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A new contained human immunodeficiency virus type 1 host cell system for evaluation of antiviral activities of interferons and other agents in vitro

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Abstract

HIV-host infection systems in vitro are important in the pre-clinical assessment of anti-retroviral drug activity. The present report describes the development of a new HIV-host model comprised of an epithelial cell line of HeLa lineage (HeLa-1), transfected with expression vectors bearing *tat* and *rev* (TART) genes of HIV-1 as well as the CD4 receptor gene, and HIV-$1^{\Delta Tat/Rev}$, a biologically contained strain of HIV-1 deleted in *tat* and *rev*. Measurement of infectivity, by syncytium formation and reverse transcriptase assay, revealed that HeLa-1 is infected with HIV-$1^{\Delta Tat/Rev}$. This virus failed to productively infect the TART-deficient CD4-positive HeLa cells, confirming its contained, non-infectious nature. The HeLa-1/HIV-$1^{\Delta Tat/Rev}$ system was used to measure the anti-retroviral activity of a human leukocyte-derived interferon (IFN-αn3) preparation, several nucleoside analogs, and protease inhibitors. The HeLa-1/HIV-$1^{\Delta Tat/Rev}$ model provides a biologically contained system for the study of the HIV pathogenesis and the relative and combined therapeutic effects of anti-retroviral agents in vitro.

Keywords: HIV; IFN; Antiviral; Epithelial cells

1. Introduction

Development of effective strategies for the clinical management of HIV has been dependent upon infection models that allow for the evaluation of the relative and combined therapeutic activities of anti-retroviral drugs in vitro. A number of HIV-host infection models have been devised in recent years. Many investigators utilize wild-type strains of HIV and peripheral blood mononuclear cells (PBMC) as the viral target cell (Blauvelt et al., 1997; Kent et al., 1997; Schwartz et al., 1997).

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Limitations associated with current systems include the dangers of handling infectious wild-type HIV, inadequate response of some cell lines to antiviral agents such as interferons (Ralph et al., 1995), and the short life span of the fully differentiated PBMCs. The recent development of CEM^{TART}, a transformed T-cell line which expresses Tat and Rev proteins of HIV-1, provides a safe system for the assay of antiviral agents when used with HIV-$1^{\Delta Tat/Rev}$, an HIV strain unable to produce Tat and Rev (Chen et al., 1992). This cell line, however, lacks sensitivity to various anti-retroviral agents.

The present study reports the development of a contained HIV-host infection system for the testing of various anti-retroviral treatments in vitro. This system bears the advantage of using a non-infectious mutant strain of HIV-1 combined with a HeLa cell line that can be cultured for long periods of time and still retain sensitivity to the virus. The host cell line, HeLa-1, was developed from the human epithelial cell line HeLa-229 and was constructed such that it expresses CD4, the T-cell surface receptor, and the HIV essential regulatory proteins, Tat and Rev. Expression of CD4 on the HeLa-1 cell surface renders the cell line susceptible to binding and entry of HIV-1 (Maddon et al., 1986). Expression of *tat* and *rev* genes allows these cells to efficiently harbor HIV-$1^{\Delta Tat/Rev}$, which is replication deficient in normal cells due to its inability to produce functional Tat and Rev. The development of the HeLa-1 cell line, demonstration and optimization of its susceptibility to infection with HIV-$1^{\Delta Tat/Rev}$, and evaluation of the sensitivity of this virus-host system to anti-retroviral agents are described here.

2. Materials and methods

2.1. Cell cultures

HeLa-229 was obtained from American Type Culture Collection (ATCC, Gaithersburg, MD). HeLa cells were grown in minimum essential medium (MEM; Gibco/BRL, Gaithersburg, MD) containing 2 mM glutamine and 5% heat-inactivated fetal bovine serum (FBS). The medium for HeLa-1 culture also contained 200 μg/ml of G418 (Gibco/BRL). CEM^{TART} was cultured in RPMI-1640 (Gibco/BRL) containing 20% heat-inactivated FBS and 200 μg/ml of G418. All cultures were maintained at 37°C under 5% CO_2 in a humidified incubator.

2.2. Antibodies

Neutralizing human antibody to interferon-α (HuIFN-α antibody, G037-501-572) was obtained from Braton Biotech, (Gaithersburg, MD), a contractor to National Institute of Allergy and Infectious Diseases, NIH (Bethesda, MD). Monoclonal anti-CD4 antibody was generated as culture supernatant of SIM.2 hybridoma (McCallus et al., 1992; Oravecz and Norcross, 1993). Goat anti-mouse IgG Fc was purchased from ICN Pharmaceuticals, (Aurora, OH). FITC-labeled rabbit anti-goat IgG was from Dynatech Diagnostic, (South Windham, MN).

2.3. Construction of plasmids

PCR amplification of *tat* and *rev* genes were performed on the genomic DNA of CEM^{TART} cells with 5′ primer ATC GTG ACC TGG GAA GCC TTG GCT TT and 3′ primer CTG GGG ACT TTC CAC ACC TGG TTG C. The PCR amplified 1.1 kb fragment was first subcloned into TA plasmid (Invitrogen, Carlsbad, CA) followed by insertion into eukaryotic expression vector, pcDEF3 (Goldman et al., 1996) to develop pc-TART. For construction of a CD4 expression plasmid (pHMGT4), the 1.7 kb CD4 gene in the plasmid pBST4 (Maddon et al., 1985) (a generous gift from Dr D.R. Littman; Howard Hughes Medical Institute, New York University, NY) was cloned into an expression plasmid, pHMG (Gautier et al., 1989).

2.4. Transfection and selection of transformants

All transfections were performed using either standard calcium phosphate co-precipitation (Wigler et al., 1977) or Lipofectin-mediated transfection (Felgner et al., 1987) methods. Isolation of $CD4^+$ transfected cells was performed by a pan-

ning method as previously described (Wysocki and Sato, 1978). Briefly, cells were incubated with anti-CD4 antibody, SIM.2, for 1.5 h, then washed and plated on bacteriological dishes pre-coated with goat anti-mouse IgG-Fc for 75 min. Plates were washed ten times with a wash buffer consisting of 2% FBS in phosphate-buffered saline (PBS) to remove the non-adherent cells. Adherent cells were resuspended in MEM containing 5% FBS and cultured at 37°C. The panning process was repeated four more times to enrich for $CD4^+$ cells. These panning procedures resulted in generation of 50% CD4 expressing cells. The mixed cell populations were then subcloned by single cell cloning method, and the expression of CD4 in single subclones was confirmed first by examining syncytia formation upon infection with HIV-$1^{\Delta Tat/Rev}$ followed by FACS and Northern blot hybridization. Among the six isolated clones five showed equivalent and homogeneous expression of CD4 receptor.

2.5. Fluorescent activated cell sorting

Expression of CD4 in HeLa-1 was assessed by FACS, using an EPICS-2000 (Beckman Coulter, Inc., Fullerton, CA). For each FACS analysis, 5×10^6 of HeLa-1 or HeLa-TART cells were first labeled with anti-CD4 antibody, SIM.2, then goat anti-mouse IgG Fc, and finally with the FITC-labeled rabbit anti-goat IgG. The cells were fixed in PBS containing 3% formaldehyde and maintained at 4°C until analyzed.

2.6. Probes

Digestion of pcTART with EcoRI generated an 800 bp fragment containing *tat* and *rev* genes. Digestion of pHMGT4 with BamHI resulted in the release of an 1.7 kb fragment coding for the CD4 protein. These fragments were isolated from agarose gel slices by Gen Elute (Pharmacia, Newark, NJ) and labeled with [α-^{32}P]dCTP (6000 Ci/mmol, 10 μCi/ml; Amersham, Arlington Heights, IL) using Radprime DNA labelling kit (Gibco/BRL). 100 ng DNA was labeled in 50 μl vol. at 37°C for 30 min. Labeled DNAs were purified with Spin Column 30 (Clontech, Palo Alto, CA) to specific radioactivity of about $1–2 \times 10^8$ cpm/μg.

2.7. Northern blot analysis

Total cellular RNA was isolated by lysing cells in the Trizol reagent (Gibco/BRL) followed by purification of mRNA using the poly-A spin mRNA isolation kit (New England Biolabs; Beverly, MA). Northern blot analysis was performed on 1 μg mRNA followed by transferring to Zeta probe membrane (Bio-Rad; Hercules, CA) according to the published method (Sambrook et al., 1989). Membranes were pre-hybridized in 2X SSPE, 50% Formamide, 7% SDS (w/v) and 100 μg/ml of sheared/denatured Herring sperm DNA (Amresco, Solon, OH) at 43°C for 30 min. Hybridization was carried out under the same conditions with 1×10^6 cpm/ml of the denatured CD4 probe for 20 h. The membranes were washed at room temperature in 1X SSPE for 15 min and then in 1X SSPE and 2% SDS for 15 min. Finally, the membranes were washed with 1X SSPE and 2% SDS at 50°C for 15 min and exposed to an X-ray film at −70°C. Subsequently, the membranes were stripped twice in 1X SSPE and 0.1% SDS at 95°C each time for 15 min and hybridized with the TART probe under the same conditions used for the CD4 probe.

2.8. Virus stock and infectivity assays

An HIV-$1^{\Delta Tat/Rev}$ (Chen et al., 1992) stock was produced by propagating the virus in CEM^{TART} cells. Briefly, 3.5 ml of actively growing CEM^{TART} cell suspension at a concentration of 6.5×10^5 cells/ml with cell viability greater than 90% was centrifuged for 10 min at $400 \times g$. The supernatant was discarded and 0.5 ml of HIV-$1^{\Delta Tat/Rev}$ was added to the cell pellet. The mixture was incubated for 1 h at 37°C and centrifuged again for 10 min at $400 \times g$. The supernatant was discarded and the pellet resuspended in 10 ml of fresh CEM^{TART} culture medium. The centrifugation and resuspension steps were repeated once more and the cells were finally placed in a T-25 flask in 10 ml of medium and incubated at 37°C for 7–9 days. The supernatant was then collected,

filtered through 0.22 μ filters (Corning, Corning, NY), and aliquoted. Viral stocks were frozen and kept in liquid nitrogen. The viral titer was determined using an infectivity assay by syncytia formation (Mahy, 1985; Johnson and Byington, 1990), and measured to be 5.1×10^3 $TCID_{50}$/ml (equivalent to 4.3×10^6 cpm/ml by reverse transcriptase assay; see next section). The HIV-1 NL4-3 stocks were prepared as described previously (Fitzgibbon et al., 1992).

2.9. Reverse transcriptase assay

A quantitative assay for detection of virus associated reverse transcriptase in the supernatant of infected cells is related to production of complete virions (Fernie et al., 1991). RT assays were performed using a modification of the published protocols (Poiesz et al., 1980; Popovic et al., 1984). Assays were typically performed in duplicates in 96 or 24 well plates by placing 5×10^3 cells in 0.2 ml or 1.2×10^4 cells in 0.5 ml, respectively, of MEM containing the appropriate concentrations of the treatment drug or no treatment for viral controls. Cells were incubated overnight at 37°C, and the following day 50 μl (equivalent to 85 $TCID_{50}$ or RT activity of 70 000 cpm) per sample in a 96 well plate or 120 μl per well in a 24 well plate of the HIV-$1^{\Delta Tat/Rev}$ suspension or, in some assays, 200 μl of HIV-1 NL4-3 per sample in a 24 well plate was added to each well. Each assay also included cell controls with no virus to determine the background. Infections were carried out for 5 days, unless otherwise indicated, and stopped by the transfer of the cell supernatants to a clean plate which was frozen and maintained at −70°C for RT assay. Aliquots of culture supernatants (5 μl) were mixed with 25 μl of RT reaction mixture containing poly-A (5 μg/ml) and oligo-dT (0.17 U/ml) (both from Pharmacia) in 50 mM Tris, pH 7.8, 75 mM KCl, 2 mM $MgCl_2$, 2 mM dithiothreitol (Sigma, St. Louis, MO), 0.5% Nonidet P-40 (Sigma), and 20 μCi/ml [α-^{32}P]dTTP (3000 Ci/mmol; Amersham, Arlington Heights, IL) and incubated for 3 h at 37°C. Reactions were stopped by placing the samples on ice and subsequently spotting 6 μl of each mixture onto individual Whatman DE81 filter discs (Fisher Scientific, Springfield, NJ). These filter discs were air dried and washed 5 times with 1X SSC (0.15 M NaCl and 15 mM sodium citrate) and twice with 70% ethanol and their radioactivity was measured using a Beckman LS 9800 liquid scintillation counter (Beckman Instruments, Fullerton, CA).

3. Results

3.1. Development of HeLa-1

HeLa-1 cell line was developed through transfection of the interferon-sensitive HeLa-229 cells with pcTART, an expression vector bearing *tat* and *rev* genes, to produce HeLa-TART, and subsequent transfection with pHMGT4, a plasmid expressing the gene for CD4. $CD4^+$ cells were isolated and further subcloned to derive HeLa-1 cells. $CD4^+$ control cells were constructed by co-transfection of HeLa-229 with pHMGT4 and pSV2neo (Southern and Berg, 1982) followed by selection for G418 resistant cells.

3.2. Expression of CD4, Tat, and Rev in HeLa-1

To confirm the expression of CD4 in HeLa-1 and compare it to that of the CD4 expression in its parent cell line, HeLa-TART, both cell lines were labeled with the anti-CD4 antibody and subjected to fluorescent activated cell sorting (FACS) analysis. Results (Fig. 1A) demonstrate that HeLa-1 cells are uniformly labeled with the anti-CD4 antibody while no labeling of the HeLa-TART was detected. Expression of CD4 as well as *tat* and *rev* genes was confirmed by Northern blot hybridization of the mRNAs (Fig. 1B, top panel) isolated from HeLa-1, HeLa-TART, HeLa-229 and CEM^{TART}. Efficient expression of TART in HeLa-1 and HeLa-TART at levels that are comparable to those in CEM^{TART} was observed (Fig. 1B, middle panel). No expression of TART was seen in untransfected HeLa-229 cells. As expected, expression of CD4 RNA in HeLa-1 was detected (Fig. 1, B bottom panel). Absence of CD4 expression was noted in both HeLa-229 and HeLa-TART.

3.3. HIV-1$^{\Delta Tat/Rev}$ infection of HeLa-1

Reverse transcriptase (RT) assays were used to measure HIV replication in HeLa-1 cell line after infection with HIV-1$^{\Delta Tat/Rev}$. In these experiments, HeLa-1 cells were seeded overnight in a 96-well plate and infected with various dilutions of HIV-1$^{\Delta Tat/Rev}$ followed by incubation for various times. Samples of supernatants were then removed and assayed for RT activity. Infectivity of HIV-1$^{\Delta Tat/Rev}$ towards HeLa-1 is dependent on virus concentration. A 1:3 dilution of the virus

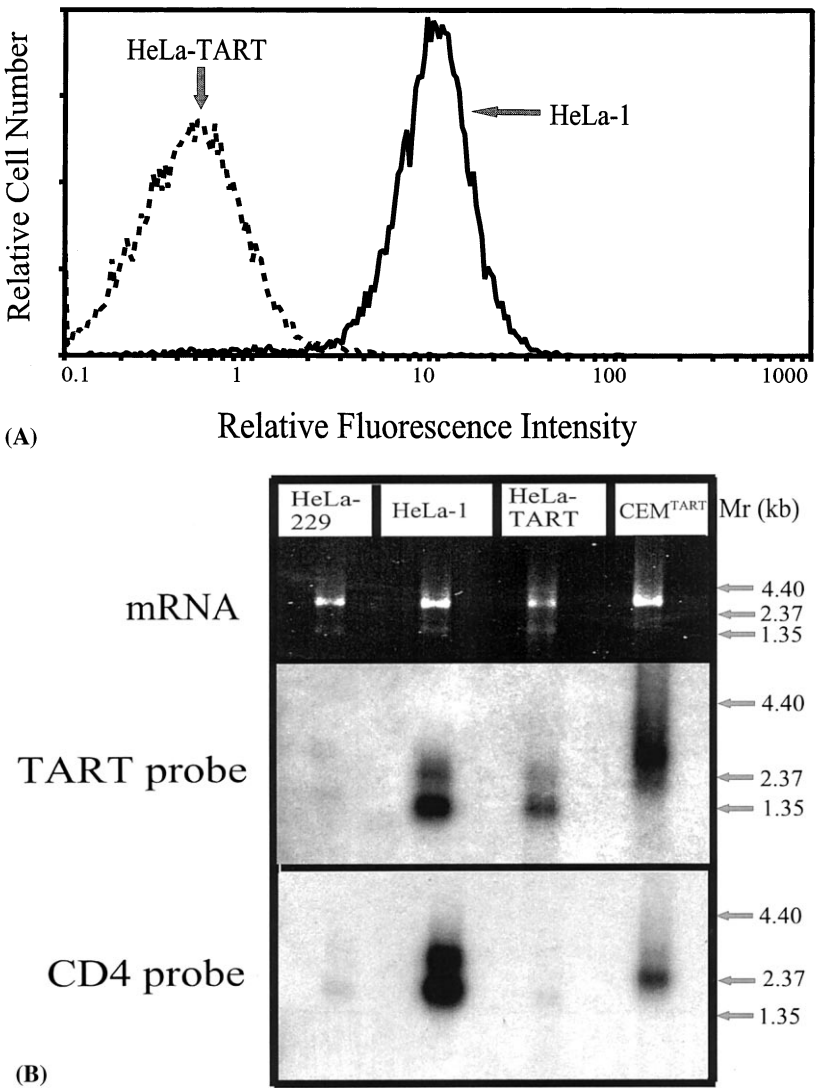

Fig. 1. FACS and Northern analysis of HeLa-1 for expression of CD4 and TART. (A) HeLa-1 and the control cell line HeLa-TART were labeled with anti-CD4 antibody, then with goat anti-mouse IgG Fc, and subsequently with FITC-labeled rabbit anti-goat IgG as described in Materials and Methods. The cells were subjected to FACS and the expression of CD4 in HeLa-1 (solid line) and its parent cell HeLa-TART (dashed line) assessed through comparison of the relative fluorescence intensities in the two cell lines. (B) Northern blot hybridization was performed on mRNA (top panel) of cells as discussed in Section 2. The positions of RNA ladder molecular weight markers (Gibco/BRL) have been labeled on the right side of the figure. Hybridization was performed with either the TART probe (middle panel) or the CD4 probe (bottom panel).

Table 1
Infectivities of HIV-$1^{\Delta Tat/Rev}$ and HIV-1 NL 4-3 towards various cell lines[a]

Cells	Infection with HIV-$1^{\Delta Tat/Rev}$		Infection with HIV-1 NL 4-3	
	RT activity (cpm/ml)	Syncytium induction	RT activity (cpm/ml)	Syncytium induction
HeLa-229	$5.9\times10^4\pm5.7\times10^3$	−	$1.4\times10^5\pm5.7\times10^3$	−
HeLa-1	$1.4\times10^6\pm4\times10^4$	++	$1.8\times10^6\pm9.1\times10^4$	++
HeLa-CD4	$1.5\times10^5\pm5.5\times10^3$	−	$1.2\times10^6\pm6.9\times10^4$	++
HeLa-TART	$6\times10^4\pm1.9\times10^3$	−	$1.3\times10^5\pm6.4\times10^3$	−
CEM^{TART}	$6.1\times10^6\pm5.7\times10^4$	+++	$4.1\times10^6\pm2.2\times10^5$	+++

[a] RT assays were carried out as described in the text. Syncytium induction was determined microscopically five days after virus infection. Relative numbers of syncytium formation are indicated as; −, no syncytium; ++, numerous syncytia; or +++, extensive syncytiation. Background RT activity was in the range of 10^4–10^5 cpm/ml.

stock appeared to be optimal (data not shown) for this assay system and was therefore used in all other experiments. The optimum HeLa-1 cell number as well as the infection time course of HIV-$1^{\Delta Tat/Rev}$ were also evaluated. Optimum productive infection was observed with 5000 cells per assay in a 5-day infection (data not shown).

3.4. Contained nature of the HeLa-1/HIV-$1^{\Delta Tat/Rev}$ system

Infectivities of HIV-$1^{\Delta Tat/Rev}$ and the wild-type HIV-1 NL 4-3 towards HeLa-1 as well as the control cell lines HeLa-229, HeLa-CD4, HeLa-TART, and CEM^{TART} were examined (Table 1). The results of the RT assays and syncytium formation demonstrated that HIV-$1^{\Delta Tat/Rev}$ productively infected HeLa-1 and CEM^{TART} but not the TART-deficient HeLa-CD4 and HeLa-229 or the CD4-deficient HeLa-TART. The infectious HIV isolate, HIV-1 NL 4-3, on the other hand, infected all cell lines except for the CD4-deficient HeLa-229 and HeLa-TART cells. These data demonstrated that the products of *tat* and *rev* genes are essential for the replication of HIV-$1^{\Delta Tat/Rev}$, thereby confirming the contained nature of HeLa-1 as a host for HIV-$1^{\Delta Tat/Rev}$.

3.5. Sensitivity of HeLa-1 versus CEM^{TART} to IFN-αn3 and the protease inhibitor, nelfinavir

The HeLa-1/HIV-$1^{\Delta Tat/Rev}$ system is responsive to a wide range of anti-retroviral agents including some to which CEM^{TART} cells proved unresponsive. Among these are IFN-αn3 and the protease inhibitor, nelfinavir. IFN-αn3 fails to inhibit RT activity in the HIV-$1^{\Delta Tat/Rev}$ or HIV-1 NL 4-3 infected CEM^{TART} cells regardless of the concentration of the cytokine (Fig. 2, panel A, and Table 2). On the other hand, as demonstrated by the IFN-αn3 dose response curve in HeLa-1, HIV-$1^{\Delta Tat/Rev}$ as well as HIV-1 NL 4-3 replications are efficiently inhibited by IFN-αn3 in a concentration-dependent manner with a 50% inhibitory effect (IC_{50}) observed at an interferon concentration of 15 IU/ml and complete inhibition at 125 IU/ml (Fig. 2, panel B, and Table 2). The anti-HIV activity of IFN-αn3 in HeLa-1 cells was further confirmed in experiments in which HeLa-1 cells were pre-treated with a mixture of IFN-αn3 and anti-IFN-a antibody or IFN-αn3 alone as control. Anti-IFN-a antibody completely neutralizes the anti-HIV activity of IFN-αn3 demonstrating the specificity of interferon action against HIV (Fig. 3).

A dose response study of nelfinavir, a protease inhibitor, on CEM^{TART} versus HeLa-1 cells again demonstrates that nelfinavir does not inhibit HIV-$1^{\Delta Tat/Rev}$ replication as measured by reverse transcriptase activity present in the culture supernatant from CEM^{TART} cells (Fig. 4). However, HIV-$1^{\Delta Tat/Rev}$ replication was efficiently inhibited by nelfinavir (IC_{50} of 8 μg/ml) when HeLa-1 cells were used as host.

3.6. Sensitivity of HeLa-1 to inhibitory effects of other anti-retroviral agents

An efficient HIV infection model requires a system that manifests susceptibility to a variety of anti-retroviral agents allowing comparative as well as combination drug studies in vitro. The antiviral sensitivity of HeLa-1 towards a number of anti-retroviral treatments is compared in a parallel experiment to CEM^{TART} on HIV-1$^{\Delta Tat/Rev}$

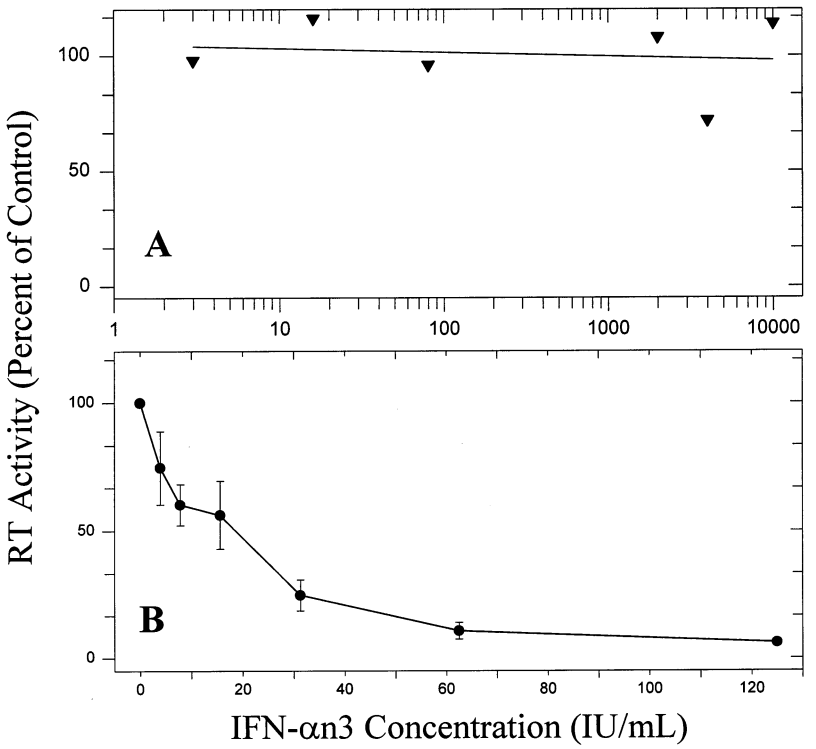

Fig. 2. IFN-αn3 dose response against HIV-1$^{\Delta Tat/Rev}$ replication in HeLa-1 and CEM^{TART}. Equal numbers of CEM^{TART} (panel A) and HeLa-1 (panel B) cells were pre-treated with various concentrations of IFN-αn3 and infected with HIV-1$^{\Delta Tat/Rev}$ the following day. RT activities were then measured at day 5 post-infection and are expressed as percent of RT activity produced by HIV-1$^{\Delta Tat/Rev}$ treated control cells. Results are averages of two to three independent experiments.

Table 2
IC_{50} for IFN-αn3 and various anti-retroviral agents on HeLa-1 or CEM^{TART} cells infected with HIV-1$^{\Delta Tat/Rev}$ or HIV-1 NL 4-3[a]

Drug	HeLa-1		CEM^{TART}	
	IC_{50} for HIV-1$^{\Delta Tat/Rev}$	IC_{50} for HIV-1 NL 4-3	IC_{50} for HIV-1$^{\Delta Tat/Rev}$	IC_{50} forHIV-1 NL 4-3
IFN-αn3	15 IU/ml	5 IU/ml	ND**	ND*
AZT	0.5 ng/ml	0.2 ng/ml	2.0 ng/ml	0.2 ng/ml
Ritonavir	80 ng/ml	20 ng/ml	400 ng/ml	10 ng/ml
Saquinavir	5 μg/ml	2 μg/ml	5 μg/ml	3 μg/ml

[a] RT assays were carried out as described in Section 2. ND, not detectable.
* For this assay maximum of 200 IU/ml of IFN-αn3 was used.
** For this assay maximum of 10 000 IU/ml of IFN-αn3 was used (also see Fig. 2A).

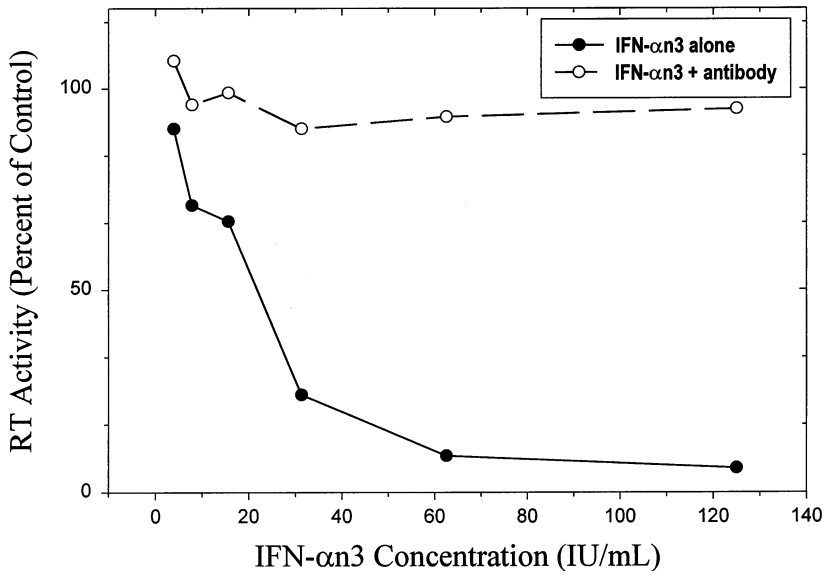

Fig. 3. Specificity of IFN-αn3 inhibition of HIV-$1^{\Delta Tat/Rev}$ infection. HeLa-1 cells were treated overnight with various concentrations of IFN-αn3 alone (solid line) or in combination with neutralizing anti-IFN-α antibody (dotted line). Cells were then infected with HIV-$1^{\Delta Tat/Rev}$. RT activities were measured on day 5 and reported as percent of viral controls.

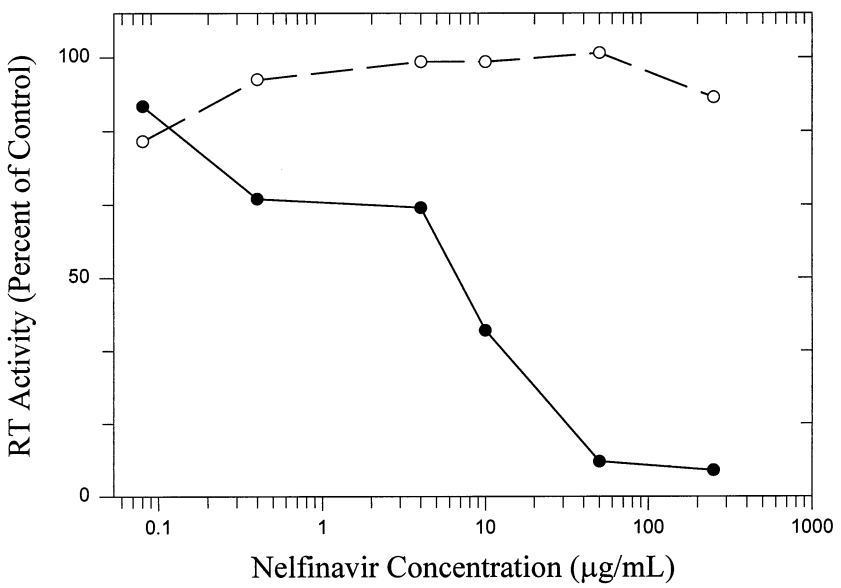

Fig. 4. Differential sensitivities of HeLa-1 and CEM^{TART} to nelfinavir. HeLa-1 (solid line) and CEM^{TART} (dotted line) were treated with varying doses of nelfinavir overnight and then incubated with HIV-$1^{\Delta Tat/Rev}$. RT activities were measured on day 5 and reported as percent of viral controls.

and HIV-1 NL 4-5 (Table 2). The IC_{50} for AZT is in the range of 0.2–2 ng/ml depending on the cell or virus used . Results also demonstrated that the protease inhibitors saquinavir inhibited the HIV-1 replication in a dose-dependent fashion, manifesting a 50% inhibitory effect at about 2–5 μg/ml.

The IC_{50} for ritonavir in these assay systems was in the range of 10–400 ng/ml. In general, the CEM^{TART} cells were least sensitive to the inhibitory activities of antiviral agents when challenged with $HIV^{\Delta Tat/Rev}$.

4. Discussion

Emergence and transmission of resistance to single or combinations of anti-retroviral agents are significant challenges faced in the treatment of HIV infection (Erice et al., 1993; Colon et al., 1994; Imrie et al., 1997; Kemp et al., 1998; Hecht et al., 1998). Development of resistance to antiviral agents limits their clinical benefit. New anti-HIV drugs must be identified to compensate resistance development. Evaluation of new anti-HIV therapies requires an in vitro infection system with a host cell line that is sensitive to the inhibitory potentials of various drugs. In addition, the assay system should be safe and preclude accidental transmission to laboratory workers. One such system has recently been developed (Chen et al., 1992) by combining CEM^{TART}, a transformed T-cell line which expresses Tat and Rev proteins of HIV-1, and HIV-$1^{\Delta Tat/Rev}$. This host cell line, however, lacks sensitivity to IFN-α, a new class of anti-retroviral agents that have demonstrated success in the treatment of HIV infection, especially in patients with high CD4 cell count (Lane, 1994; Skillman et al., 1996; Brand et al., 1998). The inability of CEM^{TART} cells to respond to the anti-retroviral activity of IFN-αn3 is not due to the absence of IFN receptor or its signal transduction pathways since significant levels of the IFN-induced protein, 2′,5′-oligoadenylate (2-5A) synthetase were measured after incubation of these cells with IFN-αn3 (data not shown). These data suggest that the anti-HIV activity of IFN-αn3 is independent of induction of intracellular 2-5A synthetase activity and may be due to other interferon-associated signal transduction pathways. Furthermore, the lack of response is not due to the absence of IFN-α induced antiviral state in CEM^{TART} cells since IFN-αn3 could completely inhibit replication of vesicular stomatitis virus (VSV) at concentrations of less than 100 IU/ml with IC_{50} of 20 IU/ml (data not shown). The observed difference in the antiviral effect of IFN-αn3 gainst HIV-1 and VSV is not yet understood. The host cell line described in this report, HeLa-1, was developed from the parental cell line HeLa-229 which possesses interferon-specific receptors and the related anti-retroviral signaling components and is therefore influenced by the modulatory effects of this class of cytokines. Such differential sensitivities in HeLa-1 and CEM^{TART} are also exhibited with respect to the anti-retroviral activity of the protease inhibitor, nelfinavir. Only the HeLa-1 cell line responds to the anti-HIV effect of nelfinavir. In general, CEM^{TART} cells are not inhibited efficiently by antiviral agents when challenged with HIV-$1^{\Delta Tat/Rev}$. The reasons for the lack of or diminished insensitivity of CEM^{TART}/HIV-$1^{\Delta Tat/Rev}$ system towards anti-retroviral agents are not known. The HeLa-1 cell line has demonstrated sensitivity to a wide spectrum of anti-HIV agents ranging from protease inhibitors to nucleoside analogs. Indeed, this infection model responds to all anti-retroviral agents tested thus far.

Infectivity assays of HeLa-1 as well as the control HeLa-TART and the TART-deficient HeLa-CD4 with both the HIV-$1^{\Delta Tat/Rev}$ and the wild type strain HIV-1 NL 4-3 showed that HIV-$1^{\Delta Tat/Rev}$ does replicate in the Tat- and Rev-deficient cell lines, thereby demonstrating the contained nature of this mutant strain. Tat and Rev proteins of HIV increase transcription of the viral mRNA and therefore, their deletion results in a replication-incompetent virus. The possibility of HIV-$1^{\Delta Tat/Rev}$ reversion by mutation or recombination to replication-competent virus is minimized by creating two physically separate mutations in the two exons of *tat* and *rev* genes (Chen et al., 1992). The biologically contained system described here provides an infection model in vitro in which the activities of various classes of anti-retroviral drugs in monotherapy as well as in combination can be safely examined.

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