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A simple human immunodeficiency virus vector system for selective infection of CD4+ cells and inducible expression of foreign genes

Yeon-Soo Kim[1]

1 Institute for Cancer Research, College of Medicine, Yonsei University, Seoul 120-752, Korea

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Abbreviations: ADA, adenosine deaminase; AIDS, acquired immunodeficiency syndrome; CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; HSV, herpes simplex virus; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside

Abstract

The alteration of T lymphocyte functions as a consequence of human immunodeficiency virus (HIV) infection is a potential target for the genetic treatment of the acquired immunodeficiency syndrome (AIDS). One approach to the gene therapy for AIDS is to block the replication of HIV–1. Tat–dependent expression of forein gene and selective infection of CD4+ cells by retroviral vector might be useful for abrogating the production of HIV–1 from cells. As part of studies to examine the feasibility of this concept, I constructed tat+ and tat- HIV–1 proviral vectors that express all HIV–1 genes except for env and/or tat gene. When tat+ or tat- HIV-1 particles were used for infection of HeLa T4 cells containing the endogenous β-galactosidase (lacZ) gene under the control of the HIV-1 promoter and transactivation response element sequences, only the tat+ HIV-1 particles transactivated the lacZ gene expression. This activation of lacZ expression following HIV infection of Tat- cells that stably contained but did not express the lacZ construct was determined to be an efficient process. I also constructed simple HIV-1 vectors that express the lacZ gene in a Tat-dependent manner or the hygromycin B phosphostransferase gene (Hygr) under the control of the SV40 early promoter. The Tat-dependent vector conferring the lacZ+ phenotype was assayed by β-gal staining after infection of Tat+ or Tat- cells. The activation of lacZ expression was observed only in tat+ cells. Another simple HIV-1 vector containing the Hygr gene was used for retroviral production from HeLa cells expressing the HIV-1 env gene and infection of CD4+ or CD4- cells, but Hygr colony was observed only from CD4+ cells. These results provide a rationale for the use of HIV-1 retroviral vector system in the design of gene therapy of HIV infection.

Keywords: HIV-1, retroviral vector, AIDS, CD4, Tat, gene therapy

Introduction

The acquired immunodeficiency sysdrome (AIDS), caused by human immunodeficiency virus (HIV), is a chronic disease primarily characterized by a decrease in CD4+ T lymphocytes. Since the discovery of HIV in 1983, significant progress has been made toward the discovery and development of anti-HIV drugs. In addition, considerable progress has been made in the development of treatments for various opportunistic infections. Although these treatments can delay desease progression, side effects associated with their use and the development of viral resistance to these drugs may inevitably lead to treatment failure. For these reasons, alternate therapies for HIV infection are needed.

Recent advances in the application of gene therapy to several diseases (Spangrude *et al.,* 1988; Drumm *et al.,* 1990; Nabel *et al.,* 1990; Rosenberg *et al.,* 1990) have stimulated interest in the therapeutic potential of nucleic acids expressed endogenously by cells. The genetic correction of T lymphocytes in the first gene therapy experiment with adenosine deaminase (ADA) deficiency has shown that "sick" T lymphocytes can be made "healthy" (Blease and Culver 1992). Since the immunodeficiency results when the HIV makes T lymphocytes sick, genetically altering HIV-infected T lymphocytes may be a useful therapy. Although several approaches have succeeded in producing anti-HIV activity in cultured cells by transfection with vectors containing diphtheria toxin A gene, HIV-specific ribozyme, herpes simplex virustype 1 thymidine kinase (HSV1-TK), interferon gene, or antisense gene (Harrison *et al.,* 1991a; Caruso and Klatzmann 1992; Rossi *et al.,* 1992; Lisziewicz *et al.,* 1995; Su *et al.,* 1995), the difficulty in obtaining sufficient expression of the desired gene in a sufficient number of cells *in vivo* has remained an obstacle.

Murine retroviral vectors also could be used for constitutive expression of potentially therapeutic genes in cells of HIV-infected individuals. Because the receptor of the commonly used amphotropic murine leukemia virus is present on a variety of cell types, these vectors provide a potential way for introducing genes into early lineage hematopoietic cells as well as mature T lymphocytes. However, because of difficulties encountered both in isolating and effectively infecting hematopoietic stem

cells and because the primary target for HIV infection appears to be helper T cells, it is possible that a vector capable of specifically infecting mature T lymphocytes might be useful. One method for targeting potentially therapeutic genes to cells suscetible to HIV infection ($CD4^+$ cells) would be to use an HIV vector. As known well, retrovirus entry into cells is dependent on recognition of the cellular receptor by the viral envelope glycoprotein. By using an HIV vector, entry of the foreign gene into cells would occur only if the cells expressed the HIV receptor, the CD4 molecule (Maddon *et al.,* 1986).

In addition, since a number of the genes that might be used to interfere with HIV may be toxic to the cell, constitutive expression of these genes would be harmful. Another method for expressing genes in specific cells is to use unique HIV *cis*- and *trans*-acting elements that control gene expression from the HIV LTR. Efficient gene expression from the HIV LTR is dependent on the viral protein Tat (Sodroski *et al.,* 1985; Cullen, 1986). Tat is an RNA-binding protein; its target sequence, the Tat activation response element (TAR), is a 59-nucleotide sequence present in the newly transcribed R region of the 5' LTR (Rosen *et al.,* 1985). Since Tat is normally found only in cells infected with HIV, this type of vector might provide a means by which foreign genes that interfere with HIV replication could be expressed specifically in HIV-infected cells. Because expression of the foreign gene is dependent on Tat, this vector system provides a way in which to introduce genes into cells where they will be maintained but not expressed until induced. This strategy forms the basis for an "inducible" gene therapy against HIV. This control of gene expression might decrease potential side effects associated with expression of the foreign gene in uninfected cells.

In the study presented here, simple HIV vectors that contain no intact viral open reading frames and that expresses foreign report genes from the HIV LTR or heterologous internal promoter were constructed. These vectors were used as markers to characterize the Tat-inducible and $CD4^+$-specific vector system and its usefulness for delivery of gene expressed from the HIV LTR.

Materials and Methods

Plasmid construction

TAR-cat was made by replacing the HIV-1 *env* gene in the TAR-env plasmid with the bacterial CAT gene. The plasmid TAR-env was constructed by deleting the *EcoR*I fragment containing the SV40 poly(A) signal from pIIIenv3.1 (Sodroski *et al.*, 1985). Thus, TAR-env still contains a Tat-responsive HIV-1 5'LTR and open reading frames for *rev* and *env* genes as well as intact HIV-1 3'LTR. Plasmid dTAR-cat are identical to TAR-cat except for containing a deletion of the entire TAR sequence from a *Bgl*II site(+20) in the R region of the HIV-1 5'LTR to an artificial *Sal*I site (Sodroski and others 1985) located upstream the initiation codon of *rev*. TAR-luc and dTAR-luc contain the *Xho*I-*Bam*HI and *Bgl*II-*Bam*HI luciferase gene fragment of pGL2-Basic Vector (Promega, Madison, WI) at the *Sal*I-*Bam*HI and *Bgl*II-*BamH*I sites in the pTat120B, respectively.

A *env*-HIV-1 proviral clone YK161 was made in two steps as follows: The infectious proviral clone pNL4-3 (Adachi *et al.,* 1986) was digested with *Stu*I and *Nhe*I, the digested DNA was treated with Klenow fragment, and the ends were religated (regenerating the *Nhe*I site) to produce clone pGB107. pGB107 thus contains a 426-bp inframe deletion in the *env* gene and can produce truncated gp120 and intact gp41. pGB107 was digested with *Nhe*I (regenerated enzyme site), the digested DNA was treated with Klenow fragment, and the ends were religated, resulting in plasmid YK161. YK152-10 was constructed by introducing a stop codon in 11st amino acid codons of *tat* gene in the HIV-1 proviral clone YK161.

To construct YK1001, the HIV-1 proviral clone pNL4-3 (Adachi *et al*., 1986) was digested with *Spe*I and *Xho*I and treated with the Klenow fragment and the ends were ligated to produce the intermediate clone YK1000. To introduce the β-galactosidase gene (*lacZ)* into YK1000, the plasmid was digested with *Bss*H II and treated with the Klenow fragment. The *lac*Z gene was obtained from LNβZ (Kim, T., unpublished data) by digestion the plasmid with *Hin*dIII and *Sal*I, treating it with the Klenow fragment, and isolating the appropriate DNA fragment. This fragment was then blunt end ligated into the *Bss*HII-digested YK1000 to produce the vector YK1001. To construct YK1002, pNL4-3 was digested with *Nsi*I and the ends were ligated to produce an intermediate clone. To introduce the hygromycin B phosphotransferase gene (Hyg^r) into the intermediate clone, the plasmid was digested with *Nhe* I. The Hyg^r gene promoted from SV40 early promoter was obtained from GB108 (Buchschacher and Panganiban 1992) by digesting the plasmid with *Nhe*I and isolating the appropriate DNA fragment. This fragment was then ligated into the *Nhe*I-digested intermediate vector to produce the YK1002. The replication-defective HIV-1 helper construct MSMΔenv50 (McBride, S. and Panganiban, A. T., unpublished data) contains a deletion in the *env* ORF but is capable of expressing other proteins necessary for viral propagation. The HIV-1 *env* expression plasmids pSV-A-MLV-*env* and pMcenvHc has been described previously (Kim and Risser 1993; Page *et al.,* 1990). Tat expressing plasmids pTat120B and pTat120K effector plasmids encode only the first exon and the intact sequences of HIV-1 *tat* gene (both exons), respectively (Kim and Risser 1993). Plasmid pUC19SXLTR was used as a negative effector plasmid (Kim and Risser 1993).

Cell culture and transfection

The CD4+ HeLa cell line HeLaT4 (Maddon *et al*., 1986), HeLa *env*-c cells (Gama Sosa *et al.,* 1989) expressing Tat, and HeLa were grown in Dulbecco's midified Eagle's medium(DME) with 7% calf serum. CD4-LTR/β-gal indicator cells (provided by M. Emerman) which is derived from HeLaT4 by introduction of *lac*Z gene promoted from the HIV-1 LTR (Kimpton and Emerman, 1992) were cultured in the same medium, but with 100 μg of hygromycin B per ml and 400 μg of G418 per ml. In preparation for the CAT assay and luciferase assay 0.6 x 10^6 HeLa cells were plated in 5 ml media in a 60-mm tissue culture dish one day before transfection. A total of 8 μg of plasmid DNA (3 or 5 μg of test plasmid and 5 μg of effector plasmid) was prepared for each plate, and coprecipitated with 10 μg of calf thymus DNA and calcium phosphate in a final volume of 0.5 ml by the calcium phosphate transfection method described by Graham and Van der Eb (1973) and modified by Hopkins *et al*. (1981). The plasmid precipitate was added to the culture medium for 4 h. At the end the incubation, the culture medium was removed, and the cells were rinsed once with serum-free DME, shocked with 15% glycerol in Hepes-buffered saline for 3 min at room temperature, after which the glycerol was removed and replaced with 5 ml culture media. Two days after transfection, the cells were harvested for the assay or labeled with [^{35}S]methionine for immunoprecipitation.

CAT and luciferase assays

CAT enzyme assays were done as described by Gorman *et al.* (1982). All extracts were made 48 h post-transfection by freeze-thawing, and reactions were run in 250 mM Tris-HCl (pH 7.8) for 15 min. Acetylated and unacetylated forms were resolved by thin-layer chromatography, located by autoradiography, and excised for liquid scintillation counting. For the lucifrase assay, cell extracts were prepared, and the luciferase activity was measured by using the Luciferase Assay System kit (Promega, Madison, WI) and a luminometer monolight 2010 (Analytical Luminescence Laboratory, San Diego, CA), under the condition supplied from the kit.

Virus production and infections

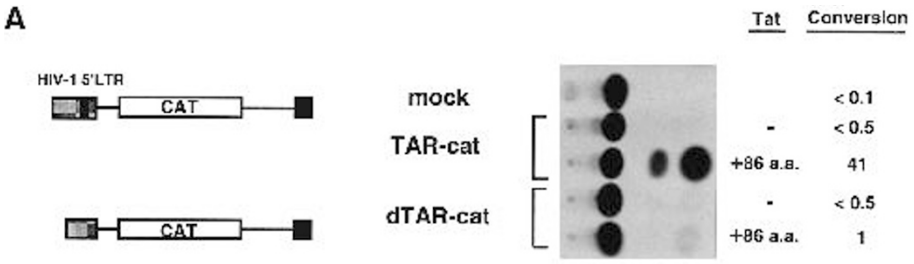
Five micrograms of each DNA was transfected into 6 x 10^5 HeLa or HeLa *env*-c cells on 60-mm-diameter tissue culture dishes by the calsium phosphate procedure. On the second day posttransfection, 5 x 10^4 HeLa, HeLa T4, HeLa *env*-c, or CD4-LTR/β-gal indicator cells were plated onto 24-well plate for infection the following day. For the cell-free infection, medium was collected from the transfected HeLa or HeLa *env*-c cells and subjected to low-speed centrifugation to remove cell debris. The top portion of the supernatant was removed, and polybrene was added to a final cencentration of 8 μg/ml. One-quarter mililiter of diluted supernatant was added to the Magi cells. After 4 h incubation, fresh medium was added. Two days postinfection, the virus titer of supernatant was quantified by scoring the number of cells that turned blue after treatment with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (Kimpton and Emerman 1992), or selection was initiated for infected cells by fluid changing with medium that contained 200 μg of hygromycin B per ml. Cells were cultured for 7 to 10 days, and the virus titer was determined by fixing and staining the cells with 0.5% crystal violet in 50% methanol and counting resistant colonies.

Results

Tat-mediated transactivation of transiently transfected heterologous genes from HIV-1 LTR

Although many earlier studies had addressed the importance of the Tat protein in *trans*-activation of gene expression from HIV-1 LTR, the HIV-1 promoter is not completely silent in the absence of Tat. When a lethal or deleterious foreign gene is used for anti-HIV gene therapy, the gene expression should be controlled stringently by Tat. Therefore, the toxic gene placed under the control of the HIV regulatory sequences will be expressed at a basal low level in noninfected cells and would be switched on in the initial phase of HIV replication. To test Tat-mediated transactivation of heterologous genes from the HIV-1 LTR by using sensitive repoter gene system, I constructed plasmids in which the bacterial CAT gene and the firefly luciferase gene are under the control of the HIV-1 5' LTR (TAR-cat and TAR-luc, respectively). Two alternative Tat expression constructs were used in an attempt to transactivate reporter gene expression; pTat120B encodes the 73 amino acid Tat peptide derived from the first *tat* coding exon, whereas pTat120K encodes the full-length 86 amino acid Tat protein. The reporter plasmids were cotransfected into HeLa cells along with plasmids expressing either the 86- or 73 amino acid version of Tat. As expected, both forms of Tat dramatically increased the expression of either CAT or luciferase from LTR containing TAR (Figure 1). Also, an easily recognizable background (Tat-independent) expression was not found. However, when analyzed under the control of the HIV-1 LTR lacking the TAR region (dTAR-cat or dTAR-luc), there was no induction of the CAT or luciferase gene by either form of Tat. These results indicate that both Tat protein and TAR element are essential for efficient expression of heterologous genes from the HIV-1 LTR promoter system.

To ensure that the effector plasmids, pUC19SXLTR, pTat120B, and pTat120K, were not affecting DNA uptake during cotransfection, an assay was used in which plasmid pCH110 (Pharmacia), which expresses β-gal gene, was cotransfected with pUC19SXLTR, pTat120B, or pTat120K. Uptake of pCH110 was quantified by scoring the number



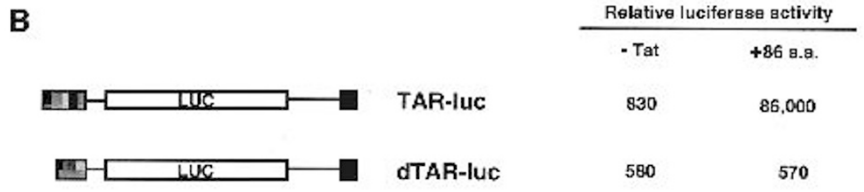
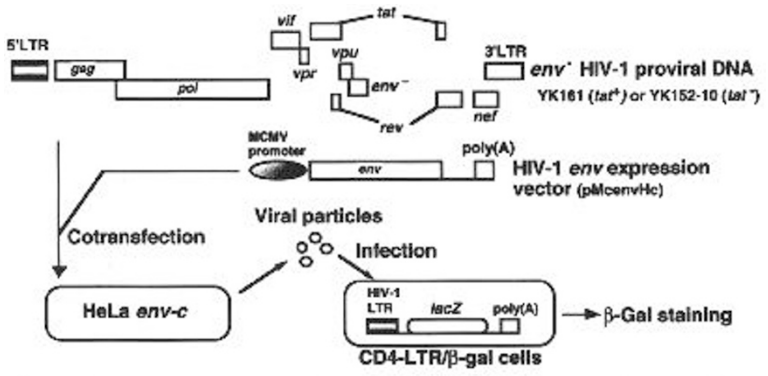


Figure 1. Tat-responsive expression of heterologous genes from HIV-1 LTR in transfected cells. HeLa cells were transfected with 3 μg of the CAT (A) or luciferase (B) expression vector in the presence of 5 μg of pTat120K (86 a.a. Tat) or pUC19SXLTR (-Tat). All extracts were made 48 h posttransfection, and the activitie of CAT enzyme or luciferase was measured as described in Materials and Methods.



Expt no.	No. of blue cells/ml ± SD	
	YK161	YK151-10
1	2,700 ± 700	< 5
2	1,800 ± 500	< 5
3	2,100 ± 400	< 5

Figure 2. Effect of exogenous Tat on the transactivation of the endogenous HIV-1 LTR. Flowchart of the blue cell assay is shown. HIV-1 proviral DNA, YK161 (intact Tat) or YK152-10 (Tat), was cotransfected with an HIV-1 *env* expression vector, pMcenvHc, into HeLa *env*-c cells, cell-free medium was collected, and the various dilutions of the virus were then used to infect the CD4-LTR/β-gal indicator cells. The infected cells were fixed and stained as described in Materials and Methods. The numbers of blue cells in the infected CD4-LTR/b-gal indicator cells were counted 2 days after infection.

of blue cell after staining (Kimpton and Emerman, 1992). Results of this analysis indicated that none of the effector plasmids inhibited DNA uptake during cotransfection (data not shown).

Tat-dependent expression of endogenous foreign viral gene fragment

I next wanted to determine whether a gene on an endogenous Tat-dependent vector could be induced by infection with HIV-1. YK161 is an HIV-1 viral DNA clone containing the 86-aa intact Tat (Kim and Panganiban, 1993). YK152-10 is a *tat*-deficient HIV1 viral clone that was used as a negative control (Kim and Panganiban, 1993). Both HIV clones contain a truncated *env* gene and so cannot produce functional envelope glycoprotein. Therefore, an *env* expression vector, pMcenvHc (Kim and Risser, 1993), was cotransfected with the viral DNA. To produce HIV-1 particles, Tat^+ cell line (HeLa *env*-c) was transfected, virus was harvested, and the virus was used for infection of CD4-LTR/β-gal indicator cells containing an endogenous *lac*Z gene under the control of the HIV-1 LTR (Kimpton and Emerman, 1992) (the experimintal strategy is shown in Figure 2A). In this study, this construct was used as a marker for expression form the HIV-1 LTR. Two days postinfection, cells were were fixed and *lac*Z gene expression was determined by treatment with X-Gal (Kimpton and Emerman 1992). Only cells that had been infected with YK161 (tat^+) were turned blue after X-Gal staining (Figure 2B). This finding indicates that the simple HIV retroviral vector system is useful in abrogation of progeny virus production and that it could be possible to introduce the construct into Tat^- cellswhere it can be stably maintained but not expressed until activated by infection.

Transactivation of foreign viral gene expression by endogenous Tat

Since uninfected cells could have Tat protein by uptaking the Tat released from infected cells (Ensoli *et al.,* 1993), I further tested whether a foreign gene in a simple HIV vector could be expressed following infection into the cells producing Tat protein. To construct a simple vector YK1001 that would be dependent upon Tat for expression of *lac*Z gene, large deletion was made in the infectious proviral clone pNL4-3 (Adachi *et al*., 1986) and the *lac*Z gene fragment was inserted into the vector. Therefore, YK1001 does not contain any intact ORF for HIV-1 protein except for sequences used in viral replication. Because *lac*Z expression from this vector was predicted to be Tat dependent, both HeLa (Tat^-) cells and HeLa *env*-c cells (Gama Sosa *et al*., 1989), which express Tat, were used as target cells for infection. Since HeLa and HeLa *env*-c cells do not express the major HIV receptor, CD4, a murine amphotropic retrovirus envelope protein was used to pseudotype the vector and thereby allow entry into these cells. Virus was produced by transfecting HeLa cells with vector DNA, a plasmid that expresses all viral proteins necessary for particle production except the envelope glycoprotein (MSMΔenv50; Figure 3), and a plasmid that expresses the envelope glycoprotein of an amphotropic murine leukemia retrovirus (pSV-A-MLV-*env*; Figure 3). Two days posttransfection, virus was harvested, and target cells were infected. The cells were then stained by treatment with X-Gal to examine the expression of *lac*Z gene. The *lac*Z gene expression indecates that a foreign gene fragment is capable of being propagated on the simple HIV-1 vector and expressed following infection of Tat^+ cells.

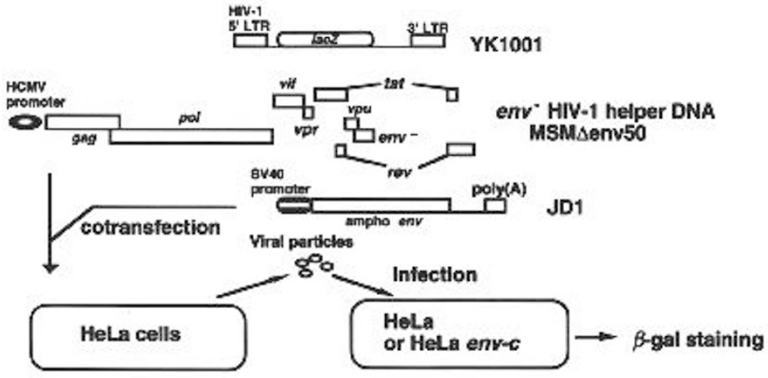
$CD4^+$ selective infection of the simple HIV-1 vector system

The HIV-1 system is intriguing because of the natural tropism for $CD4^+$ cells which may be exploited as a means of targeting gene therapy for HIV and other disorders of $CD4^+$ cells. To examine whether the simple HIV-1 vector system can be used for selective infection of mature $CD4^+$ cells, I constructed another simple HIV-1 vector, YK1002, which contains the *Hyg*^{r} gene under the control of an internal promoter, SV40 early promoter. Virus was produced by transfecting HeLa cells with the vector DNA, the helper DNA (MSMΔenv50), and the plasmid pMcenvHc that expresses the HIV-1 envelope glycoprotein. Two days posttransfection, virus was harvested, and HeLa cells ($CD4^-$) and HeLa T4 cells ($CD4^+$) were infected. The cells were then selected with hygromycin B, and the infection of the vector virus produced was determined by fixing, staining, and counting resistant colonies.

When tested by this assay, YK1002 consistently gave the Hyg^r colonies only when HeLa T4 cells were infected (Figure 4). No Hyg^r colony resulted from infection of HeLa cells with YK1002, demonstrating the $CD4^+$-specific infection of the HIV vector system by the HIV-1 envelope glycoprotein.

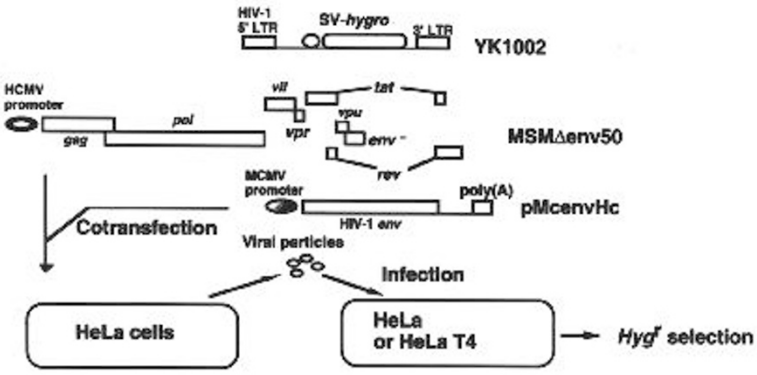
Discussion

In most gene therapy strategies to treat HIV infection, it would be necessary to introduce into cells susceptible to HIV infection a gene that specifically interferes with virus replication of that causes the death of an infected cell, thereby preventing virus spread. The construction of a simple Tat-dependent HIV vector might be useful for anti-HIV gene therapy of individuals previously infected



Expt no.	No. of blue cells/ml ± SD	
	HeLa *env-c*	HeLa
1	1,600 ± 200	< 5
2	1,800 ± 500	< 5
3	1,600 ± 500	< 5

Figure 3. Effect of endogenous Tat on the transactivation of *lac*Z gene from HIV-1 LTR after infection. Flowchart of the blue cell assay is shown. A simple HIV vector containing *lac*Z, YK1001, was cotransfected with a helper HIV-1 DNA (*env*$^-$) and an amphotropic *env* expression vector into HeLa cells, cell-free medium was collected, and the various dilutions of the virus were then used to infect HeLa or HeLa *env*-c cells. The infected cells were fixed and stained as described in Materials and Methods. The numbers of blue cells were counted 2 days after infection.



Expt no.	No. of Hygr colonies/ml ± SD	
	HeLa T4	HeLa
1	300 ± 70	< 1
2	200 ± 40	< 1
3	200 ± 50	< 1

Figure 4. Selective infection of CD4+ cells by recombinant HIV-1 retroviral vector system. Flowchart of the Hygr selection assay is shown. A simple HIV vector containing Hygr gene expressed from an internal promoter, YK1002, was cotransfected with a helper HIV-1 DNA (*env*-) and an HIV-1 *env* expression vector into HeLa cells, cell-free medium was collected, and the various dilutions of the virus were then used to infect HeLa or HeLa T4 cells. The cells were then selected with hygromycin B, and the infection of the vector virus produced was determined by fixing, staining, and counting resistant colonies.

by HIV. If an antiviral gene expressed from the HIV LTR can be introduced into a subset of the HIV target cells prior to HIV infection, gene expression might be specifically activated by *de novo* Tat expression from an incoming virus (Figure 2). Since this type of strategy involves the expression of genes which are potentially toxic, stringent control of gene expression may be necessary for such an approach to be successful as a therapeutic modality. In the experiments presented here, Tat-dependent gene expression from the HIV-1 LTR was measured by the production of an easily recognizable phenotype (Figure 1 and 2). The significance of background (Tat-independent) expression is unclear and would likely depend on the particular foreign gene on the vector, the cell type infected, or the presence of other factors known to affect LTR expression (Nabel *et al.,* 1988). It is conceivable that minimal expression of particular genes has no detrimental effect while a similar level of expression of an alternative gene is toxic to the cell. Addition of the *cis* acting Rev-responsive element (*rre*) to the vector may offer increased control of gene expression and act as an additional safe-guard against inappropriate gene expression (Harrison *et al.,* 1991b).

During acute HIV-1 infection or after transfection of the *tat* gene, Tat protein is released into the cell culture supernatant (Frankel and Pabo 1988; Ensoli *et al.*, 1993). In this extracellular form, Tat stimulates both HIV-1 gene expression and the growth of cells derived from Kaposi's sarcoma (KS) lesions of HIV-1-infected individuals (AIDS-KS cells). Tat-containing supernatants stimulate maximal AIDS-KS cell growth but only low to moderate levels of HIV-1 gene expression. Tat, released into the serum from infected cells, might be taken up by other cells, causing transactivation of the HIV promoter in cells harboring latent virus of causing altered expression of cellular genes. In addition, recently, it was proposed that HIV-1 Tat strongly sensitizes gp120/CD4-induced T-cell apoptosis by accelerating the physiological process of activation-induced CD95-mediated T-cell apoptosis, a mechanism that may contribute to CD4+ T-cell depletion in AIDS (Westendorp *et al.,* 1995). The effects of Tat could occur in both infected and non-infected cells. Since AIDS patients do show antibodies to Tat (Barone *et al.,* 1986; Franchini *et al.,* 1987), Tat is probably present extracellularly at some time. If cellular uptake is important *in vivo*, this will have important implications for gene therapy and drug design. Therefore, introduction of the simple HIV-1 vectors encoding various anti-HIV genes into the Tat+ non-infected cells (Figure 3) may allow new therapeutic interventions in AIDS.

Because cells of the hematopoietic system seem to be the primary targets for HIV infection, it might be advantageous to target multipotent stem cells for introduction of a foreign gene. This gene would then be present in mature hematopoietic cells following proliferation and differentiation of the stem cells. However, because of difficulties encountered both in isolating and effectively infection hematopoietic stem cells and because the primary target for HIV infection appears to be helper T cells, it is possible that a vector capable of specifically infecting mature T lymphocytes might be useful, although repeated administration probably then would be necessary. One method for targeting potentially therapeutic genes to cells susceptible to HIV infection (CD4+ cells) would be to use an HIV vector system using the HIV-1 envelope glycoprotein which is essential for the natural tropism for CD4+ cells. In the experiments presented here, a simple HIV-1 vector, YK1002, which constitutively express marker genes from an internal promoter, was constructed and is capable of being propagated when viral proteins

are provided using transient cotransfection protocol and was used to study the HIV-1 envelope glycoprotein-mediated selective infection of $CD4^+$ cells (Figure 4).

Currently, murine retroviral vectors and packaging cell lines are the best characterized systems for introducing foreign genes into cells (Miller, 1990). However, the development of HIV vector system including an HIV packageng cell line might be not easy because of the greater complexity of the HIV genome and replication cycle relative to those of th avian and murine retroviruses for which effecient packaging systems exist. A major obstacle in the development of a high-titer, vector-producing packaging cell line has been difficultyies encountered in obtaining cell lines that stably express the HIV proteins. It has been hypothesized that this difficulty results from toxicity of one or more viral proteins (Kim, Y.-S., unpublished data). In addition, to inhibit the possibility of generating replication competent HIV via DNA recombination or encapsidation of helper viral RNA in packaging cells, the packging signal sequences (Ψ) should be mapped accurately.

Taken together, simple HIV-1 vectors that contain no intact viral open reading frames and that expresses foreign genes from the HIV-1 LTR or an internal promoter have been described. Because expression of the foreign gene is dependent on Tat and infection is specific for CD4 receptor, this vector system provides a way in which to introduce genes into cells they will be maintained but not expressed until induced. However, though this vector system is useful for studying the feasibility of this strategy, currently the titer of vector virus produced is far too low to be of *in vivo* practical use. Development of an efficient HIV packaging cell line will improve efficacy and safety of the HIV gene therapy using this system.

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