Detection of human immunodeficiency virus type 1 after infection of unstimulated peripheral blood mononuclear cells

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Application of a highly sensitive PCR-based reverse transcriptase (RT) assay to the analysis of the infection of CD4+ cell lines with human immunodeficiency virus type 1 (HIV-1) demonstrated that virus production can be detected as early as 24 h after infection. Most of the signal at 24 h was due to virus production, as it could be substantially reduced by prior treatment with the RT inhibitor zidovudine. Virus production at 24 and 48 h was unaffected by the protease inhibitor indinavir. Infection of unstimulated peripheral blood mononuclear cells (PBMC) with a macrophage-tropic HIV-1 isolate yielded increasing virus production for 2–3 weeks, while infection with a T-cell line-tropic isolate yielded only low and sporadic virus production. Productive infection of unstimulated PBMC by the macrophage-tropic virus required functional Gag matrix and Vpr proteins; therefore, the monocyte-derived macrophage is probably the virus-producing cell in these cultures.

Studies on human immunodeficiency virus type 1 (HIV-1) replication in vitro most commonly use a p24 antigen ELISA or reverse transcriptase (RT) assay to quantify virus released into the culture supernatant from infected cells. The p24 assay is capable of detecting HIV-1 between the pg and ng range, while conventional RT assays are about 10 to 100 times less sensitive. Several highly sensitive RT assays have been described that are approximately 104-fold more sensitive than conventional RT assays (Heneine et al., 1995; Pyra et al., 1994; Silver et al., 1993). These assays rely on the ability of a retroviral RT to copy an exogenous RNA template of known sequence into a complementary DNA (cDNA). This cDNA is then amplified by PCR, and the PCR product can be detected and quantified. These assays have the potential to detect a single retroviral virion (Heneine et al., 1995; Maudru & Peden, 1997; Pyra et al., 1994; Silver et al., 1993) and thus should be ideal to detect low levels of any type of retrovirus. We have used a PCR-based RT (PBRT) assay to determine whether the virus produced from a single round of infection of CD4+ cell lines could be detected. In addition, we have used the assay to see if virus production could be detected after infection of unstimulated peripheral blood mononuclear cells (PBMC).

PM1 cells (Lusso et al., 1995), CEMx174 (Salter et al., 1985), Jurkat cell clone E6-1 (Weiss et al., 1984) and Jurkat-CCR5 (Alkhatib et al., 1996) were maintained in RPMI-1640 growth medium supplemented with 10% (v/v) foetal bovine serum (RPMI-10). Stocks of the T-cell line (TCL)-tropic viruses HIV-1 LA1 (Peden et al., 1991) and HIV-1 NL4-3 (Adachi et al., 1986) and the macrophage (M)-tropic virus HIV-1 AD (Theodore et al., 1996) and its mutants were prepared by transfection (Peden & Martin, 1995) of human 293 cells with proviral molecular clones. The plasmid pADvpr1, a Vpr mutant derivative of HIV-1 AD, has been described (Theodore et al., 1996). The matrix (MA) mutants of HIV-1 AD were constructed by replacing the BssHI–SphiI fragment of pAD, the molecular clone of HIV-1 AD, with the corresponding one from pLAI MA1 (Bukrinsky et al., 1993) to produce pAD-MA1. The MA1 and Vpr double mutant was constructed by replacing the MA1 BssHI–SphiI fragment in pADvpr1 to produce pADvpr1.MA1.

Cells (2 × 105 to 8 × 105 per ml) were infected in 200 μl with virus corresponding to between 2 × 103 to 8 × 104 c.p.m. standard RT activity (Peden & Martin, 1995) depending on the experiment; this corresponds to approximately m.o.i. of 0.002 to 0.01, respectively. Virus adsorption was for 2 h at 37 °C, after which the cells were diluted in RPMI-10 and washed three times by centrifugation for 5 min at 250 g at room temperature followed by resuspension in RPMI-10. Cultures were established by adding 2 × 105 infected cells in 1 ml RPMI-10 to a well of a 24-well tissue culture plate. The plates were centrifuged for 5 min at 250 g at room temperature before 100 μl supernatant samples were collected. The remaining culture medium was then removed and replaced with 1 ml fresh medium. Cultures were maintained at 37 °C, cultures were sampled and the medium was replaced daily; supernatant samples were stored at −40 °C.

Virus production was measured by a conventional RT (Peden & Martin, 1995) or by a PBRT assay (Maudru & Peden, 1997) except that the read-out was modified. Amplified products of the PCR were subjected to electrophoresis in 2%
PM1 cells (2 x 10^5 cells) were infected with HIV-1_LAI at an approximate m.o.i. of 0.1. Samples were taken at initiation of infection (lane a) and daily for the next 8 days (lanes b–i). Lanes j–o contain 4-fold increasing amounts of AMV RT (1, 3, 9, 15, 6, 62, 5, 250 and 1000 nU, respectively). (B) Infection kinetics of HIV-1_LAI in PM1 cells in the absence [square brackets] or presence of the antiretroviral drugs zidovudine (10 µM; Glaxo Wellcome) and indinavir (1 µM; Merck), added immediately after the zero time-point sample was taken and for the next 5 days. RT activity increased in parallel with that in the untreated culture for the first 2 days but then decreased (Fig. 1 B). At no time was RT activity detected in the supernatants of mock-infected cell cultures.

To ascertain whether the PBRT assay can be used with different HIV-1 strains and cells, three CD4+ cell lines were infected with two TCL-tropic viruses (HIV-1_LAI and HIV-1_NL4-3) and one M-tropic virus (HIV-1_AD). Both HIV-1_LAI and HIV-1_NL4-3 were able to establish productive infections in all three T-cell lines, as expected for TCL-tropic viruses (Fig. 2). In PM1 and CEMx174 cells (Fig. 2 A and B, respectively), HIV-1_NL4-3 replicated to higher levels than HIV-1_LAI. In Jurkat cells, replication of both TCL-tropic viruses was similar (Fig. 2 C). In contrast, the M-tropic HIV-1_AD only established a productive infection in PM1 cells (Fig. 2 A). These results agree with published reports with these cell lines and analogous viruses and were qualitatively similar to those obtained with conventional RT assays (data not shown).

Because the infections of mitogen-stimulated PBMC and T-cell lines in vitro are unlikely to be equivalent to infection of the resting or partially activated CD4+ lymphocytes in vivo, we tested whether virus production following infection of PBMC in the absence of mitogen stimulation could be detected by the PBRT assay. PBMC were infected with the TCL-tropic LAI strain of HIV-1 or the M-tropic AD strain, and virus production was assessed by PBRT assay of the culture medium. A small amount of LAI production was detected, whereas infection with the M-tropic HIV-1 strain AD produced increasing amounts of RT activity over time (Fig. 1 B). In the presence of the HIV-1 protease inhibitor indinavir (1 µM; Merck), added immediately after the zero time-point sample was taken and for the next 5 days, RT activity increased in parallel with that in the untreated culture for the first 2 days but then decreased (Fig. 1 B). At no time was RT activity detected in the supernatants of mock-infected cell cultures.

To determine the utility and sensitivity of the PBRT assay in quantifying virus production after infection, cells were infected with HIV-1_LAI, a TCL-tropic virus, at an approximate m.o.i. of 0.01. Following virus adsorption, the cells were washed three times to remove input virus. The PBRT assay detected increased virus in the cell culture medium as early as 24 h after infection when compared with the zero time-point (Fig. 1 A, lanes a and b). Virus production increased on ensuing days, peaking between days 3 and 5 (Fig. 1 A, lanes c–f) before decreasing. These results are presented graphically in Fig. 1 (B). On the peak days of virus production, the amount of RT activity is above the linear range of the PBRT assay.

To be sure that this increase in RT was due to synthesis of virus rather than the release of bound input virus from the cells over time, antiretroviral drugs were employed. The potent HIV RT inhibitor zidovudine (10 µM; Glaxo Wellcome) was added 1 h prior to infection and was maintained in the culture for 5 days. In the presence of zidovudine, there was no increase in RT activity over time (Fig. 1 B). In the presence of the HIV-1 protease inhibitor indinavir (1 µM; Merck), added immediately after the zero time-point sample was taken and for the next 5 days, RT activity increased in parallel with that in the untreated culture for the first 2 days but then decreased (Fig. 1 B). At no time was RT activity detected in the supernatants of mock-infected cell cultures.

Fig. 1. Infection of PM1 cells with HIV-1_LAI. (A) Agarose gel analysis of the PBRT assay product from AMV RT dilutions and supernatants of HIV-1-infected cells. PM1 cells (2 x 10^5 cells) were infected with HIV-1_LAI at an approximate m.o.i. of 0.1. Samples were taken at initiation of infection (lane a) and daily for the next 8 days (lanes b–i). Lanes j–o contain 4-fold increasing amounts of AMV RT (1, 3, 9, 15, 6, 62, 5, 250 and 1000 nU, respectively). (B) Infection kinetics of HIV-1_LAI in PM1 cells in the absence [square brackets] or presence of the antiretroviral drugs zidovudine (10 µM; Glaxo Wellcome) and indinavir (1 µM; Merck), added immediately after the zero time-point sample was taken and for the next 5 days) measured by the PBRT assay. The amount of RT activity was calculated from a standard curve.
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Fig. 2. Infection of T-cell lines with HIV-1LAI (●), HIV-1NL4-3 (△) or HIV-1AD (●). Cells were infected and the amount of virus produced on the ensuing days was determined by the PBRT assay. (A) PM1 cells (2 × 10⁴) were infected with HIV-1AD (5 × 10⁴ c.p.m. RT activity), HIV-1LAI (5 × 10⁴ c.p.m. RT activity) or HIV-1NL4-3 (1.25 × 10⁵ c.p.m. RT activity). (B) CEMx174 cells (2 × 10⁵) were infected with HIV-1AD, HIV-1LAI or HIV-1NL4-3 (each with 2 × 10⁵ c.p.m. RT activity). (C) Jurkat cells (2 × 10⁵) were infected with HIV-1AD, HIV-1LAI or HIV-1NL4-3 (each with 1.25 × 10⁵ c.p.m. RT activity).

Fig. 3. Infection of unstimulated PBMC and Jurkat-CCR5 cells. PBMC were obtained from healthy donors and purified by centrifugation through a Ficoll–Paque (Pharmacia Biotech) cushion; before use, they were washed twice with PBS and once with RPMI-10. (A) PBMC (8 × 10⁵ cells) were infected without mitogenic stimulation with the T-cell line-tropic LAI strain (■) or the M-tropic AD strain (■) of HIV-1 (8 × 10⁴ c.p.m. RT activity for each virus). Virus production was followed using the PBRT assay. (B) The effect of mutations in the MA, vpr or both MA and vpr genes of HIV-1AD on the ability to establish a productive infection in unstimulated PBMC. Unstimulated PBMC were infected with AD (■), ADvpr1 (△), AD.MA1 (○) or ADvpr1.MA1 (▲) (4 × 10⁴ c.p.m. RT activity for each virus). Virus production was followed using the PBRT assay. (C) Infection of Jurkat-CCR5 cells with AD (■), ADvpr1 (△), AD.MA1 (○) or ADvpr1.MA1 (▲) (2 × 10⁴ c.p.m. RT activity for each virus for 2 × 10⁶ cells). Virus production was measured in the culture medium by an RT assay (Peden & Martin, 1995).

amounts of RT activity in the culture supernatant for several days (Fig. 3 A). PBMC from each of four different donors gave similar results, and zidovudine eliminated the production of virus (data not shown).

The amounts of RT activity even in the AD infections of unstimulated PBMC were considerably lower than those found in the supernatants of mitogen-stimulated PBMC, which were detectable with conventional RT assays (Theodore et al., 1996; data not shown).

The difference between LAI and AD strains in their ability to establish a productive infection in unstimulated PBMC is probably related to the phenotypes of these viruses. Because freshly isolated PBMC are resistant to the establishment of a productive infection by HIV-1, although they become susceptible to infection as they differentiate in culture to macrophages (Sonza et al., 1996), and because viruses that can infect macrophages can also infect CD4 lymphocytes but not the converse, we predicted that it was the differentiation of the monocytes in the PBMC to macrophages during culture that provided the permissive cells for the M-tropic virus. To test this hypothesis, mutants known to have reduced capacity to infect monocyte-derived macrophages (MDM) were tested for their ability to establish productive infections in the cultures of unstimulated PBMC. Mutations in either the MA protein or
the Vpr protein have been shown to decrease the ability of M-tropic viruses to infect MDM (Bukrinsky et al., 1993; Heinzinger et al., 1994) and the double mutant is severely impaired (Connor et al., 1995; Freed et al., 1995; Freed & Martin, 1994; Heinzinger et al., 1994; von Schwedler et al., 1994).

Unstimulated PBMC were infected with AD, AD.MA1, ADpbr1 or ADpbr1.MA1. Virus production was substantially reduced by inactivation of either the MA gene or the vpr gene, and was further reduced after removal of both genes (Fig. 3 B). Nevertheless, these same mutants were able to infect dividing cells, for example Jurkat-CCR5 cells, although the mutations decreased the replication capacity (Fig. 3 C).

These results are consistent with the following scenario. Although quiescent CD4 lymphocytes are resistant to the establishment of a productive HIV-1 infection (Bukrinsky et al., 1991; Zack et al., 1990), PBMC isolated from normal individuals will always include a small proportion of in vivo-activated lymphocytes. Activated normal CD4 lymphocytes have on their surface some of the second receptors needed by both TCL-tropic and M-tropic isolates of HIV-1 and are permissive for both types of virus (Moore, 1997). These cells are probably the source of virus produced early in both the HIV-1AD and HIV-1LAI cultures and then sporadically in the HIV-1LAI culture. During cultivation, the monocytes adhere to the surface and differentiate into macrophages. MDM have a co-receptor used by M-tropic but not TCL-tropic viruses, most likely CCR5, which suggests that the expansion of virus seen in the HIV-1AD cultures is due to virus replication in macrophages.

In conclusion, the PBRT assay is sensitive enough to detect early events following HIV infection in culture and can also be used to measure virus production under conditions that may be more similar to those in vivo.

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