A Lentiviral Vector That Activates Latent Human Immunodeficiency Virus-1 Proviruses by the Overexpression of Tat and That Kills the Infected Cells

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Abstract

Despite the efficient HIV-1 replication blockage achieved with current highly active antiretroviral therapy (HAART) therapies, HIV-1 persists in the body and survives in a latent state that can last for the entire life of the patient. A long-lived reservoir of latently infected CD4+ memory T cells represents the most important sanctuary for the virus and the greatest obstacle for viral eradication. In this work, we present an initial step toward a gene therapy approach aimed at the activation of latent provirus to induce the death of latently infected T cells. Latent HIV-1 infection is characterized by the failure of viral gene expression as a consequence of uninitiated or aborted transcription. We have constructed an HIV-1-based lentiviral vector (p5p53RTAT3) that expresses the viral trans-activating protein Tat in a drug-regulated manner and p53 in a Rev-dependent manner. We have demonstrated that the Tat-expressed protein from p5p53RTAT3 vector reactivates latent HIV-1 proviruses in J1.1 and ACH-2 cell lines and promotes p53-induced apoptosis in the presence of Rev. Our system was able to trigger the trans-activation of the provirus 5' long terminal repeat (LTR), stimulate the expression of the Rev protein from a tat-defective provirus, and provoke apoptosis selectively in the cells transfected with a tat-defective HIV-1 provirus in contrast to those with no HIV-1 provirus. However, the Rev-dependent p53 killing of latently infected cells was not effective enough for complete elimination of the awakened HIV-1 viruses. In summary, we have developed a vector system that is efficient in activating latent HIV-1 proviruses but that needs further improvement to kill infected cells.

Overview Summary

To test the feasibility of a gene therapy scheme aimed at the destruction of latently HIV-1-infected cells, we have constructed an HIV-1-based lentiviral vector (p5p53RTAT3) that overexpresses Tat in a drug-regulated manner and the p53 gene in a Rev-dependent manner. Transfection with the p5p53RTAT3 construct induced massive cell death only in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG, which induces Tat expression) and Rev protein. Transduction of latently infected cells lines J1.1 and ACH-2 with p5p53RTAT3 strongly reactivated the latent proviruses. This indicated that the overexpression of Tat, in the absence of any other stimulus, was sufficient to achieve full reactivation of the latent proviruses. However, these data also indicate inefficient Rev-dependent killing of HIV-1-infected cells, because the p24 level rose with time. It is noteworthy that the drug-induced expression of Tat caused nearly 10-fold higher activation of a latent 5' LTR than did the strong cell and provirus activator tumor necrosis factor-α.

Introduction

HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV-1) viral genes are expressed from the 5' long terminal repeat (LTR) promoter, which contains binding sites for a number of cellular transcription factors involved in T cell activity such as SPI, NF-κB, AP-1, and NF-AT (Garcia et al., 1987; Nabel and Baltimore, 1987; Kawakami et al., 1988; Li et al., 1991; Ross et al., 1991). For this reason the provirus is expressed in response to cellular activation (Siekevitz et al., 1987; Tong-Starksen et al., 1987; Stevenson et al., 1990b). The integrated
provirus is expressed in a full-length RNA that contains several splicing donors and acceptor sites. Fully spliced RNA encodes three proteins, Tat, Rev, and Nef, which in the case of Tat and Rev accumulate in the cell nucleus. Tat binds a 5′ stem–loop of the nascent RNA (TAR) (Berkhout et al., 1989) that recruits to the HIV-1 promoter the cellular F-TEFb kinase complex, composed of cyclin T1 and cyclin-dependent kinase-9 (CDK9) (Zhu et al., 1997), which in turn induces serine-2 phosphorylation of the RNA polymerase II complex (Parada and Roeder, 1996). This Tat-induced modification promotes efficient transcriptional elongation (Kao et al., 1987). Tat also stimulates transcription by direct, TAR-independent activation of the viral promoter (Brady and Kashanchi, 2005). Rev protein is accumulated until a threshold is reached, and then multiple Rev proteins bind a sequence present inside the env gene (RRE) and at the same time associate with the host nuclear export protein CRM1 (Fornerod et al., 1997; Henderson and Percipalle, 1997; Neville et al., 1997). Therefore, Rev is involved in nuclear export of unspliced or single-spliced transcripts and in this way allows a change in the pattern of gene expression from early (tat, rev, and nef) to late genes (gag, pol, env, vif, vpu, and vpr) (Felber et al., 1989; Hammarskjold et al., 1989; Malim et al., 1989). There is also ample evidence showing that nonintegrated HIV-1 cDNA can also be transcribed; in this case, only Tat, Rev, and Nef proteins have been detected (Ansari-Lari et al., 1995; Cara et al., 1995; Engelman et al., 1995; Spina et al., 1995; Stevenson et al., 1990a,b; Wiskerchen and Muesing, 1995; Wu and Marsh, 2001).

At present, virus replication is often quite efficiently blocked by highly active antiretroviral therapy (HAART). However, viral sanctuaries of replication-competent HIV-1 proviruses (Folks et al., 1987; Laughlin et al., 1993; Popik and Pitha, 1993; Poli et al., 1994; Prins et al., 1999; Kulkosky et al., 2001; Managlia et al., 2006), but the adverse effects of these therapies and the poor clinical benefit found have shown that this approach is not useful (Stellbrink et al., 1998; Dybul et al., 2002). Non-tumor-promoting phosphoribosyl esters such as prostratin and derivatives are under study because they can activate the provirus without complete activation of the cell (Korin et al., 2002; Bocklandt et al., 2003; Marquez et al., 2008). The effect of phosphoribosyl esters is mediated by activation of NF-kB (Williams et al., 2004). Amphotericin-B and interleukin (IL)-7 have been also tested for the activation of silent proviruses (Jones et al., 2005; Wang et al., 2005). However, various activators of proviral latency may deplete only specific subsets of the latent proviruses (Wang et al., 2005). In addition, elimination of the cells infected with latent proviruses is not yet adequate, and much work is needed to address the possible side effects of these drugs.

Initial reports suggested that integration into inactive heterochromatin was responsible for low basal levels of transcription (Jordan et al., 2001). The absence of host factors as well as chromatin structure in the integration site have been proposed as possible mechanisms to explain why there is no transcription of the viral genes. However, data indicate that a large proportion of proviruses are silent in vitro, even in actively dividing cells (Jenninga et al., 2008), suggesting that it may be an intrinsic property of the viral promoter system in dividing cells. The finding that >90% of the viral integration events in CD4+ memory T cells of HAART-suppressed patients occurred in the exons or introns of genes actively expressed in memory T cells (Han et al., 2004) supports the idea that transcriptional host gene interference may be the major mechanism to control HIV-1 latency (Han et al., 2008; Lenasi et al., 2008; Duverger et al., 2009). On the other hand, reactivation of latent HIV-1 integrated in a highly transcribed gene was achieved when chromatin-reassay factors were depleted (Vanti et al., 2009). In this context, Tat protein may play a role in remodeling the chromatin structure by facilitating the recruitment of the SWI/SNF complex to the HIV-1 promoter activating the transcription (Mahmoudi et al., 2006).

To achieve total virus clearance, we need to reactivate latent proviruses in a controlled manner and selectively kill infected cells to prevent the release of viral particles. This reactivation requires the active transcription of full viral RNA, which depends on Tat activity. In fact, the addition of exogenous Tat protein enhances the activation of transcription of latent HIV-1 proviruses (Lin et al., 2003). Therefore, high Tat protein activity might be used as a way to activate silent proviruses. In addition, the infected cell with the reactivated provirus should be killed as soon as possible to prevent sustained release of infectious viral particles while the immune system or the viral activity destroys the cell. Several lethal genes have been used to destroy HIV-1-infected cells (reviewed in Luque et al., 2005). However, their use can damage healthy cells and this represents a limitation of this strategy. The physiological protein p53, if expressed in a sustained manner at a high level, induces apoptosis of cells. Meanwhile, low levels of p53 expression are harmless to normal cells and the protein does not accumulate because it is subjected to proteolytic turnover. In fact, this protein is widely known for tumor suppression activity. Therefore, it may be a good choice to induce the selective death of infected cells.

For the selective killing of infected cells, the apoptotic gene should be expressed only if an HIV-1 provirus is present in the cell. The Rev protein is involved in the rapid transport of transcripts to the cytoplasm, avoiding or reducing the rate of splicing of introns and transcript maturation (Hammarskjold et al., 1989). Thus, Rev-dependent expression of the apoptotic gene could be used to achieve its selective HIV-1-dependent expression. In the search for a strategy of latent provirus reactivation and killing of infected HIV-1 cells, we have developed a lentiviral vector that expresses Tat protein in a drug-inducible way and p53 in a Rev-dependent way.
Materials and Methods

Cell lines

All cell lines were supplied by the NIH AIDS Research and Reference Reagent Program (Germantown, MD). HeLa-CD4-LTR-βgal cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), and supplemented with antibiotics G-418 at 0.2 mg/ml and hygromycin-B at 0.1 mg/ml in a 5% CO₂ atmosphere. Jurkat, J-Lat GFP clone A72, J1.1, and ACH-2 cells were cultured in RPMI 1640 with 10% FCS in a 5% CO₂ atmosphere.

Construction of lentiviral vector

The vector was constructed using three DNA fragments amplified by polymerase chain reaction (PCR) from HIV-1 clone pNL4-3. The primers were as follows: R1 (5'-AAC TGC AGT GGA AGG CCT AAT TAC-3') and R2 (5'-GCT CTA GAC CTG CTG GAA GGG ATG GTG GCT GT-3'); R3 (5'-GCT CTA GAG CTG CTA TTA ACA AGA GAG ATG GTG GT-3') and R4 (5'-GGG GTA CCC AGA AGT GCC ACA ATC TTC GTT A-3'); R5 (5'-CAG GTA CCT TTA ACA GCA ATC GAT CAC TAC-3') and R6 (5'-GAA TTC TAG AGA TTT TCC ACA ATG A-3'). A regulated cat construction and the p53 gene were inserted into the backbone as shown in Fig. 1. To detect any artifact during the construction of the vector, we checked each new inserted fragment by DNA sequencing. Sequencing reactions were performed in an automatic capillary sequencer (CEQ 2000XL; Beckman Coulter, Fullerton, CA).

Transfections

Transfections were made by electroporation of exponentially growing cells with 1 µg of plasmid DNA per million cells in a GenePulser II electroporation system (Bio-Rad, Hercules, CA). Electroporation efficiency was determined in each experiment with the plasmid pCMV-eGFP (Clontech, Mountain View, CA), which expresses the enhanced green fluorescent protein.

RT-PCR

Accumulation of mRNA of the apoptotic gene p53 was analyzed by RT-PCR. RNA was extracted from 1.2 million cells, using the Perfect RNA kit for eukaryotic cells (Eppe-ndorf, Hamburg, Germany). The cDNA was synthesized from 3 µg of RNA, using a 1st strand cDNA synthesis kit for RT-PCR (Roche Diagnostics, Indianapolis, IN). The reaction mixture was incubated for 10 min at 25°C, 60 min at 42°C, and for a final step of 5 min at 99°C. PCRs were carried out in an iCycler thermal cycler (Bio-Rad). Two microliters of cDNA was used for amplification in 50 µl of a mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTPs, 2.5 U of FastStart Taq polymerase, and 50 pmol of each primer. The primers were designed to amplify 300 bp corresponding to the upstream region and 5' end of the p53 gene in the vector. No amplification of the endogenous p53 transcripts was achieved with this primer pair. Primer sequences were as follows: p53outF (5'-GAC AAA TAC TGG GAC AAC TAC AAT C-3') and p53inR (5'-TTG AGT GGA CCA TTT TTT TTA ATC GT-3'). Amplification was performed by incubation at 95°C for 4 min; followed by 30 cycles, each of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1.5 min; and a final extension of 3 min at 72°C. The results of PCR were visualized by electrophoresis in a 2% agarose gel.

Western blot

Accumulation of p53 protein was analyzed by Western blot. Transfected cells were maintained in culture medium for 24hr. Protein extracts were obtained by incubation for 20 min in buffer containing 25 mM Tris phosphate (pH 7.8), 8 mM MgCl₂, 1 mM Triton X-100, 1% dithiothreitol (DTT), 15% glycerol, 2 µM leupeptin, pepstatin (2 µg/µl), and 100 µM phenylmethylsulfonyl fluoride (PMSF). Cell extracts were centrifuged at 2000 × g for 3 min and supernatants were transferred to a clean tube. Protein concentration was determined by the Bradford method. Thirty micrograms of
protein was loaded into 12% denaturing polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes by electrotransfer and blocked with Tris-buffered saline (TBS) plus 5% nonfat milk for 2 hr. Blots were incubated with anti-p53 antibody (diluted 1:5000) overnight at 4°C followed by incubation with peroxidase-labeled anti-rabbit IgG (diluted 1:10,000) for 1 hr. Finally, a film was exposed to an enhanced chemiluminescence system.

**Apoptosis detection**

Apoptosis was analyzed 48 hr posttransfection in HeLa cells, and 36 hr posttransfection in Jurkat cells, in samples with cell numbers ranging from $1 \times 10^5$ to $1 \times 10^6$ by flow cytometry on an EPICS Elite ESP flow cytometer (Beckman Coulter). Viral p24 antigen concentration was determined by enzyme immunoassay (Genetic Systems HIV-1 antigen EIA; Bio-Rad) in supernatants of transduced J1.1 and ACH-2 cells. The cells were incubated with 5 mM isoprropyl-$\beta$-thiogalactopyranoside (IPTG). Tumor necrosis factor (TNF)-$\alpha$ was added at 20 ng/ml.

**Results**

**Lentiviral vector construction**

A lentiviral vector (p5p53RTAT3) was constructed to test the feasibility of activating latent proviruses and killing infected cells. The vector backbone contains all cis-acting elements of HIV-1 needed to package it into viral particles but it lacks the trans elements (Fig. 1). The vector contains the cDNA tat gene under a regulated expression system derived from the LacSwitch II inducible mammalian expression system (Stratagene, La Jolla, CA), so that the tat gene is repressed by the LacI protein that binds the operator sequences placed just after the Rous sarcoma virus (RSV) LTR promoter. The tat gene is expressed in response to the presence of the inducer IPTG, which releases it from the LacI protein repression. A p53 gene, placed between splicing signals and next to an RRE element, is expressed from the 5' LTR, but it requires the presence of the Rev protein to be expressed by exporting the transcripts before the splicing occurs.

**p53 expression from p5p53RTAT3 is regulated by IPTG and Rev protein**

The 5' LTR activity is enhanced by Tat trans-activation, and high levels of Tat are expected only in IPTG-induced cells transfected with p5p53RTAT3. The 5' LTR/Tat-driven expression of p53 was analyzed by transient transfection of HeLa-CD4-LTR-$\beta$gal cells with the vector and with or without Rev expressed from a cytomegalovirus (CMV) promoter. Specific RT-PCR that amplifies only the vector mRNA and Western blot detection of the p53 protein were performed. Weak mRNA amplification was found in the presence of Rev and absence of IPTG, and no amplification was detected in the absence of both. However, as expected, strong amplification was observed in the presence of both IPTG and Rev (Fig. 2A). Western blot analysis confirmed that the p53 protein was overexpressed only in the presence of both IPTG and Rev protein (Fig. 2B).

**Apoptosis induced by p5p53RTAT3 needs the presence of Tat and Rev**

The capacity of p5p53RTAT3 to induce apoptosis in the presence of Tat induced by IPTG and Rev protein was tested in transient transfection experiments of HeLa-CD4-LTR-$\beta$gal cells. Cells were transfected with p5p53RTAT3 and treated with various IPTG concentrations. A construct lacking the p53 gene (p5RTAT3) was used as a control of basal apoptosis induced nonspecifically by experimental cell manipulation. Average results from four experiments are shown in Fig. 3. The apoptosis level found with p5p53RTAT3 in the absence of Rev was similar to the basal level found with the p53-deficient construct, and was independent of the IPTG concentration, except for a slight effect at the highest concentration of IPTG (7 mM). This indicates that p5p53RTAT3 is harmless for the cells in the absence of Rev. Rev was
expressed from two different promoters: the CMV promoter and the HIV-1 LTR promoter. In both cases a similar apoptosis induction pattern dependent on IPTG concentration was noted. However, at low IPTG concentrations the LTR promoter gave better results than did the CMV promoter. In the presence of Rev, a high apoptosis level of about 40% was detected in the absence of IPTG. This finding may be due to basal expression of the 5' LTR in response to cellular transcription factors such as NF-κB. The highest apoptosis level reached was nearly 80% with at least 5 mM IPTG. Taking into account that transfection efficiency reached 65%, we can assume that in the presence of IPTG and overexpression of Rev, killing efficiency approached 100% (Fig. 3).

Cotransfection of HeLa cells with p5p53RTAT3 and a Dtat, Dgag–pol, Denv defective provirus also induced a similar high level of apoptosis, 81.2 ± 10.6%, whereas cells transfected only with p5p53RTAT3 registered 25.0 ± 10.6% apoptosis, and cells transfected only with the defective provirus registered 25.1 ± 6.1% apoptosis. Therefore, the Tat protein expressed from p5p53RTAT3 induced expression of the provirus Rev, indicating that Tat-deficient proviruses can be induced by p5p53RTAT3. The induction of apoptosis in Jurkat T cells was analyzed with this defective provirus, confirming that p5p53RTAT3 was able to induce apoptosis quite efficiently in those cells (Table 1 and Fig. 4).

**Ability of p5p53RTAT3 to awaken latent HIV-1 proviruses and to kill infected cells**

To assess the ability of p5p53RTAT3 to awaken latent HIV-1 proviruses and to kill infected cells, we transduced latent provirus-containing J1.1 and ACH-2 cells with p5p53RTAT3, p5RTAT3 (p53−), and p5R3 (p53+, tat−) lentiviral particles. The titer obtained when packaging these vectors ranged from 8×10⁵ to 3×10⁶. This low titer was a consequence of the size of the vectors, more than 9 kb. To package the p5p53RTAT3 vector we collected supernatants only at 48 hr after transfection, because at later times the cell culture had undergone extensive p53-induced apoptosis. At longer incubation times such as 56–72 hr, no effective packaged vector was obtained because of the extensive p53-induced apoptosis observed. Strong activation of the p24 content occurred in both cell lines when transduced with p5p53RTAT3 and p5RTAT3 lentiviral vectors, indicating strong reactivation of the latent proviruses (Fig. 5). Therefore, Tat expression was adequate to activate these latent proviruses at high efficiency. The p24 content was similar in cell lines transduced with p5p53RTAT3 and p5RTAT3 lentiviral vectors in the first 48 hr (Fig. 5A). However, induction of apoptosis by p53 strongly reduced viral production at a longer incubation time (Fig. 5B).

The p24 content was higher in latently infected cells transduced with the p53− p5RTAT3 lentiviral vector than in the absence of IPTG. This finding may be due to basal expression of the 5' LTR in response to cellular transcription factors such as NF-κB. The highest apoptosis level reached was nearly 80% with at least 5 mM IPTG. Taking into account that transfection efficiency reached 65%, we can assume that in the presence of IPTG and overexpression of Rev, killing efficiency approached 100% (Fig. 3).

Cotransfection of HeLa cells with p5p53RTAT3 and a Dtat, Dgag–pol, Denv defective provirus also induced a similar high level of apoptosis, 81.2 ± 10.6%, whereas cells transfected only with p5p53RTAT3 registered 25.0 ± 10.6% apoptosis, and cells transfected only with the defective provirus registered 25.1 ± 6.1% apoptosis. Therefore, the Tat protein expressed from p5p53RTAT3 induced expression of the provirus Rev, indicating that Tat-deficient proviruses can be induced by p5p53RTAT3. The induction of apoptosis in Jurkat T cells was analyzed with this defective provirus, confirming that p5p53RTAT3 was able to induce apoptosis quite efficiently in those cells (Table 1 and Fig. 4).

**Table 1. Apoptosis Induced by p5p53RTAT3 in the Presence of a tat-Defective Provirus in Jurkat T Cells**

<table>
<thead>
<tr>
<th>Cotransfected plasmids</th>
<th>Mean average apoptosis level (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>def-HIVb</td>
<td>18.1 ± 3.2</td>
</tr>
<tr>
<td>def-HIVb + p5RTAT3</td>
<td>20.9 ± 4.9</td>
</tr>
<tr>
<td>def-HIVb + p5p53RTAT3</td>
<td>52.0 ± 7.1</td>
</tr>
</tbody>
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*The apoptosis level was analyzed in Jurkat T cells cotransfected with the indicated plasmids. Cells were incubated for 12 hr before the addition of 5 mM IPTG and then incubated for another 24 hr. The apoptosis level was analyzed in Jurkat T cells cotransfected with the indicated plasmids. Cells were incubated for 12 hr before the addition of 5 mM IPTG and then incubated for another 24 hr. The apoptosis level was analyzed in Jurkat T cells cotransfected with the indicated plasmids. Cells were incubated for 12 hr before the addition of 5 mM IPTG and then incubated for another 24 hr.*

![Image](image-url)
with the \( p53^+ \) \( p5p53RTAT3 \) lentiviral vector, indicating that Rev-dependent \( p53 \)-induced apoptosis was able to reduce viral particle release. However, the increment in p24 levels found with \( p5p53RTAT3 \) also indicates that, on activation, infected cells were not sufficiently killed to completely block the escape of infectious HIV-1 particles. This may be because \( p5p53RTAT3 \)-induced apoptosis was not quick enough to avoid some viral particles from being released in the J1.1 and ACH-2 cell lines.

**FIG. 4.** Flow cytometry graphics of a single experiment involving Jurkat T cells cotransfected with the indicated plasmids and incubated for 12 hr before the addition of 5 mM IPTG followed by incubation for another 24 hr; the apoptosis levels analyzed are shown.

Tat protein expressed from \( p5p53RTAT3 \) vector induces integrated silent LTR at a higher level compared with TNF-\( \alpha \).

To determine the strength of the induction of the silent integrated LTR achieved by Tat expressed from \( p5p53RTAT3 \), the J-Lat GFP clone A72 cell line was transduced with this vector and the results compared with the effect of the TNF-\( \alpha \). This cell line contains an integrated LTR-GFP in a silent state, thus allowing the level of activation of this LTR by GFP fluorescence and cytometry to be measured (Jordan et al., 2003). A nearly 10-fold higher fluorescence level was attained in the \( p5p53RTAT3 \)-transduced cells compared with the TNF-\( \alpha \)-induced cells (Fig. 6). This indicates that Tat is a more potent activator of this silent integrated LTR than the cell activator TNF-\( \alpha \).

**Discussion**

HAART reduces HIV-1 in plasma to levels below the limit of detection. Despite the efficient HIV-1 replication blockage achieved with current HAART therapies, they are unable to eradicate the virus and are far from being a real cure for the infection (Chun et al., 1997b). Instead, HIV-1 persists in the body and survives in a latent state, as replication-competent forms but transcriptionally silent, not affected by HAART. These latently infected cells represent a viral reservoir that leads to plasma viremia reemergence after withdrawal of the drugs (Finzi et al., 1997; Wong et al., 1997; Chun et al., 2000). In fact, T cells harboring latent proviruses can last for the entire life of the patient (Siliciano et al., 2003). Thus, the need for new therapeutic strategies that target viral latency is evident.

Latent HIV-1 infection is characterized by integration of the provirus into the host DNA, followed by temporary silencing of viral gene expression. HIV-1 latency has a multifactorial nature (reviewed in Lassen et al., 2004), but regardless of the underlying mechanism, there is a lack of Tat protein in cells with latent proviruses, a situation that may explain the absence of high viral gene expression in latently infected cells. Therefore, Tat is required to induce the acti-

**FIG. 5.** Supernatant p24 concentration in culture medium of latently infected cell lines J1.1 and ACH-2 after (A) 48 hr and (B) 96 hr of incubation with TNF-\( \alpha \) (positive control), with nothing added (C-, negative control), or after transduction with p5R3, p5RTAT3, or p5p53RAT3. Results are the average of three independent experiments.
viation of HIV-1 proviruses and, in fact, it has been shown that Tat enhances the effect of other stimuli to activate latent proviruses (Lin et al., 2003; Han et al., 2008; Lenasi et al., 2008). In addition, infected cells with reactivated proviruses should be killed as soon as possible to avoid the sustained release of new virions. In this work, we present an initial step toward a gene therapy approach aimed at activating latent proviruses by Tat overexpression in order to induce the death of latently infected T cells.

To test the feasibility of a gene therapy scheme targeted toward the activation of latently infected cells, we have constructed the p5p53RTAT3 HIV-1-based lentiviral vector, which overexpresses Tat in a regulated manner to avoid any unwanted side effect after prolonged overexpression in healthy transduced cells. In addition, infected cells with reactivated proviruses should be killed as soon as possible to avoid the sustained release of new virions. In this work, we present an initial step toward a gene therapy approach aimed at activating latent proviruses by Tat overexpression in order to induce the death of latently infected T cells.

To test whether lentiviral vectors that overexpress Tat were able to activate latent proviruses, we packaged them to transduce latently infected cells. These cells are rare, with a low frequency on the order of 1 in 10^6 resting CD4+ T cells, and lack a distinctive surface marker differentiating infected and uninfected cells (Chun et al., 1997a, 2000). In addition, their manipulation in vitro changes the activation status of these cells, making the direct study of latency almost impractical. For this reason, latency models have been developed. We have used two well-established models to study latency: the chronically HIV-1-infected T cell clone (J1.1) derived from Jurkat T cells, for which the provirus can be activated with TNF-α (Perez et al., 1991); and the ACH-2 T cell line (Clouse et al., 1989; Folks et al., 1989). A genetic alteration has been described in the TAR element of the latent provirus of the ACH-2 cell line (Emili et al., 1996). However, the ACH-2 latent provirus can be activated by stimuli that target the NF-κB pathway, such as TNF-α (Folks et al., 1989; Pomerantz et al., 1990; see Fig. 5). Therefore, both cell lines represent reasonably good models, although not identical to the in vivo latent proviruses. The transduction of both cell lines with the p53+ p5RTAT3 lentiviral vector prompted the same strong reactivation of latent proviruses (Fig. 5A). This indicated that overexpression of Tat, in the absence of any other stimuli, was sufficient to achieve full reactivation of latent proviruses. When the cells were transduced with the full lentiviral vector p5p53RTAT3, which contains the p53 gene, a clear reduction of viral particles resulted with incubation times longer than 48 hr (Fig. 5B). However, the apoptotic p53 gene was unable to fully block viral particles from escaping in the first 48 hr (Fig. 5A). The short-term release of viral particles should not be a major problem in patients receiving highly efficient antiretroviral therapy when compared with the benefit of destroying the latent reservoir.

To analyze the strength of overexpressed Tat in the activation of a latent 5' LTR, we measured green fluorescent protein in the latency-model cell line J-Lat GFP clone A72 (Jordan et al., 2001) in response to transduction with p5p53RTAT3 and found that activation of the 5' LTR promoter by overexpressed Tat was nearly 10-fold stronger than that by TNF-α.
As the p5p53RTAT3 lentiviral vector contains intact LTRs, it could potentially be mobilized \textit{in vivo} on infection of the transduced cells by HIV-1. This possibility should be deemed not just a major concern, but also a positive characteristic because it could amplify the antiviral effects. However, self-limiting mobilization is expected because the size of the lentiviral vector makes it poorly competitive with the HIV-1 to be packaged into transducing particles, and the apoptotic effect of p53 will also limit the \textit{in vivo} HIV-1-driven mobilization.

These results, together with the previously described role for Tat as a viral protein able to recruit cellular host factors for remodeling chromatin (to promote the elongation of transcription as well as some positive effects on the initiation of transcription), support further studies to assess its possible use in eliminating the pool of latently infected cells. In addition, the HIV-1-dependent induction of apoptosis by p53 overexpression represents another key element to prevent long-term virus production by the reactivation of latently infected cells. Therefore, p5p53RTAT3 is a vector containing elements that might be useful for gene therapy focused on provirus activation to make latently infected cells visible and to eliminate them as a way to destroy the latent HIV-1 reservoir.

Acknowledgments

The following cell lines were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: J1.1, cat. no. 1340 from Dr. Thomas Folks and Dr. Salvatore Butera; J-Lat GFP clone 72, cat. no. 9856 from Dr. Eric Verdin; and ACH-2, cat. no. 349 from Dr. Thomas Folks and Dr. Salvatore Butera; J-Lat GFP clone 72, cat. no. 9856 from Dr. Eric Verdin; and ACH-2, cat. no. 349 from Dr. Thomas Folks. The DNA sequencing and flow cytometry were performed in the Centro de Instrumentación Científico-Técnica, Universidad de Jaén, Spain. The authors thank Francisco José Sánchez Luque for help with the experiments.

Author Disclosure Statement

The vector is protected by patent P200301398 of 13/06/2003.

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