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High SDF-1 Expression in HIV-1 Carriers Does Not Correlate with CD8$^+$ T-Cell-Mediated Suppression of Viral Replication

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Stromal cell-derived factor 1 (SDF-1) inhibits T-cell tropic (T-tropic) HIV-1 infection *in vitro*. In this study, we examined the regulatory role of SDF-1 on HIV-1 replication in peripheral blood mononuclear cells (PBMC) of HIV-infected individuals. We found that the amount of SDF-1 mRNA in freshly isolated PBMC of HIV-1 carriers was higher than in healthy donors. Moreover, PBMC from some asymptomatic carriers (ACs) exhibited high levels of SDF-1 mRNA expression. The level of SDF-1 expression in PBMC did not correlate with the magnitude of CD8$^+$ T cell-mediated suppression of HIV-1 among ACs. SDF-1 inhibited HIV-1 replication at the viral entry step, whereas a single-cycle HIV-1 infection system showed that the major part of the CD8$^+$ T-cell-mediated suppression occurs after intracellular penetration of the virus. Our results suggest that SDF-1 acts as a suppressor of virus replication in a CD8$^+$ T-cell-independent mechanism in HIV-infected individuals.

Key Words: AIDS; asymptomatic carriers; latent period; viral replication; HIV-1 carriers.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), causes a slow and progressive destruction of the immune systems and a long asymptomatic latent period extending over 10–15 years (Fauci, 1993). The mechanisms involved in the long asymptomatic latent period are not fully understood, but probably include host immune responses against the virus, such as cytotoxic T lymphocytes (CTLs), neutralizing antibodies, and antibody-dependent cellular cytotoxicity (Weiss *et al.,* 1986; Ljunggren *et al.,* 1987; Ojo-Amaize *et al.,* 1987; Walker *et al.,* 1987).

In addition to these antiviral responses, a number of studies have shown that CD8$^+$ T cells from HIV-infected individuals are capable of suppressing viral replication in a noncytolytic, MHC-nonrestricted manner (Walker *et al.,* 1986; Levy *et al.,* 1996). This CD8$^+$ T-cell-mediated suppression is effective against multiple laboratory HIV-1 strains, indicating that CD8$^+$ T cells of asymptomatic carriers (ACs) may suppress HIV-1 replication regardless of the dominant HIV-1 strain *in vivo* (Kannagi *et al.,* 1990). A similar CD8$^+$ T-cell-mediated suppression of viral replication has also been detected in SIV-infected macaques and HIV-1-infected chimpanzees (Kannagi *et al.,* 1988; Castro *et al.,* 1991).

Two host cell molecules are required for HIV-1 entry into the cells: the first is CD4, while the other is a member of chemokine receptors (Sattentau and Weiss, 1988; Feng *et al.,* 1996). CD4 mediates the binding of the virus to cells, and chemokine receptors are thought to be involved in the virus entry process into cells. Two types of viruses have been identified based on the host infected cells (cell tropism), such as macrophage-tropic (M-tropic) virus and T-cell-tropic (T-tropic) virus. Cell tropism is determined by the chemokine receptor used by the respective virus. Chemokine receptor CC-CKR5 and fusin/CXCR4 act as entry cofactors for M-tropic virus (Al-Khatib *et al.,* 1996; Bleul *et al.,* 1996a; Deng *et al.,* 1996; Dragic *et al.,* 1996) and T-tropic virus (Feng *et al.,* 1996), respectively. Consistent with the chemokine receptor usage, the ligands of CC-CKR5, such as MIP1α, MIP1β, and RANTES, inhibit infection of cells by M-tropic HIV-1 (Cocchi *et al.,* 1995; Oberlin *et al.,* 1996). Similarly, SDF-1 inhibits infection by T-tropic virus through fusin/CXCR4 (Feng *et al.,* 1996).

SDF-1 was initially identified as a bone-marrow stromal cell-derived factor (Tashiro *et al.,* 1993), and as a pre-B-cell stimulatory factor (Nagasawa *et al.,* 1994). SDF-1 is also known as highly efficient lymphocyte chemoattractant (Bleul *et al.,* 1996b). Although SDF-1 blocks

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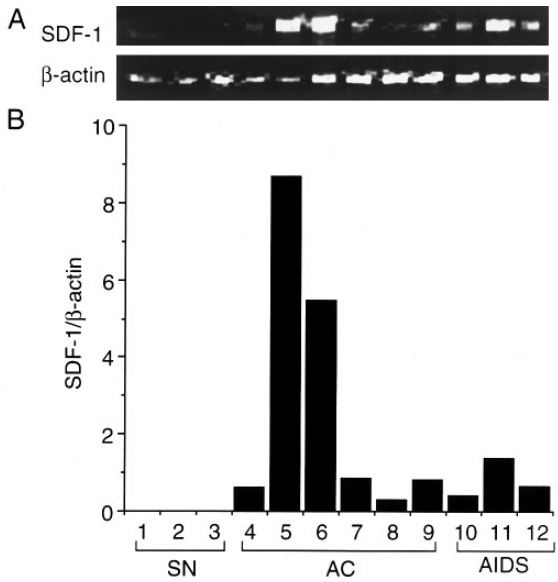

FIG. 1. SDF-1 mRNA expression in freshly isolated peripheral blood mononuclear cells (PBMC). Total RNA was prepared from PBMC freshly isolated from three seronegative subjects (SN), six asymptomatic carriers (AC), and three patients with AIDS. The expression of SDF-1 mRNA in each sample was measured semiquantitatively by RT-PCR analysis. The amount of PCR-amplified fragments separated by polyacrylamide gel was analyzed by a sequencer. (A) The visualized image of amplified fragments of SDF-1 and β-actin genes detected by a sequencer. (B) SDF-1/β-actin ratio representing the amplified SDF-1 fragment relative to that of β-actin.

the entry of T-tropic HIV-1 to cells *in vitro* (Bleul *et al.,* 1996a; Oberlin *et al.,* 1996), it remains to be determined whether SDF-1 is expressed in peripheral blood mononuclear cells (PBMC) of HIV-infected individuals and whether it actually plays a role as a suppressor of HIV-1 replication *in vivo.*

In this study, we show a high expression of SDF-1 in freshly isolated PBMC from HIV-1 carriers, especially in ACs. Our results also suggest that SDF-1 is not involved in the suppressive effect of $CD8^+$ T cells, which is the major mechanism of suppression of HIV-1 replication in ACs.

RESULTS

Enhanced expression of SDF-1 mRNA in unstimulated PBMC of asymptomatic carriers

Using the RT-PCR method, we measured the expression of SDF-1 mRNA in PBMC from HIV-infected individuals with different stages of the disease. As shown in Fig. 1, SDF-1 mRNA was not detected in PBMC of seronegative individuals (SNs). In contrast, SDF-1 mRNA expression was detected in PBMC of ACs and AIDS patients. Among the HIV-1 carriers tested, two ACs showed markedly high levels of SDF-1 expression. These results suggest that SDF-1 might act as an inhibitor of HIV-1 replication in carriers, particularly ACs.

Lack of correlation between SDF-1 mRNA expression in PBMC and inhibitory effects of HIV-1 replication

Exogenous HIV-1 replicate efficiently in phytohemagglutinin (PHA)-activated PBMC obtained from most SNs, whereas both endogenous and exogenous HIV-1 poorly replicate in PBMC of ACs. The latter inhibition of HIV-1 replication is mediated by $CD8^+$ T cells (Kannagi *et al.,* 1990). We next assessed HIV-1 suppressive activities of PBMC from ACs by examining the correlation with SDF-1 mRNA levels. In this assay, we used MHC-I mismatched $CD4^+$ and $CD8^+$ T cells to exclude CTL activity which may suppress HIV-1 replication. Consistent with previous reports, $CD8^+$ T cells from ACs exhibited significant levels of HIV-1 inhibitory activity (average suppression: 81.4%) compared with those from SNs (average suppression: 7.1%, Fig. 2A). The $CD8^+$ T cells from a representative AC inhibited HIV-1 replication more effectively in autologous than in allogenic $CD4^+$ T cells (Fig. 2B). In addition, $CD8^+$ T cells from a representative SN showed minimal levels of inhibition against both autologous and allogenic $CD4^+$ T cells, suggesting that the anti-virus activity of $CD8^+$ T cells in ACs was not due to allogenic stimulation.

Since all HIV-1 replication assays were carried out with PHA-activated PBMC, we next examined the expression of SDF-1 mRNA in PHA-stimulated PBMC together with the $CD8^+$ T-cell-mediated inhibitory activity of HIV-1 replication. As shown in Figs. 3A and 3B, all four PBMC samples from SNs expressed SDF-1 mRNA 7 days after the addition of PHA, and the level of expression was equivalent or more than that of ACs. However, $CD8^+$ T cells from only one of four SNs showed a significant inhibition of HIV-1 p24 production in $CD4^+$ T cells (Fig. 3C). In addition, case 2 of SNs had the highest level of SDF-1 expression among 8 cases tested, but had no inhibitory activity against HIV-1 production. On the other hand, p24 production by $CD4^+$ T cells was inhibited to 25% in all four ACs. In case 4 of the same group, we observed a significant level of HIV-1 inhibition with the lowest levels of SDF-1 mRNA expression. Thus, these results indicate that the expression level of SDF-1 mRNA does not correlate with HIV-1 suppressive activities in these HIV-infected individuals *in vitro.*

$CD8^+$ T cells from ACs block HIV-1 replication after virus entry

To examine the inhibitory mechanism of $CD8^+$ T cells, we used pseudotype HIV-1 carrying a luciferase gene. NLlucΔBgl is a derivative of NL-4-3, an infectious molecular clone of T-tropic HIV-1, and has a partial deletion of the envelope and nef genes, but instead has the luciferase gene. NLlucΔBgl was cotransfected into 293T cells together with an expression plasmid of the HIV-1 envelope gene derived from NL-4-3. The pseudotype virus (NLluc/LET) in a supernatant of 293T cells can

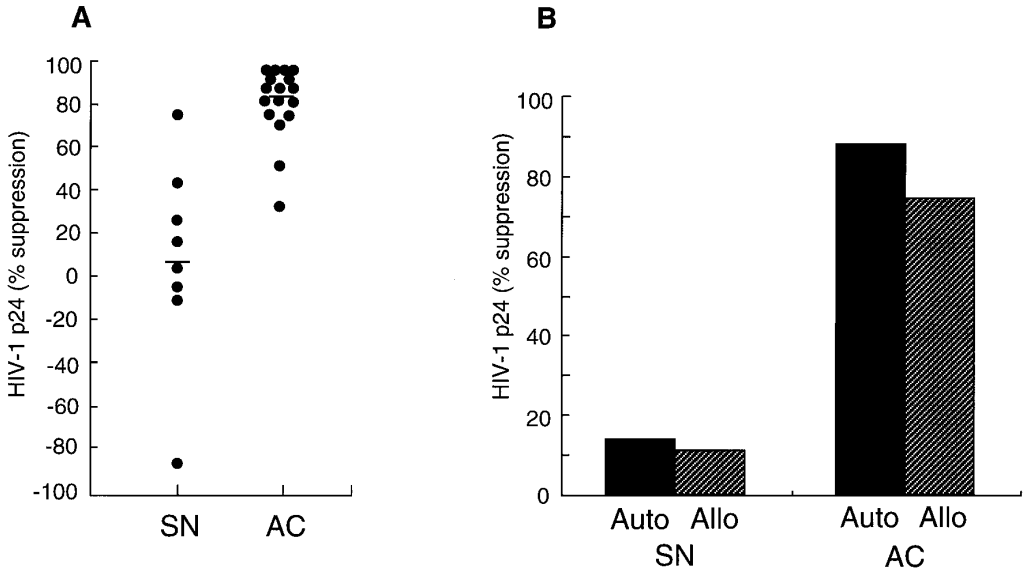

FIG. 2. HIV-1 suppressive effects of $CD8^+$ T cells derived from asymptomatic carriers (AC). (A) $CD4^+$ T cells isolated from seronegative subjects (SN) were infected with HIV-1 LAI and cocultured with $CD8^+$ T cells of SNs or ACs. The amount of HIV-1 p24 in the culture supernatant 4 days after *in vitro* HIV-1 infection was indicated as percentage suppression as described under Materials and Methods. Horizontal bars indicate the mean values of percentage suppression. The $CD8^+$ cells used in this experiment were isolated from randomly selected donors. (B) $CD8^+$ T cells from a representative AC and SN were cocultured with autologous (Auto) or allogenic (Allo) $CD4^+$ T cells exogenously infected with HIV-1 LAI. The amount of HIV-1 p24 in the culture supernatant was measured 4 days after *in vitro* HIV-1 infection and indicated as percentage suppression against controls without $CD8^+$ cells.

infect only cells in a single cycle. In addition, replication of this pseudotype virus can be monitored by measuring the luciferase activity. $CD4^+$ T cells from SN were infected with this pseudotype virus and then cultured with or without $CD8^+$ T cells from AC. We detected 4000 counts per second (CPS) of luciferase activity in $CD4^+$ T cells without $CD8^+$ T cells. However, the activity was reduced to 400 CPS following the addition of $CD8^+$ T cells prior to viral infection (90% suppression; Fig.4A, lane 3). Slightly decreased but still compatible levels of inhibition (70% suppression) of luciferase activity were also observed when $CD8^+$ T cells were mixed with $CD4^+$ T cells after viral infection (Fig. 4A, lane 2). These data indicate that $CD8^+$ T cells from ACs can effectively inhibit HIV-1 replication even after the virus binds to susceptible cells. We next used NLluc recombinant virus with a heterologous envelope protein of amphotropic MuLV. This pseudotype virus can infect cells independent of CD4 and chemokine receptors. As shown in Fig. 4B, $CD8^+$ T cells from AC inhibited the replication of this pseudotype virus by 80%. These results indicated that the inhibition of HIV-1 replication by $CD8^+$ T cells mainly occurs at the postbinding steps of the HIV-1 life cycle.

DISCUSSION

The major findings of the present study were the enhanced expression of SDF-1 mRNA in fresh PBMC isolated from HIV-infected individuals and the high levels of SDF-1 mRNA expression in some asymptomatic carriers relative to those in patients with AIDS. Since the expansion of T-tropic HIV-1 is associated with a rapid decline of $CD4^+$ T cells and progression of the disease (Tersmette *et al.,* 1989), the high levels of SDF-1 in ACs may contribute to the suppression of T-tropic HIV-1 replication. However, it is still not clear whether the levels of SDF-1 mRNA can be equated to the levels of protein and protein activity or whether the enhanced expression of SDF-1 detected in the present study is sufficient for HIV-1 suppression *in vivo.* Further studies are required to clarify these questions.

M-tropic HIV-1 plays an important role in primary HIV-1 infection. The expression of MIP1α, MIP1β, and RANTES (inhibitors of replication of the M-tropic virus) is upregulated in HIV-1 infected individuals, but there is no significant difference in expression between nonprogressor and AIDS (McKenzie *et al.,* 1996). Thus, further analysis is also required for the defensive role of these CC chemokines against the onset of the disease *in vivo.*

Our results showed that treatment with PHA induced the expression of SDF-1 in PBMC of SNs (Fig. 3). Recently, we also showed that the expression of SDF-1 mRNA is upregulated in T cells infected with human T-cell leukemia virus type I (HTLV-I) and that HTLV-I transcriptional activator Tax, which mimics activation signal in T cells, is responsible for the upregulation (Arai *et al.,* 1998). In addition, PBMC isolated from some patients with active autoimmune diseases also had elevated SDF-1 mRNA levels (Arai *et al.,* unpublished observa-

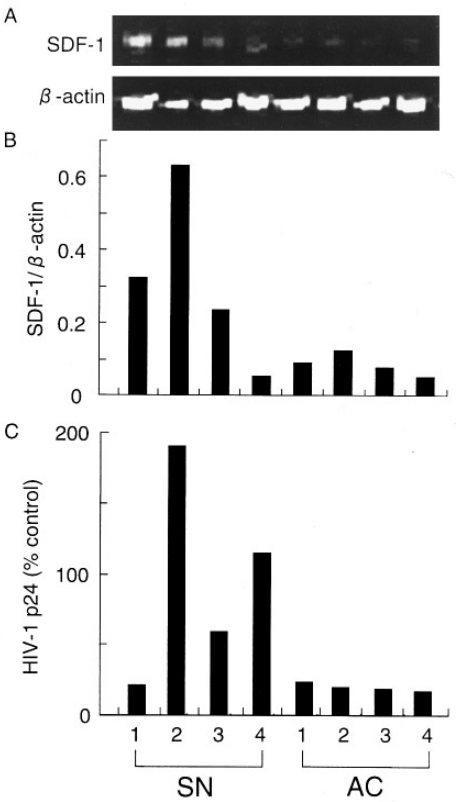

FIG. 3. SDF-1 mRNA expression in PHA-stimulated PBMC did not correlate with HIV-1-suppressive activity of $CD8^+$ T cells. (A) Total RNA was prepared from PHA-stimulated PBMC of four seronegative subjects (SN) and four asymptomatic carriers (AC) and the expression of SDF-1 mRNA in each sample was measured semiquantitatively by RT-PCR analysis. The PCR-amplified fragments separated by a polyacrylamide gel were analyzed by a sequencer. The visualized image of amplified fragments of SDF-1 and β-actin genes detected by a sequencer are shown. (B) SDF-1/β-actin ratio representing the amplified SDF-1 fragment relative to that of β-actin. (C) HIV-1 suppressive effects of $CD8^+$ T cells derived from the same individuals. Experiments were carried out in a manner similar to that described in the legend of Fig. 2A.

tions). These findings suggest that the high level of SDF-1 expression in HIV-1 carriers is due to immune reaction activated by viral infection.

$CD8^+$ T cells are known to secrete CC chemokines including MIP1α, MIP1β, and RANTES (Cocchi *et al.,* 1995). These chemokines are at least partially responsible for $CD8^+$ T-cell-mediated suppression of M-tropic HIV-1 but not T-tropic virus. Another major finding of the present study was that SDF-1 is not involved in $CD8^+$ T-cell-mediated anti-HIV activity (Fig. 3). This is in agreement with the recent report of Lacey *et al.* (1997) indicating that SDF-1 is not responsible for $CD8^+$ T cell suppression and that $CD8^+$ T cells contain extremely low levels of SDF-1.

Using a pseudotype HIV-1 carrying luciferase, we were able to show that $CD8^+$ T cells affect HIV replication mainly after the virus binds to its receptors (Fig. 4). These observations are compatible with previous findings demonstrating that $CD8^+$ T-cell-derived antiviral activity inhibits the HIV-1 life cycle at the transcription level (Chen *et al.,* 1993; Powell *et al.,* 1993). It should be noted that a slightly stronger inhibition against HIV-1 replication was detected when $CD8^+$ T cells were cocultured with $CD4^+$ T cells before viral infection than after infection (Fig. 4A). This may indicate that $CD8^+$ T cells partly inhibit viral replication at the viral entry step. Alternatively, preculture of $CD4^+$ T cells with $CD8^+$ T cells before viral infection may enhance the effectiveness of inhibition at the postbinding steps of the HIV-1 life cycle.

In conclusion, expression of SDF-1 transcripts in PBMC of HIV-1 carriers is significantly high, particularly in ACs, which may, at least in part, contribute to the suppression of HIV-1 *in vivo.* However, SDF-1 is unlikely to be involved in $CD8^+$ T-cell-mediated suppression of HIV-1 in PBMC of ACs.

MATERIALS AND METHODS

Isolation of peripheral blood mononuclear cells

Blood specimens were obtained from 17 HIV-1 asymptomatic carriers with 200–500/μl of peripheral $CD4^+$ T lymphocytes and from 3 AIDS patients with $<200/\mu l$ of $CD4^+$ T cells. Control specimens were obtained from 11 healthy SNs. PBMC were isolated on a Ficoll–Paque (Pharmacia Biotech, Uppsala, Sweden) gradient and immediately used for isolation of total RNAs or stimulated with PHA (Difco Laboratories, Detroit, MI) for 24 h. Cells were then washed and cultured in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 2 ng/ml of recombinant IL-2 (Shionogi, Osaka, Japan) and 10% FCS (Whittaker, Walkersville, MD) and used for experiments after 7 days of PHA stimulation.

$CD8^+$ T cells were prepared by depleting $CD4^+$ cells from cultured PBMC using a magnetic particle concentrator and Dynabeads M-450 (Dynal A. S., Oslo, Norway) coated with anti-CD4 antibody. $CD4^+$ T cells were isolated from PBMC by depleting $CD8^+$ cells using Dynabeads coated with anti-CD8 antibody.

Semiquantitative reverse transcriptase–polymerase chain reaction analysis

RNA was extracted from PBMC according to the Isogen method described previously (Chomczynski, 1993). For reverse transcriptase–polymerase chain reaction (RT-PCR) analysis, rTth DNA polymerase with reverse transcriptase activity was used for reverse transcription and sequential reaction in the presence of sense and antisense primers (Perkin–Elmer). Primers used for the amplification of SDF-1 and β-actin were 5′-CCGCGCTCTGCCTCAGCGACGGGAAG-3′ (sense primer), 5′-CTTGTTTAAAGCTTTCTCCAGGTACT-3′ (antisense primer) and 5′-AAGAGAGGCATCCTCACCCT-3′ (sense primer), 5′-TAGATGGCTGGGGTGTTGAA-3′ (antisense primer), respec-

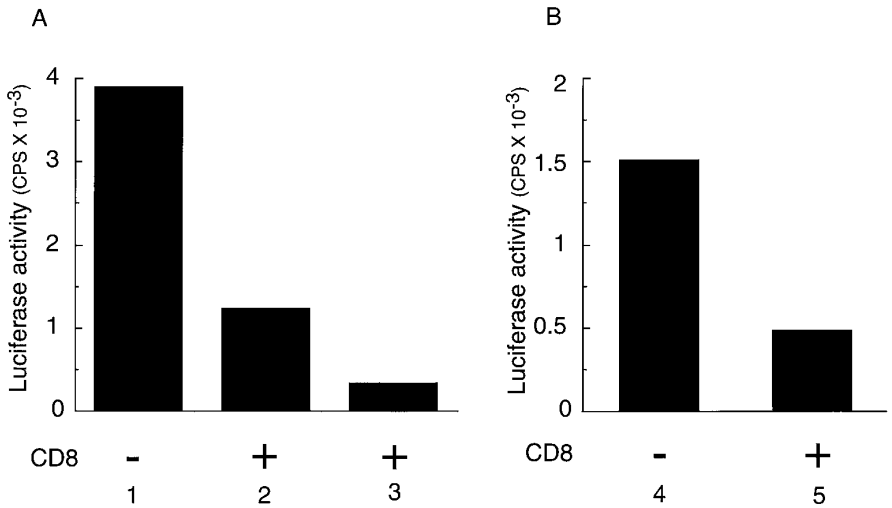

FIG. 4. Luciferase activity following infection of $CD4^+$ T cells in seronegative subjects (SN) by luciferase-expressing HIV-1, NL-luc. (A) $CD4^+$ T cells were infected with NL-luc pseudotype with T-tropic envelope (pLET) for 6 h, washed three times with PBS, and cultured in the absence (lane 1) or presence (lane 2) of $CD8^+$ T cells for 2 days. Alternatively, $CD4^+$ T cells were cocultured for 2 days, then infected with the virus for 6 hours, and cultured for 2 days (lane 3). (B) $CD4^+$ cells were infected with NL-luc pseudotyped with amphotropic MuLV envelope (pJD-1) for 6 hours, washed three times with PBS, and cultured in the absence (lane 4) or presence (lane 5) of $CD8^+$ T cells for 2 days. After culture, cell lysates were prepared and luciferase activity was assayed as described under Materials and Methods.

tively. The sense primers for SDF-1 and β-actin were labeled with 6-carboxyfluorescein (6-FAM) and 4, 7, 2′, 7′-tetrachloro-6-carboxyfluorescein (TET), respectively. The amounts of expected 227-bp (SDF-1) and 218-bp (β-actin) fragments were quantified by measuring their absorbency at 532 nm (6-FAM) and at 543 nm (TET) with a sequencer (Prism 377, Applied Biosystems) according to the instructions provided by the manufacturer. The relative amount of SDF-1 RNA represented the amount of the SDF-1 fragment relative to that of β-actin after amplification at linear phases.

Viruses

HIV-1 LAI infectious supernatants were obtained from infected MOLT-4/HIV-1 LAI C-3 cells (Harada *et al.,* 1987). Luciferase-expressing viruses capable of a single replication cycle were generated by cotransfection of 293T cells with pNLlucΔBgl encoding env-defective NL-4-3 HIV-1 sequences (Planelles *et al.,* 1995) and pLET encoding the env gene of HIV LAI (NLluc/LET) (Langlade-Demoyen *et al.,* 1988) by using the calcium phosphate method. Amphotropic MuLV envelope-expressing pJD-1 (NLluc/JD-1) was also used (Dougherty *et al.,* 1989). These plasmids were kindly provided by Dr. Irvin S. Y. Chen, from the University of California at Los Angeles. Culture supernatants were collected after 48 h of transfection and used immediately for infection.

In vitro HIV-1 infection of PBMC

$CD4^+$ T cells separated from PBMC as described above were incubated with culture supernatants containing HIV-1 LAI (MOI 0.1–0.01), NLluc/LET, or NLluc/JD-1 at 37°C for 3 h and washed extensively. The virus-infected $CD4^+$ T cells (1×10^5) were cocultured with $CD8^+$ T cells (1×10^5) in a round-bottom 96-well plate for 4 days for p24 assay or for 2 days for luciferase assay. In some experiments, $CD4^+$ and $CD8^+$ T cells were cocultured for 2 days, then infected with NLluc/LET, and assayed for luciferase activity after 2 days of infection.

Quantitation of HIV-1 p24

HIV-1 p24 antigen in the culture supernatants was measured with an enzyme immunoassay system, EIA-II (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany), according to the instructions provided by the manufacturer. Percentage suppression was calculated using the formula [(1 − test p24 value /medium control p24 value) × 100].

Luciferase assay

After two days of infection, 100 μl of cell suspension was lysed with 25 μl of 5× luciferase lysis buffer (Promega). A 20-μl section of each lysate was assayed for photon emission with a luminometer (LUMAT; EG&G Berthold, Bad Wildbad, Germany) by using the Promega Luciferase Assay System. Luciferase activity was indicated as counts per second.

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