTSN, or plXSN. Soluble 24 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).

pIIIBAT/Rev Infection. Following cotransfection of COS cells with 2.5 mg of either plIIB or pIIIBAT/Rev and 2.5 mg of plTARTSN, COS cells were maintained in culture for 72 hr and then cocultivated by the addition of 2.5 x 10^6 CEM or CEMTART 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 x 10^6 cells per ml at 37°C in 5% CO2 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 pIIIBAT/Rev-infected CEMTART cells (CEMTART/IIIBAT/Rev) were assayed for soluble p24 concentration and used to infect permissive cells. Infection of fresh CEMTART was performed by collecting supernatant from CEMTART/IIIBAT/Rev after 48 hr, passing the supernatant through a 22-μm filter, and incubating 1 ml with 10^5 CEMTART for 1 hr at 37°C. Infected CEMTART cells were then washed twice, counted, and resuspended in fresh medium at 2 x 10^6 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. CEMTART cells were then resuspended in fresh medium at 2 x 10^6 cells per ml. Every 7 days, culture supernatants were used to infect fresh CEMTART or either CEM or PBLs. Culture supernatants from CEMTART infected with progeny virus were serially passed every 7 days on fresh CEMTART, and the progeny vIIIBAT/Rev produced after passage no. 4 were assayed for infectivity on CEMTART, CEM, and PBLs as described.

DNA Analysis. Oligonucleotides corresponding to the 5' and 3' sequences flanking the mutations in vIIIBAT/Rev were designed to allow PCR amplification of the mutated tat and rev genes in the integrated IIIIBAT/Rev provirus (tat and rev exon 1 mutation 5' primer, CAAAACACTGCTGTTTAGTCTCA; tat and rev exon 1 mutation 3' primer, CATGACACCATGGTGAAGAAACT; tat and rev exon 2 mutation 5' primer, TGGCCTGTGTATATAAATTA; tat and rev exon 2 mutation 3' primer, CATAAGCCCGTAAAAGGCGACACGGCT). Following genomic DNA extraction of CEMTART infected with vIIIBAT/Rev and maintained in culture for 9 weeks or infected with vIIIBAT/Rev recovered after four serial passages in CEMTART, PCR amplification and dideoxynucleotide sequencing were performed as described (11). In addition, genomic DNA was extracted from CEM exposed to vIIIBAT/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 CEMTART with vIIIBAT/Rev was performed in the presence of various concentrations of AZT (13). On day 0, 0.5 x 10^5 CEM or CEMTART 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% CO2 containing humidified air. One milliliter of a filtered (22-μm) 72-hr culture supernatant from COS cells cotransfected with either 2.5 μg of plIIB or pIIIBAT/Rev and 2.5 μg of plTARTSN, 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

Table 1. Mutations in Tat and Rev of vIIIBAT/Rev

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutations</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Exon Mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tat</td>
<td>Ile→Ser</td>
<td>ATCTCTATGCG</td>
</tr>
<tr>
<td>Rev</td>
<td>Met</td>
<td>ACCAGAGCGC</td>
</tr>
<tr>
<td>Second Exon Mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tat</td>
<td>Glu→Glu</td>
<td>GAGGTTGAGG</td>
</tr>
<tr>
<td>Rev</td>
<td>Arg→Trp</td>
<td></td>
</tr>
</tbody>
</table>

GATACTGCTGTTTAGTCTCA; Tat and rev exon 1 mutation 5' primer, CAAAACACTGCTGTTTAGTCTCA; Tat and rev exon 1 mutation 3' primer, CATGACACCATGGTGAAGAAACT; Tat and rev exon 2 mutation 5' primer, TGGCCTGTGTATATAAATTA; Tat and rev exon 2 mutation 3' primer, CATAAGCCCGTAAAAGGCGACACGGCT. Following genomic DNA extraction of CEMTART infected with vIIIBAT/Rev and maintained in culture for 9 weeks or infected with vIIIBAT/Rev recovered after four serial passages in CEMTART, PCR amplification and dideoxynucleotide sequencing were performed as described (11). In addition, genomic DNA was extracted from CEM exposed to vIIIBAT/Rev and maintained in culture for 10 days. PCR amplification (AmpliTaq; Cetus) and dideoxynucleotide sequencing (Sequenase; United States Biochemical) were performed as described (12).
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^ATat/Rev*, pL^TARTSN, and CEM^TART.

The full genome of HIV-1 (IIIB) is depicted in Fig. 1A. 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^ATat/Rev* that functionally delete tat and rev.

pC-TART is a cDNA expression vector that encodes both exons of tat and rev (Fig. 1B) (31). The retroviral vector LXSN is also depicted in Fig. 1B. The construction of L^TARTSN is depicted by brackets surrounding cTART and the arrow pointing at the Xho site of LXSN. The L^TARTSN vector contains the Sal I–Xho I fragment from pC-TART 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 pC-TART- and pL^TARTSN-transfected COS cells (data not shown).

After CEM cells were cultured 48 hr with vL^TARTSN (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).

vIIIB^ATat/Rev Infection.

Supernatants of COS cells transfected with pIIIB for 72 hr contained >200 pg of p24 per ml. When pIIIB was cotransfected with pC-TART, pC-TAT, pC-REV, or pL^TARTSN, similar levels (200–800 pg/ml) of p24 were produced. In contrast, supernatants of COS cells transfected with pIIIB^ATat/Rev for 72 hr contained no detectable p24. When pIIIB^ATat/Rev was cotransfected with pC-TART or pL^TARTSN, 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^ATat/Rev with pL^TARTSN leads to higher p24 levels than cotransfection with pC-TART. This is partially due to transcription from a single SV40 origin of replication in COS cells transfected with pIIIB or pIIIB^ATat/Rev with pL^TARTSN, whereas COS cells containing pIIIB or pIIIB^ATat/Rev with pTART contain competing SV40 origins of replication, leading to a decrease in transcription. COS cell supernatants 72 hr after cotransfection of pIIIB^ATat/Rev with pC-TART alone or pC-REV alone were shown to contain no detectable p24, confirming that pIIIB^ATat/Rev rescue required functional Tat and Rev.

CEM and CEM^TART were then infected with vIIIB^ATat/Rev produced by cotransfection of COS cells with pL^TARTSN and pIIIB^ATat/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^ATat/Rev typically have a decrease in cell viability (<50%) after 72 hr, with a return to >90% viability after days 7. CEM cells exposed to vIIIB^ATat/Rev did not demonstrate any cytopathology. Culture supernatants of CEM^TART cells infected with vIIIB^ATat/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^ATat/Rev.

Flow cytometric analysis of CEM^TART infected with vIIIB^ATat/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^ATat/Rev did not demonstrate any decrease in expression of CD4 (data not shown). Western blot analysis of CEM^TART infected with vIIIB^ATat/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^ATat/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.
sulfate and didexoycytidine, inhibited vIIIB\textsuperscript{Tat/Rev} infection of CEM\textsuperscript{TART} at levels similar to their inhibition of vIIIB infection of CEM (data not shown).

Because CEM\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{TART} (denoted as circles) with vIIIB, the concentration of p24 in culture supernatants of infected CEM\textsuperscript{TART} after 9 days was \( \approx \)1000-fold higher than p24 concentrations in infected CEM.

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

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

To determine if vIIIB\textsuperscript{Tat/Rev} infection and proviral integration occurred in CEM cells, genomic DNA from CEM cells 10 days after exposure to vIIIB\textsuperscript{Tat/Rev} was extracted. PCR amplification and DNA sequencing of the IIIIB\textsuperscript{Tat/Rev} provirus present in the genomic DNA demonstrated the presence of proviral IIIIB\textsuperscript{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\textsuperscript{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.
Table 2. Detection of replication-competent vIIIBATat/Rev

<table>
<thead>
<tr>
<th>vIIIBATat/Rev source</th>
<th>Target cells</th>
<th>vIIIBATat/Rev*</th>
<th>vIIIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEMTART infected with</td>
<td>CEM</td>
<td>0</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>IIIBATat/Rev for 9</td>
<td>PBLs†</td>
<td>0</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEMTART infected with</td>
<td>CEM</td>
<td>112,000 ± 1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>IIIBATat/Rev after</td>
<td>PBLs†</td>
<td>0</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>four passages</td>
<td>CEMTART</td>
<td>150,000 ± 1500</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

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, intra-vaginal, or intra-naural 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 vIIIBATat/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, CEMTART 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 standardize 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 vIIIBATat/Rev infecting CEMTART parallel results obtained using wild-type HIV-1 infecting CEM. Other antiviral agents have been similarly tested, yielding comparable results.