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Activation of a β-Galactosidase Recombinant Provirus: Application to Titration of Human Immunodeficiency Virus (HIV) and HIV-Infected Cells

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A quantitative bioassay for human immunodeficiency viruses has been developed on the basis of the ability of the *tat* gene to transactivate the expression of an integrated β-galactosidase gene in a HeLa-CD4$^+$ cell line. Infection by a single virion of HIV-1 or HIV-2 corresponds to a unique blue syncytium or a cell cluster detected after fixation and addition of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (a β-galactosidase substrate). The number of infected lymphoid cells in a culture (stimulated human peripheral blood lymphocytes and cell lines) can also be quantified by cell-to-cell transmission of HIV into the HeLa-CD4$^+$–β-galactosidase monolayer. Infections by simian immunodeficiency viruses are similarly detected. This assay has been used to determine the dose response of drugs, the half-life of HIV at 37°C, and the appearance of infectious particles after virus infection.

Human immunodeficiency virus (HIV)-infected cells and free virions are difficult to detect and quantitate by using the currently available tests. The reverse transcriptase assay (1) and p24^{gag} antigen capture (23) do not measure directly the number of virions or HIV-infected cells. Adaptation of these methods by using endpoint dilution gives quantitative results, but they are still indirect and time-consuming. Plaque assays (14, 15, 22, 25) that use leukemia cell lines are useful, but their limitation lies in their inability to detect wild-type HIV isolates (5). Recently, Chesebro and Wehrly (5) have developed a focal immunoassay that uses adherent HeLa cells expressing T4 receptor (HT4) as the target. The assay is highly efficient with both culture-adapted and wild-type HIV isolates.

We have modified the test by taking advantage of the property of the human and simian immunodeficiency viruses (SIV) to transactivate their own expression by virally encoded control elements (32). The product of the *tat* gene of HIV positively regulates the level of full-length transcription from the long terminal repeat (LTR) by acting through sequences called *tar*, localized in the 5′ transcribed sequences of the virus (2, 28, 30). Recombinant genes containing an LTR with intact *tar* are responsive to Tat. We have introduced an nlsLacZ (with a modified β-galactosidase reporter gene) recombinant HIV-1 provirus (4) into HT4 (5). We report that integrated copies of a recombinant *lacZ* provirus in HT4 cells (HT4LacZ-1) are transactivated following infection by HIV-1, HIV-2, SIV_{mac} and SIV_{agm}, but not human T-cell leukemia virus type 1 (HTLV-1). A single virion or a single infected lymphoid cell leads to a cluster of cells or a syncytium expressing a high level of β-galactosidase. In nlsLacZ, β-galactosidase has been modified by the addition of a nuclear locating signal (18); therefore, the enzyme is concentrated in the nucleus (4). Concentration of β-galactosidase in the nucleus reduces to zero the false positives due to the low level of endogenous β-galactosidase in the cytoplasm of the cell. Detection of transactivated cells or syncytia and enumeration of nuclei in syncytia are easy to perform. Also, because this test is based on transactivation of LacZ, there is no difficulty detecting viruses which are poor syncytium formers and distinguishing syncytia attributable to HIV-induced fusion from syncytia attributable to spontaneous cell fusion, a situation already recognized as highly misleading (5). The transactivation is optimally detected after 60 h by the simple 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) histochemical assay (4), which is easy to perform, rapid, can be done in microplates, and can be scored macroscopically or at a low magnification (×20).

We demonstrate here the use of this assay to identify antiviral agents, to quantify the production of virions during the time course of infection, to determine the half-life of infectious particles, and to quantify the number of infected cells in a suspension culture. This β-galactosidase assay (β-gal assay) for HIV infections would be beneficial for a number of in vitro and in vivo studies, including clinical aspects of the acquired immunodeficiency syndrome (AIDS; for a review, see reference 11). A similar approach could be applied to other RNA or DNA viruses coding for a transactivator.

MATERIALS AND METHODS

Cell lines, cell culture, and cell sorting. HT4 and clone HT4-6C (5) were cultivated in Dulbecco modified Eagle medium–10% fetal calf serum. SupT-1, a human T-cell lymphoma cell line (16), was cultivated in RPMI 1640 medium–10% fetal calf serum. Human peripheral blood lymphocytes (PBL) were cultivated in RPMI 1640 medium–10% fetal calf serum–10% interleukin-2 (Biotest). The cultures were previously stimulated by 0.1% phytohemagglutin (PHA; Difco Laboratories) for 3 days. HT4 and HT4LacZ-1 were cultivated and dissociated as described previously (17). For cell sorting, the procedure, which consisted of fluores-

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cence-activated cell sorter and fluorescein di-β-D-galactopyranoside staining was as described previously (26).

Plasmids, transfections, Southern analysis, and RNA dot blots. pHIVSVnlsLacZ*tat* (4), pSV*tat*, pKP125, and pLet (10) were amplified, and the DNA was purified as described previously (21). Plasmids were transfected as described previously (29), except that the calcium phosphate precipitate was added directly to the culture medium. Preparation of cellular DNA, enzymatic restrictions, electrophoresis, Southern analysis, RNA preparations, and dot blots were performed as described previously (21).

Virus, infection, and coculture. HIV-1 BRU (3), HIV-2 ROD (6), and simian immunodeficiency viruses (SIV) SIV_{mac}, SIV_{agm5}, and SIV_{agm14} (8, 13) were all produced on SupT-1 cells. In brief, fresh SupT-1 cells were infected by viruses at a low multiplicity of infection at day zero, and the virus was harvested in the supernatant starting 7 days later. Alternatively, virus was harvested from the chronically infected cells maintained in culture. It was stored at −80°C. HTLV-1 was produced in C10/MJ2 cells (27).

Coculturing of HT4LacZ-1 and SupT-1 or PHA-PBL-infected cells was done in RPMI 1640 medium–10% fetal calf serum. Infected cells were washed twice in culture medium before coculturing.

X-Gal assay and ONPG assay. For the X-Gal assay, cells were briefly fixed (2 to 5 min) in 1% formaldehyde–0.2% glutaraldehyde in 150 mM NaCl–15 mM sodium phosphate (PBS), pH 7.3, washed two times in PBS, and incubated at 37°C for 10 to 60 min in a reaction mixture containing 1 mg of X-Gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM $MgCl_2$ per ml in PBS. X-Gal was dissolved at 40 mg/ml in dimethyl sulfoxide. The enzymatic reaction was stopped by removing the X-Gal reaction mixture. Stained cells were stored in PBS or water. Blue syncytia were scored macroscopically or at low magnification (×20). The *o*-nitrophenyl-β-D-galactopyranoside (ONPG) assay on protein extracts was performed as described previously (29).

Standard procedure of the β-gal assay of infection. HT4LacZ-1 cells were plated at 1.5×10^5 cells per cm^2 in 24-well microplates the day before use. Virus infection was in presence of 10 μg of DEAE-dextran per ml. Cultures were incubated for 3 days at 37°C in standard conditions for tissue culture, which corresponds to 1 day after confluency. The X-Gal assay was then developed for 10 to 60 min. Cell-to-cell infection was by coculture of the infected cells (no more than 10^5 cells per well) with HT4LacZ-1 in the conditions described above. Presence of Polybrene was avoided.

RESULTS

HT4LacZ-1 clone. To develop an assay with maximum sensitivity, an adherent human cell line was chosen, as it was supposed that syncytium formation induced by the virus would be more efficiently detected than with cells in suspension. HT4 is a clone of HeLa cells which expresses the CD4 molecule (7) and is therefore infectable by HIV (5). In the first series of experiments, pHIVnlsLacZ, a plasmid coding for β-galactosidase under the control of the HIV-1 LTR (4), was cotransfected with pSVtkneoβ in HeLa cells. Thirty-three clones resistant to G418 were next isolated and tested for *lacZ* transactivation of β-galactosidase by transfection of a plasmid expressing Tat (pLet [9]). In only seven clones, a low percentage of transactivated cells expressing a low level of β-galactosidase was detected. It was not possible to further purify a stably transactivatable cell line from these clones.

To test whether integrated recombinant HIV could remain transactivatable by maintaining transcriptional activity and/or keeping certain *trans*-acting control elements of the virus, pHIVSVnlsLacZ*tat* was transfected into HT4. pHIVS VnlsLacZ*tat* (Fig. 1c) differs from pHIVnlsLacZ by the presence of the simian virus 40 (SV40) early promoter 5′ to nlsLacZ and by the presence of a functional *env-tat* region (4). The internal promoter is in the plasmid to maintain transcription of the inserted material and the *env-tat* region to eventually provide regulators for the LTR. To isolate stable transformants, the cells were cultivated for 2 weeks after transfection and clones of cells positive for β-galactosidase were isolated by cell sorting following detection of β-galactosidase activity on living cells (26; Fig. 2A). Sixty clones were analyzed for transactivation of β-galactosidase by HIV-1 infection (see below) by direct examination of the level of β-galactosidase after X-Gal staining. Five clones were highly transactivatable. One (HT4LacZ-1) was chosen for further analysis.

The structure of the integrated material was determined by Southern blotting of cellular DNA. HT4LacZ-1 has only one copy of pHIVSVnlsLacZ*tat*. Digestion of the DNA by the *Eco*RV restriction enzyme demonstrated that the structure of the 5′ part of the plasmid (Fig. 1a and c) is unrearranged. It includes in particular the 5′ LTR. The *Xho*I restriction site between the end of *env* and the 3′ LTR is, however, clearly missing, as the 5.5-kilobase *Eco*RV-*Xho*I fragment is the same size as the *Eco*RV fragment (Fig. 1, lanes 1 and 3). To more precisely define the extent of the rearrangement in the *env-tat* region, various digestions were probed with a labeled *env* DNA fragment (Fig. 1b). The *Sac*I site (lane 1) and the *Bgl*II site (lane 3, 1.4-kilobase fragment) in the 3′ LTR as well as the *Xho*I (lane 5), *Bgl*II (lane 3) and *Hin*dIII (lane 2) sites in *env* are all missing. Therefore, the insert corresponds to an HIVSVnlsLacZ structure lacking functional *tat* and *env*. It suggests that the expression of elements of the *env-tat* region is not necessary to obtain highly transactivatable inserted copies.

To test more directly this conclusion, pHIVSVnlsLacZ lacking all of *tat* and *env* was transfected in HT4. The same proportion of stable clones as with pHIVSVnlsLacZ*tat* was transactivated by Tat. Therefore, no viral element other than the 5′ LTR is required for transactivation of integrated HIV recombinant, but a high transactivation level may necessitate the presence of a potent enhancer (SV40) active in HeLa cells. The properties described in the next paragraphs for HT4LacZ-1 have also been observed for these HIVS VnlsLacZ clones.

Transactivation of HT4LacZ-1 by Tat. HT4LacZ-1 is a clone which does not fuse spontaneously and has a basal level of β-galactosidase activity in exponentially growing culture (Fig. 3a) but exhibits an undetectable level of β-galactosidase when confluent after 1 h of X-Gal staining (Fig. 4C). Expression of *lacZ* in the clone and its subclones is stable for at least 50 generations in the absence of selective pressure for β-galactosidase (Fig. 3a to c).

The transactivation of the integrated *lacZ* gene by exogenously introduced viral control products was analyzed by transfection of plasmids into the HT4LacZ-1. pSV*tat* (9), a plasmid which expressed *tat*, induces the expression of *lacZ* which is readily scored by examination of X-Gal-stained cells and confirmed by the quantitative measure of enzymatic activity with ONPG as a substrate (Fig. 3e). As the efficiency of transfection is 5 to 10%, the transactivation index estimated is about 50- to 100-fold above that of the background because of expression from the SV40 promoter.

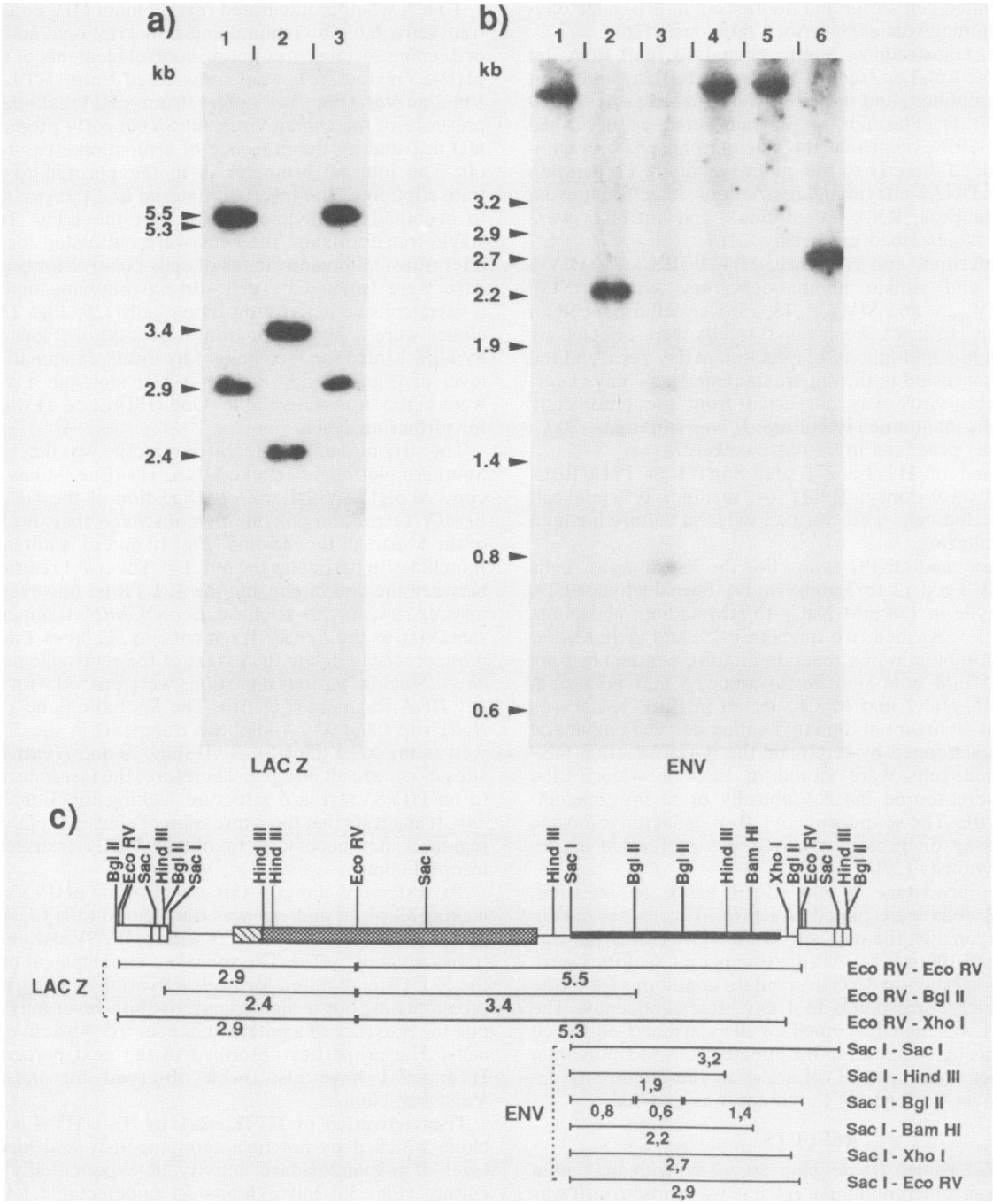

FIG. 1. *env-tat* deletion in the HIVSVnlsLacZ insert of HT4LacZ-1 (Southern blot analysis). (a) *lacZ* probe. Lanes: 1, *Eco*RV digestion; 2, *Eco*RV and *Bgl*II double digestion; 3, *Eco*RV and *Xho*I double digestion. Size markers (arrowheads) are from the same digestions of pHIVSVnlsLacZ*tat*. (b) *env* gene fragment probe from nucleotide 5289 to 8032 in the virus. Lanes: 1, *Sac*I digestion; 2, *Sac*I and *Hin*dIII double digestion; 3, *Sac*I and *Bgl*II double digestion; 4, *Sac*I and *Bam*HI double digestion; 5, *Sac*I and *Xho*I double digestion; 6, *Sac*I and *Eco*RV double digestion. Size markers are from the same digestions of pHIVSVnlsLacZ*tat*. (c) Structure of pHIVSVnlsLacZ*tat*. Symbols: ▭, HIV LTR; ▨, SV40 early promoter; ▩, nlsLacZ; ■, *env-tat* region. The sizes of the expected fragments following appropriate digestions and hybridization with the *lacZ* probe (a) and *env* probe (b) are indicated under the figure of the plasmid. kb, Kilobases.

To determine whether the HT4LacZ-1 clone expresses the viral receptor CD4 on its cellular membrane at a level compatible with the formation of syncytia, a plasmid (pKP125 [9]) which expresses the envelope gene in addition to *tat* and *rev* was transfected in HT4LacZ-1. Dramatic cell-to-cell fusion occurred (Fig. 4A), resulting in syncytia with more than 100 blue nuclei. The same result was also obtained with pLet (9), a plasmid which expresses *tat*, *rev*, and the envelope gene at a level below that detectable by immunoprecipitation (M. Emerman, unpublished data).

To determine whether HT4LacZ-1 can be transactivated by viral infection, stocks of HIV-1 (BRU isolate) were used.

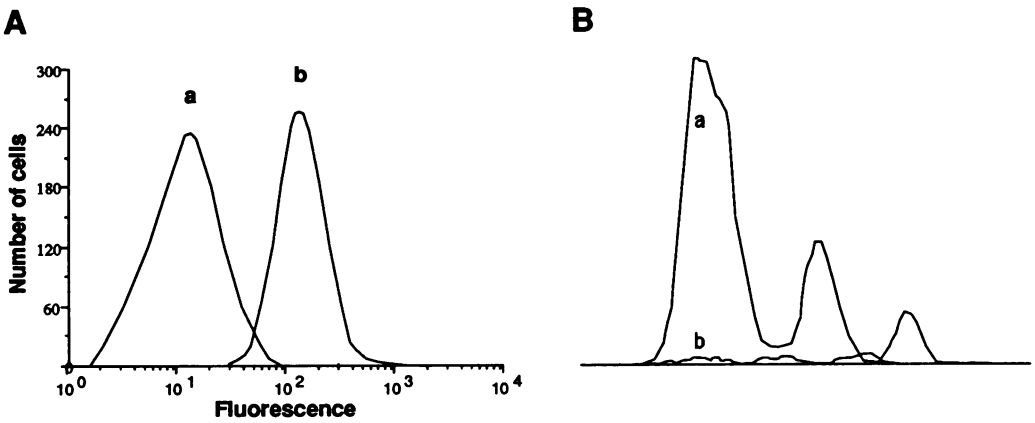

FIG. 2. Isolation of the HT4LacZ-1 cell line by selection using fluorescence-activated cell sorting and characterization. (A) Fluorescence profile of HT4LacZ 2 weeks after transfection with the nlsLacZ construct (a) and of one of the selected clones after amplification in culture (b). (B) Densitometric tracings of bands corresponding to the RNA dot blot of HIV-1-infected (a) and uninfected (b) HT4LacZ-1. Infection was 3 days before at a multiplicity of 1. The probe is *lacZ*. Maximum RNA was 20 μg, with serial fivefold dilutions.

X-Gal staining was performed 3 days after infection. Syncytia with blue nuclei were observed (see below). Dot blot hybridization of total RNA extracted from the infected cells demonstrates a transactivation effect of at least 50- to 100-fold (Fig. 2B).

Titration of HIV-1. To determine the parameters of the infection of HT4LacZ-1, X-Gal staining was performed at various times after infection by HIV-1 (BRU isolate). Syncytia with blue nuclei were observed only after a lag period of 40 to 50 h. The number of β-galactosidase-positive syncytia reached a maximal value at 60 to 80 h (Fig. 5A). They had a mean of 5 to 20 nuclei. As the larger syncytia have a tendency to round up and detach from the culture dish, the number of β-galactosidase-positive syncytia decreases at late times after infection in wells with a high density of syncytia (Fig. 5A).

The effect of polycations on infection efficiency was analyzed with HIV-1. At 10 μg of DEAE-dextran per ml, there was a fivefold increase of the number of β-galactosidase-positive syncytia. In contrast, the addition of 10 μg of Polybrene per ml decreased to 1% the number of syncytia observed with no polycations (Fig. 6A), a result also reported by Chesebro and Wehrly (5). The density of HT4LacZ-1 cells at the time of infection also has an effect on the sensitivity of the method. The optimal number of β-galactosidase-positive syncytia was observed at 1.5×10^5 cells per cm^2 (Fig. 6B). All further experiments were therefore done in presence of DEAE-dextran at this cell density.

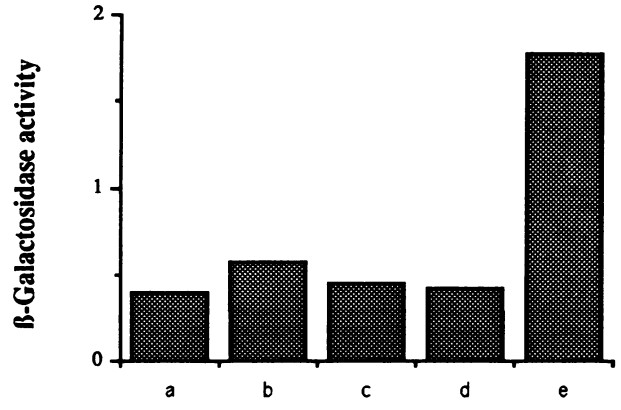

FIG. 3. Expression of β-galactosidase and transactivation by Tat in HT4LacZ-1. β-galactosidase activity was measured by ONPG hydrolysis by protein extracts as described previously (29). β-galactosidase activity in HT4LacZ-1 cells was measured at the origin of the culture (a) and after being maintained in culture for 15 generations (b) and 50 generations (c). HT4LacZ-1 cells were transfected (as described in Materials and Methods) 3 days before protein extraction with 10 μg of carrier DNA (pGEM) (d) and 8 μg of pGEM plus 2 μg of pSV*tat* (e).

There is a linear relationship between the number of β-galactosidase-transactivated syncytia and the dilution used for up to 2×10^3 positive syncytia per well (Fig. 5B). The linear portion of the curve corresponds to one-hit kinetics. Therefore, each β-galactosidase-positive cell results from the interaction of only one viral particle with one cell. By extrapolation, such curves give the number of transactivating particles per milliliter.

A comparison of the sensitivity of the direct titration of virus by the β-gal assay with methods based on amplification of lymphoid cells in culture (titers derived from limited dilution) was performed. SupT-1 cells were infected with serial dilutions of HIV-1 stock and cultured. At days 4 and 7, the presence of amplified virus was checked by testing 1/10th of the supernatants (100 μl) on HT4LacZ-1. After 4 days of amplification, the titer derived from limited dilution was 10^2 to 10^3, and it was 10^4 to 10^5 at day 7. Direct titration of the same viral stock as in Fig. 5B gave a titer of 10^4. Therefore, the direct titration of virus by the β-gal assay is as sensitive as the assays based on amplification of lymphoid cell lines in culture.

The stability of HIV-1 in culture medium at 37°C was measured (Fig. 5E). The half-life of the virus is 6.5 h.

Detection and titration of related retrovirus. The LTR of HIV-1 can be transactivated by the product of the *tat* gene of several human and simian retroviruses. HIV-2 (ROD isolate) and SIV_{agm5}, SIV_{agm14}, SIV_{mac}, and HTLV-1 stocks have therefore been used to infect HT4LacZ-1. All viruses except HTLV-1 gave blue syncytia. A more intense staining was obtained with the control HIV-1, but all were easily detected. This observation is in agreement with the finding that the HIV-2 *tat* transactivates the HIV-1 LTR less efficiently than does HIV-1 *tat* (9). It also suggests that SIV_{agm} transactivates the HIV-1 LTR at about the same level as does HIV-2. In addition, the mean size of SIV-induced syncytia

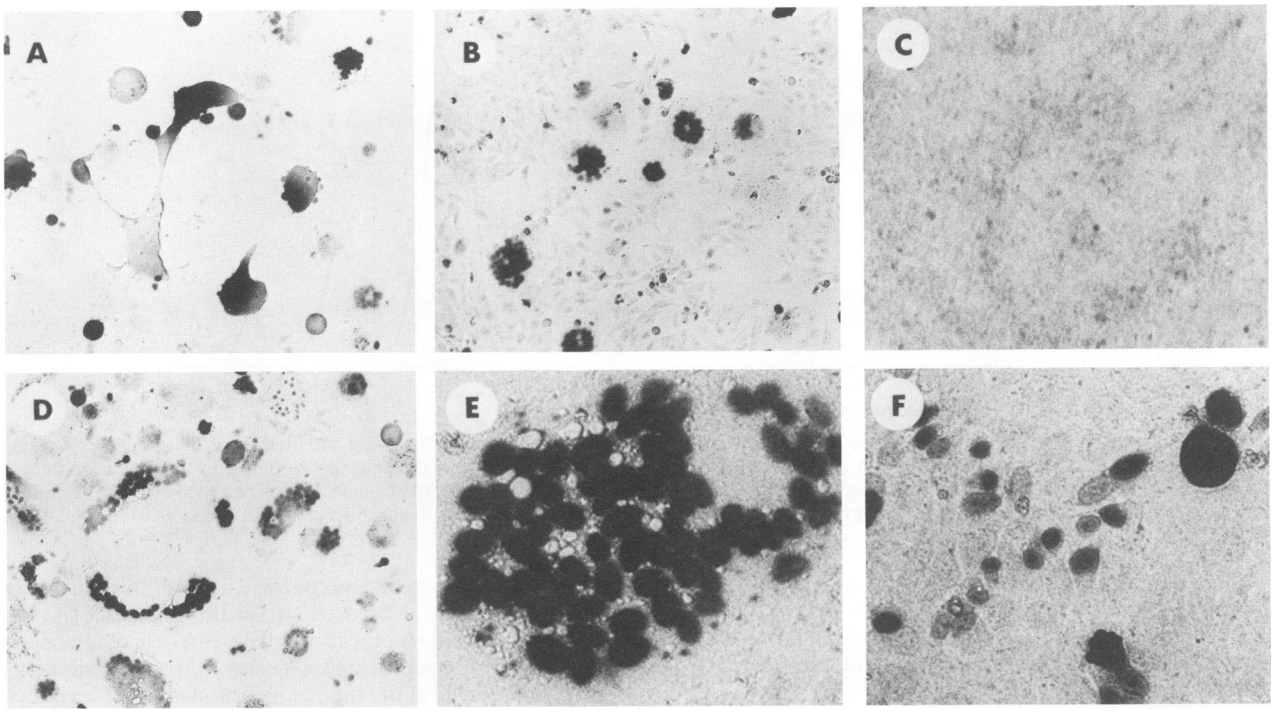

FIG. 4. HIV and HIV-infected cells detected by the β-gal assay. (A) Syncytia induced by transfection with pKP125 (note the gigantic syncytia). (B) HT4LacZ-1 3 days after infection by HIV-1 (note the elevated β-galactosidase activity in syncytia and the absence of detectable background). (C) Control plate for panel B. AZT (10 μM) was added to the cells at the time of infection (no β-galactosidase-positive syncytia). (D) HT4LacZ-1 3 days after cocultivation with PHG-PBL-infected cells in presence of 10 μg of AZT per ml (note the larger sizes of β-galactosidase-positive syncytia than those in panel B). (E) High magnification of a blue syncytium induced by HIV-1. (F) Syncytia and clusters of β-galactosidase-positive cells induced by SIV. In all cases, the fixed cells were stained with X-Gal for 30 min at 37°C.

(Fig. 4F) is lower than with HIV-1 and HIV-2, although they are all produced on SupT-1. Finally, the failure to detect HTLV-1 by cocultivation of HT4LacZ-1 with the C10-MJ2 producer line suggests an inability of HTLV-1 to infect and/or transactivate the HIV-1 LTR.

By using the same protocol as with HIV-1, the titer of viral stocks was deduced from the curves presented in Fig. 5B. In all cases, the demonstration that the number of β-galactosidase-positive syncytia corresponds to retroviral infection was confirmed by the absence of foci in control wells in which 10 μM of azidothymidine (AZT), an inhibitor of reverse transcription (24), was added (Fig. 7A). Other inhibitors such as CD4-Mμ (31) can also be used (Fig. 7B).

Detection and titration of HIV-infected cells. Lymphoid cells infected by HIV express the envelope gene products gp41 and gp120 on their surfaces. They are involved in the formation of syncytia by cell-to-cell fusion together with the viral receptor CD4 (7, 20). Because cell-to-cell transmission of HIV presumably has a role in the progression of AIDS, it is important to have an assay to detect cells involved in this kind of spread of infection and to evaluate the efficiency of drugs in vitro.

To determine whether infected cells can induce cell-to-cell HIV transmission to HT4LacZ-1, chronically HIV-1-infected SupT-1 and HT4LacZ-1 cells were cocultivated. AZT was present at all times at 10^{-5} M in the culture under conditions which inhibit the contribution to syncytium formation of free virions produced by the cells (Fig. 7A; 24). X-Gal staining was performed at different time intervals during a 5-day period. The maximum sensitivity was obtained between 40 and 60 h. An example of titration of HIV-1-infected SupT-1 cells is presented in Fig. 5C. There is a direct correlation between the number of SupT-1 cells and β-galactosidase-positive syncytia up to 10^3 β-galactosidase-positive foci per well (Fig. 5C). Therefore, the test can be used to calculate the number of cells capable of fusing with and transactivating HT4LacZ-1. With this chronically HIV-1-infected SupT-1 preparation, about 20% of the SupT-1 cells were detected positive. The fact that these syncytia are resistant to the action of AZT clearly indicates that the transactivation of HT4LacZ-1 occurs without previous requirement for reverse transcription and most probably involves the transfer of HIV DNA, *tat* RNA, or Tat by heterologous cell-to-cell fusion.

A comparison of the titers obtained on HT4LacZ-1 infected with HIV-1 and HT4LacZ-1 cocultivated with SupT-1 cells infected 4 days before with the same virus dilution was done to compare the sensitivity of both assays. Direct titration on HT4LacZ-1 and indirect titration via SupT-1 gave similar titration curves (Fig. 5D). This result is in agreement with the experiment showing that the β-gal assay has the same sensitivity as the amplification-based assays described above.

To determine when lymphoid cells can be detected after infection and when they start to produce virus, the culture supernatant of stimulated human PBLs (PHA-PBL) was checked for virus at daily intervals after an infection by HIV-1. PHA-PBL produced a detectable quantity of virus after only 70 h at an initial multiplicity of infection of 10^{-4} and after 50 h at an initial multiplicity of infection of 10^{-3} (Fig. 8). Infected PHA-PBL cells were also detectable early

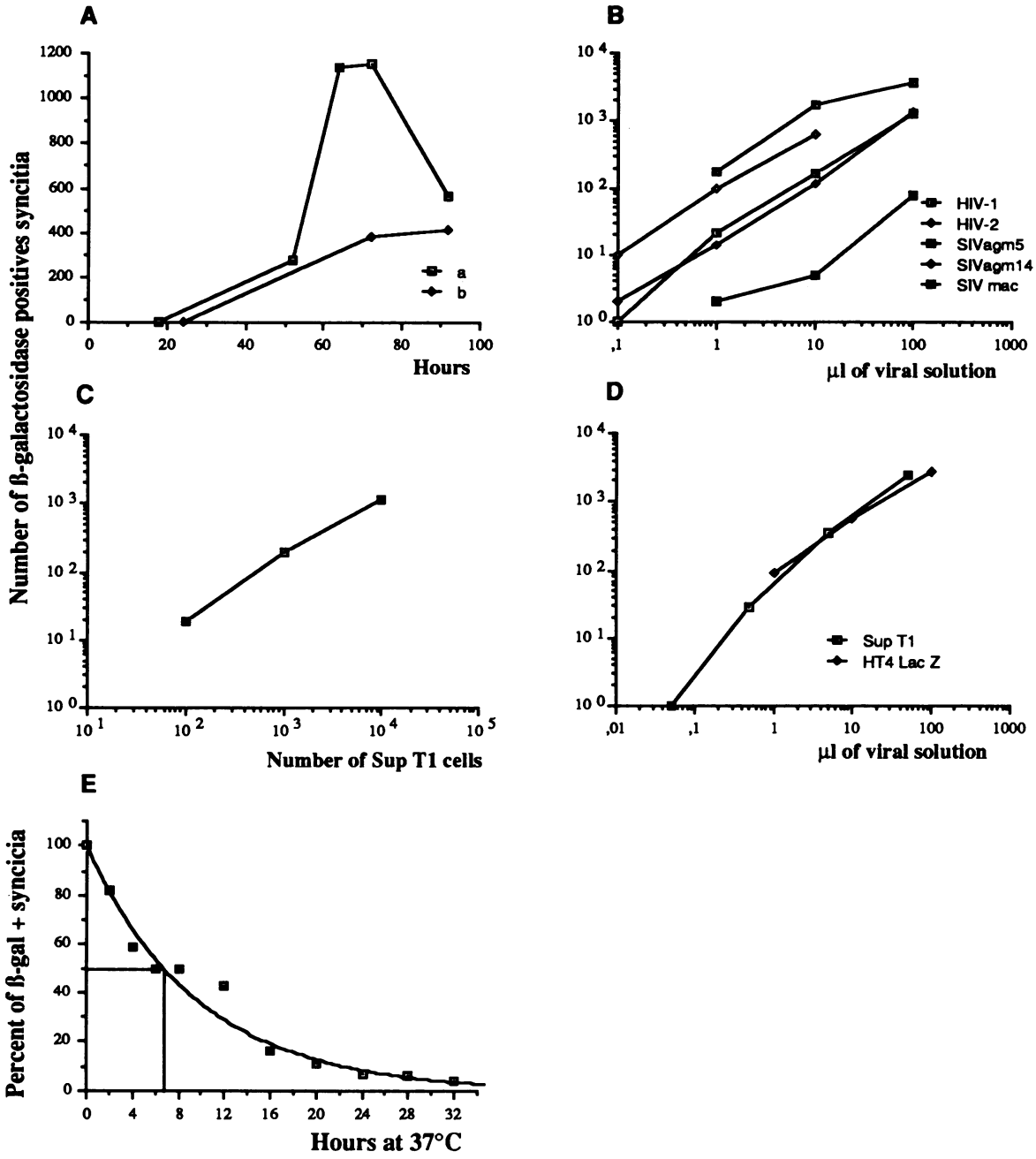

FIG. 5. Titration curves of HIV, SIV, and HIV-infected cells. (A) Time-response curve of HT4LacZ-1 to viral infection. At time intervals after the initial infection by HIV-1 (BRU), HT4LacZ-1 cells were stained with X-Gal and the number of transactivated syncytia was measured. Infection was with 30 (a) and 10 (b) µl of HIV solution. (B) Titration curves of HIV-1 (BRU isolate), HIV-2 (ROD isolate), SIV_{agm5}, SIV_{agm14}, and SIV_{mac} stocks. (C) Titration curve of HIV-1-infected SupT-1 cells in presence of 10 µM AZT and no DEAE-dextran. (D) Comparison of direct titration on HT4LacZ-1 and indirect titration with SupT-1 cells infected 4 days before being cultivated with HT4LacZ-1 in the presence of 10 µM AZT. (E) Half-life of HIV-1 (BRU) at 37°C. The virus was maintained at 37°C for 32 h in culture medium. At intervals of 2 h, 50-µl samples were used to infect HT4LacZ-1. The result is presented as the percentage of β-galactosidase-positive syncytia in the viral stock at time zero. The 50% decrease is at 6.5 h.

after infection. SupT-1 was found to be less sensitive than PHA-PBL.

In conclusion, lymphoid cells infected by HIV-1 (PHA-PBL and SupT-1) are detected following cell-to-cell transmission of HIV in the β-gal assay as soon as 2 to 3 days after the infection. This constitutes the basis of an indirect titration assay using PHA-PBL or SupT-1. In addition, these cells produce enough virus 3 to 4 days after the infection to be detected in the supernatant by the direct titration of virus by the β-gal assay.

Quantification of the effect of antiretroviral compounds. To illustrate how HT4LacZ-1 can be used in a rapid and sensitive screening of antiretroviral compounds (24), an inhibitor of the reverse transcription step of the viral cycle

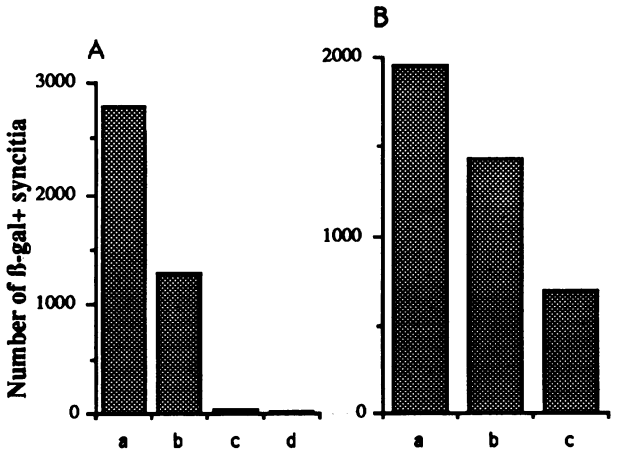

FIG. 6. Effects of polycations and HT4LacZ-1 density on HIV-1 infectivity. (A) HIV-1 was added to HT4LacZ-1 in presence of 10 μg of DEAE-dextran per ml in absence of polycations (a) or in presence of 2 (c) or 10 (d) μg of Polybrene per ml. The number of β-galactosidase-positive syncytia was counted 3 days later. (B) HIV-1 was added to HT4LacZ-1 plated the day before at 1.5×10^5 (a), 0.5×10^5 (b), or 2.5×10^4 (c) cells per cm^2. The number of β-galactosidase-positive syncytia was measured 3 days later.

(AZT) and inhibitors of the viral entry (the CD4 molecule produced as a CD4-immunoglobulin chimera [31]) were tested. Two days later, the 50% inhibitory dose was calculated from the number of *lacZ*-transactivated syncytia and found to be similar to the ones obtained by other methods (10^{-7} M for AZT [24], 10 μg/ml for CD4-Mγ2a, and less than 1 μg/ml for CD4-Mμ [31]) (Fig. 7A and B).

DISCUSSION

The HT4LacZ-1 cell line was selected after transfection with HIVSVnlsLacZtat DNA. HT4LacZ-1 is a clone which had a low level of β-galactosidase expression and did not undergo spontaneous fusion. Because the *lacZ* gene in HT4 is susceptible to transactivation by Tat and the cells undergo syncytium formation in the presence of envelope proteins, HT4LacZ-1 is an appropriate indicator cell for HIV infectivity. The integrated copy kept intact the HIV 5′ LTR and the SVnlsLacZ gene. The *env-tat* region of the molecule was deleted from the second *Bgl*II site (Fig. 1), making the genes of this region inactive. As we did not succeed in developing a highly transactivatable line with a construct in which nlsLacZ is under the control of only the HIV-1 LTR (HIVnlsLacZ [4]), we postulate that the internal promoter which maintains permanent expression of *lacZ* may be required for stably transfected cell lines. This hypothesis is in accordance with the demonstration that HT4LacZ lines which derive from a construct in which the *env-tat* region was deleted exhibit all the properties of HT4LacZ-1. There is no clear explanation for this requirement.

Because of the presence of the internal promoter, HT4LacZ-1 exhibits β-galactosidase background activity. This background is undetectable when the assay is done at a cell density of $1.5 \times 10^5/cm^2$. As the detection of the viral transactivation is obvious after only 10 to 30 min of X-Gal staining for both HIV and SIV, the signal-to-noise ratio is still very high. A similarly high level of Tat transactivation in human cells has been obtained in transient assays (for a review, see reference 32).

HT4LacZ-1 can be used to detect HIV-1, HIV-2, SIV_{mac}, SIV_{agm5}, and SIV_{agm14}. Cells which are infected with HIV-1 and HIV-2 (and presumably the other viruses) can be similarly detected. The β-gal assay measures the number of viral particles capable of entering HT4LacZ-1 cells and executing all of the steps of the viral life cycle through integration and expression of the *tat* gene (for transactivation of nlsLacZ). When syncytium formation is observed, it shows that, in addition, the envelope gene is expressed and processed. More generally, any retrovirus capable of infecting HT4LacZ-1 and transactivating the HIV-1 LTR region would be detected. AZT, CD4 (this paper), or neutralizing antibodies (19) can be used as a control for specificity for virus, as it decreases to zero the number of β-galactosidase-positive syncytia. These results suggest that the test is suitable for direct assays of human or monkey samples. In

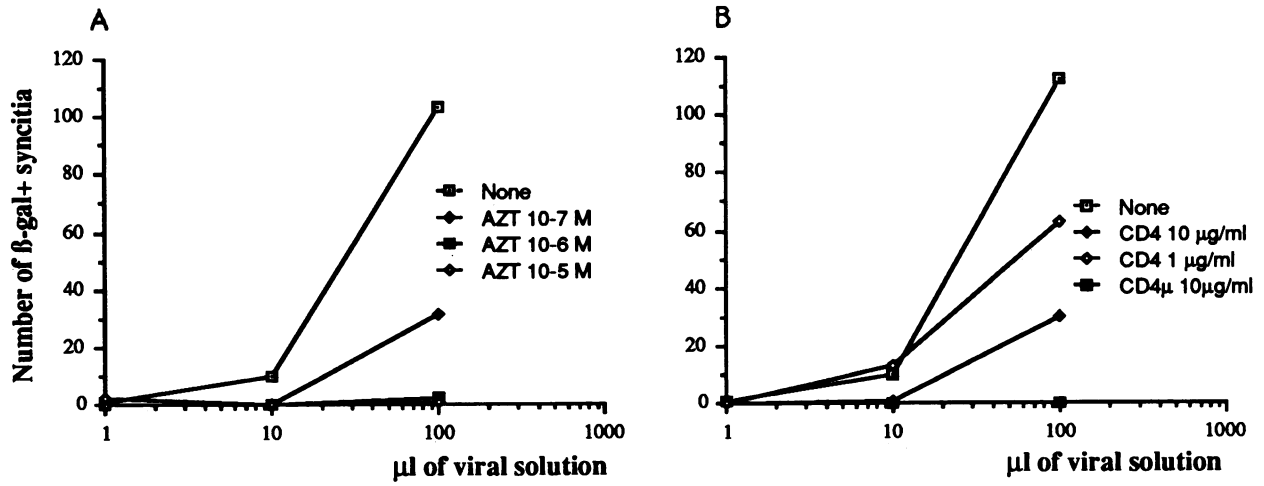

FIG. 7. Dose-response curves of antiretroviral drugs upon HT4LacZ-1 infection by HIV-1. (A) AZT (10^{-5} to 10^{-7} M, final concentration) was added at the time of infection with HIV-1. (B) CD4-Mγ2a (1 and 10 μg/ml, final concentrations) and CD4-Mμ (1 μg/ml) were preincubated for 1 h with HIV-1, and the virus-containing solution was next incubated with HT4LacZ-1 and no DEAE-dextran. The number of β-galactosidase-positive syncytia was counted 3 days later.

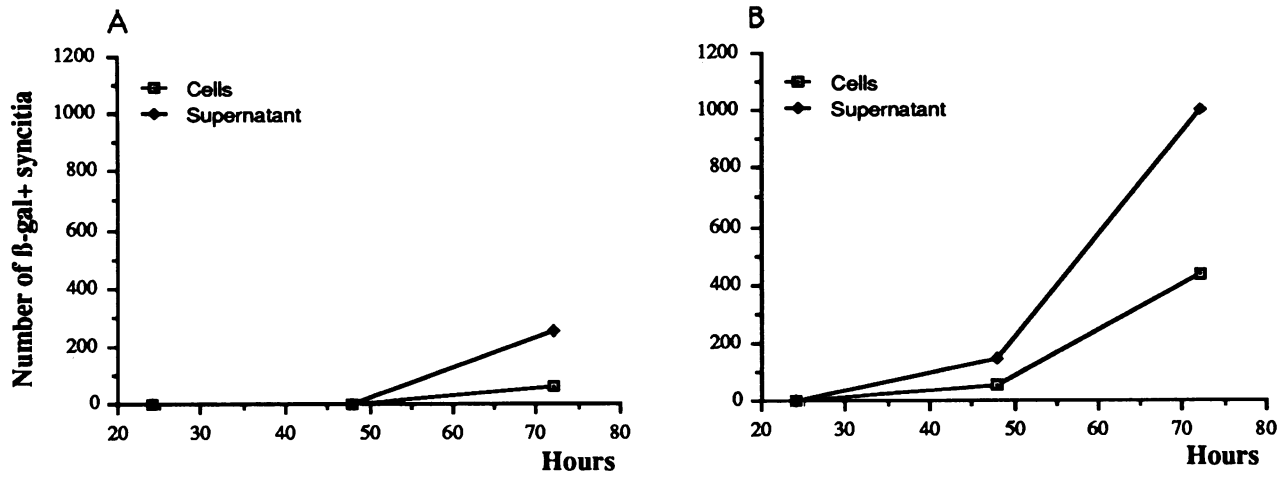

FIG. 8. Kinetics of virus production of HIV-1 by PHA-stimulated human PBLs. Cells were infected by HIV-1 (BRU) at a multiplicity of 10^{-4} (A) or 10^{-3} (B). Samples of supernatant (50 μl) depleted of cells by centrifugation (Supernatant) or 1/20th of the original 10^6 cells (Cells) were titrated at the indicated time intervals. Titration of the PHA-PBL cells was in presence of 10 μM AZT.

addition, it has been shown that HT4 is effective for detection of most wild-type HIV isolates (5).

The detection assay is rapid (60 h) compared with reverse transcriptase assays or $p24^{gag}$ antigen detection, which necessitates a week of previous amplification. Compared with other plaque assays (14, 22, 25), it is unambiguous, since it combines the criteria of syncytium formation and the specific transactivation of *lacZ*. Compared with other methods based on the utilization of reporter molecules not detectable in single cells (12), it gives the number of particles (or cells) capable of transactivating *lacZ* and therefore is more suitable for quantitative analysis. The sensitivity of the assay is very high, as the titers of particles obtained with HT4LacZ-1 are identical or even slightly higher than the titers obtained by the 7-day amplification-reverse transcription method on SupT-1 which detects replication-competent particles. The assay is similar to the immunofocal detection of Chesebro and Wehrly (5), but it is easier to perform as it overcomes the difficulties inherent to immunofluorescence and circumvents the need for specific antisera. In addition, the X-Gal assay itself is done in only a few minutes, as is the screening of an entire well. Therefore, it is particularly adapted for large-scale experiments and screening. Finally, the assay gives specific information not obtained by other methods, such as the sizes of the syncytia (compare the syncytia of HIV to those of SIV [Fig. 4F]) and an estimate of the level of transactivation.

Three assays for the detection of transactivating virus or provirus in cells have been presented. They are as follows: (i) direct titration of viral particles, (ii) direct titration of cells producing the virus and/or expressing a provirus, and (iii) indirect detection of the virus following a passage on a highly infectable intermediary cell (SupT-1 and PHA-stimulated human PBLs). This last assay may circumvent restrictions due to low infectivity on HT4LacZ-1 of certain HIV or SIV variants (although HT4 is very potent in detecting wild-type HIV isolates [5]). The titration of virus or cells follows one-hit kinetics, indicating that a single viral particle or cell is sufficient to obtain transactivation of integrated *lacZ*. The ease of the assay allows the rapid obtaining of parameters of the viral cycle otherwise difficult to derive. For instance, we found that HIV-1 has a half-life of 6.5 h at 37°C and that PHA-stimulated human PBLs produce retroviruses only 1 to 2 days after infection. Furthermore, clear demonstration of cell-to-cell transfer of HIV on which AZT has no effect was obtained. Therefore, the β-gal assay may be particularly adapted to the detection of cells involved in this manner of spreading the virus, as well as to the testing of inhibitory compounds.

Uses of HT4LacZ-1 include the following: (i) a rapid test for antiretroviral drugs capable of interfering with virus entry, maturation of the particle, integration with syncytium formation, transactivation and envelope processing, and cell-to-cell transmission of HIV; (ii) the survey of HIV infections in serum samples and lymphocytes from seropositive individuals during the progression of the disease; (iii) titration of viral interfering substance present in serum samples of patients by inhibition of HT4LacZ-1 infection by HIV; (iv) the survey of therapeutic protocols and immunization in patients and in the monkey model system using SIV_{mac} or SIV_{agm}; (v) the rapid characterization of a virus modified in the laboratory; and, finally, (vi) the possibility of detecting living β-galactosidase-positive cells and of selecting them by fluorescence-activated cell sorting (26) or visually, thus facilitating plaque purification from fresh isolates before selection of HIV and SIV variants.

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