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Interference with Human Immunodeficiency Virus (HIV) Replication by CD8+ T Cells in Peripheral Blood Leukocytes of Asymptomatic HIV Carriers In Vitro

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A long asymptomatic period is one of the characteristics of human immunodeficiency virus (HIV) infection, despite its fatal consequences. Antiviral defense in HIV-infected individuals controls viral replication during this period. In the present study, we demonstrate that peripheral blood leukocytes (PBL) of asymptomatic HIV-1 carriers, following exogenous HIV-1 infection in vitro, do not support viral replication. These cells do not produce detectable amounts of reverse transcriptase or accumulate unintegrated proviral DNA. This is a striking contrast to the behavior of HIV-1-infected PBL of seronegative individuals, which produce large amounts of RT and unintegrated DNA. Such resistance to HIV-1 replication is not seen in PBL of patients with advanced disease. Since the binding of HIV-1 to CD4 molecule is not impaired in PBL of asymptomatic carriers, the interference with HIV replication must occur after the stage of virus binding. PBL lose their resistance when CD8+ lymphocytes are removed. In addition, these PBL are not resistant to an exogenous infection with HIV-2. These observations suggest that certain populations of CD8+ lymphocytes of asymptomatic HIV-1 carriers operate on the target cells in PBL to block viral replication in an HIV-1-specific manner. Such CD8+ lymphocyte-mediated interference with HIV replication could play an important role in the maintenance of the period of disease latency.

Human immunodeficiency virus (HIV), the etiological agent of the acquired immunodeficiency syndrome (AIDS), causes slow, progressive destruction of the human immune system (8). Two peaks of antigenemia typically occur during the course of infection with HIV: the first peak at the time of primary HIV infection, and the other when signs of disease finally appear (27). This suggests that the development of disease might be a consequence of an increase in HIV replication. Anti-HIV drugs which reduce viral replication, such as reverse transcriptase (RT) inhibitors, prolong the survival time of patients with AIDS (45). Recently, soluble recombinant CD4 has been shown to induce a therapeutic response in simian immunodeficiency virus (SIV)-infected macaque monkeys (23, 44). Such observations suggest that the protection of CD4+ lymphocytes from exposure to HIV might be essential for the treatment of AIDS.

The estimated average length of the asymptomatic period between primary HIV infection and the later emergence of viral replication is approximately 7 to 8 years (1). It is still unclear to what extent HIV replicates in vivo during this period of disease latency and what prevents disease evolution. Low levels of viral DNA and RNA can be detected in peripheral blood leukocytes (PBL) of asymptomatic HIV carriers by gene amplification methods (10, 26), indicating that viral DNA integrated into the cellular DNA of PBL is not completely silent. A recent report (15), documenting the ability to detect HIV p24 antigen in vitro by using stimulated PBL and plasma from HIV-seropositive individuals, also supports the possibility of in vivo HIV replication in these individuals. However, viral antigens expressed by PBL are limited, and infectious HIV titers are low in the PBL and plasma of the asymptomatic HIV carriers. Several possibilities have been proposed to explain the maintenance of the low levels of viral replication during this period of disease latency: (i) the regulatory HIV genes might control the expression of viral DNA, either positively or negatively (12); (ii) defective viruses and helper viruses might interact to generate more pathogenic viruses (2, 11); (iii) infected cells might be eliminated by immune mechanisms such as T-cell-mediated HIV-specific cytotoxicity (28, 40, 41) and antibody-dependent cell-mediated cytotoxicity (5, 24, 33); and (iv) CD8+ lymphocytes might directly suppress spontaneous viral replication in PBL of asymptomatic HIV and SIV carriers (17, 42).

In the present study, we demonstrate that PBL of asymptomatic HIV-1 carriers do not support the replication of exogenously added HIV-1. This interference of PBL with HIV replication is dependent on CD8+ lymphocytes in culture. Such CD8+ lymphocyte-mediated suppression of HIV replication in vitro may be an important phenomenon in understanding the controlled HIV replication in vivo during the asymptomatic period.

MATERIALS AND METHODS

Blood donors. Five healthy HIV-1-seronegative laboratory workers, five asymptomatic HIV-1 carriers (Walter-Reed stages 1 and 2) (32), and two patients with AIDS-related complex (ARC) and AIDS (Walter Reed stages 4 to 6) served as blood donors. The asymptomatic HIV-1 carriers included two homosexual men, one heterosexual woman, and two hemophiliacs.

In vitro infection of PBL with HIV-1. PBL from donors

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were isolated on a Ficoll-Conrey gradient, stimulated with 1% phytohemagglutinin (PHA) (Difco Laboratories, Detroit, Mich.) for 3 days, and incubated in HIV-1-containing medium (2×10^4 cpm of RT activity per ml) at a cell concentration of 2×10^6/ml for 2 h at 37°C. A filtered culture supernatant from H9 cells chronically infected with HTLV-III_B (H9/III_B) (30) was used as the virus source. Cells were then washed with phosphate-buffered saline and adjusted to 10^6/ml in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Whittaker Bioproducts, Walkersville, Md.) and 200 IU of recombinant interleukin-2 (Shionogi, Osaka, Japan) per ml. These cells were then cultured at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was changed every 3 to 4 days, and the cell concentration was adjusted to 10^6/ml. Culture supernatants were passed through filters (pore size, 0.45 μm) and stored at −70°C for RT assay and further characterization. Culture supernatants of CEM cells chronically infected with an HIV-2 isolate (CEM/LAV-2) (7) and H9 cells chronically infected with an HIV-1 isolate from a patient with AIDS (H9/ysm) (M. Kannagi, unpublished data) were also used as virus sources in experiments whose results are shown in Fig. 5.

RT assay. RT activities of culture supernatants were measured as previously described (9). Briefly, 1 ml of each supernatant was centrifuged at 60,000 rpm in a rotor (TL-100.3; Beckman Instruments, Inc., Fullerton, Calif.) for 40 min. The pelleted virus was incubated on ice for 10 min with 20 μl of dissociation buffer (5 mM Tris hydrochloride [pH 8.1], 0.1% Triton X-100, 1 mM dithiothreitol, 0.5 M KCl). Then 10 μl of dissociated-virus solution was mixed with 40 μl of assay mixture {0.05 mM Tris hydrochloride (pH 8.4), 0.01 M $MgCl_2$, 2 mM dithiothreitol, 0.06 mg of poly(rA)-oligo(dT) template primer per ml; 2.5 μCi of [*methyl*-^{3}H] thymidine-5′-triphosphate (The Radiochemical Centre, Amersham, England)} and incubated at 37°C for 60 min in a 96-well microdilution plate. Then 200 μl of 10% trichloroacetic acid was added to each well, and the wells were incubated for 20 min at 4°C. Acid-insoluble precipitates were then harvested onto glass filters through a semiautomatic cell-harvesting system (Skatron, Lier, Norway). The filter was rinsed in 5% trichloroacetic acid–2% sodium PP_i and dried, and the radioactivity in each well was measured.

Coprecipitation of CD4 with viral envelope. Radiolabeled HIV-1 proteins were prepared as described elsewhere (16). MOLT-4 cells (10^7) chronically infected with HTLV-III_B (MOLT-4/III_B) were incubated for 16 h at 37°C in 5 ml of cysteine- and methionine-free RPMI 1640 medium containing 10% dialyzed fetal calf serum and 50 μCi of [^{35}S]cysteine and [^{35}S]methionine (ICN Radiochemicals, Irvine, Calif.) per ml. Cells were washed three times, solubilized with 1 ml of lysis buffer (0.01 M Tris hydrochloride [pH 8.0], 0.14 M NaCl, 3 mM dithiothreitol, 0.5% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride) for 20 min at 4°C, and then centrifuged at 13,000 × *g* for 10 min. CD4 was removed from this lysate by two adsorptions with protein A-Sepharose CL-4B beads (Pharmacia, Uppsala, Sweden) conjugated to OKT4 antibody (Ortho Diagnostics, Inc., Raritan, N.J.). Equal volumes of this lysate and lysates from unlabeled PBL were mixed at 4°C for 2 h and then immunoprecipitated for 1 h at 4°C with protein A-Sepharose beads conjugated to OKT4 antibody. Beads were washed sequentially in high-salt washing buffer (0.02 M Tris hydrochloride [pH 7.6], 0.5 M NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 1% sodium deoxycholate, 0.1% bovine serum albumin) and low-salt buffer (0.1 M Tris hydrochloride [pH 7.6], 0.1 M NaCl). Immunoprecipitates were eluted from the beads in 20 μl of sample buffer (0.06 M Tris hydrochloride [pH 6.8], 2% sodium dodecyl sulfate [SDS], 4% 2-mercaptoethanol, 10% glycerol, 25 μg of bromophenol blue per ml) at 100°C for 5 min and analyzed on 12.5% polyacrylamide–SDS gels by the discontinuous buffer system (22).

Isolation of unintegrated DNA from PBL. A modified (6) Hirt extraction procedure (14) was used for isolation of unintegrated DNA from PBL. At appropriate times after initiation of culture, 10^7 cells were washed three times and suspended in 1 ml of 0.1 M Tris hydrochloride (pH 7.5)–0.1 M EDTA–20 μg of RNase per ml. The cells were incubated at 65°C for 15 min and then lysed in 0.6% SDS for 20 min at 65°C. NaCl was added to 1 M, and the samples were kept at 4°C overnight. After centrifugation at 13,000 × *g* for 10 min, the supernatants were extracted with phenol and chloroform and precipitated with a double volume of ethanol at −20°C.

Agarose gel electrophoresis and Southern blot hybridization. DNA samples were separated by horizontal electrophoresis in 0.7% agarose slab gels and transferred to nylon membranes (Amersham Corp.) by the Southern transfer procedure (36). Viral DNA on the filter was detected by molecular hybridization to ^{32}P-labeled HIV-1 DNA probe prepared by nick translation. *Sac*I-digested 9.4-kilobase fragments of λBH10 (31) were used as an HIV-1-specific DNA probe. Hybridization was performed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50% formamide–5× Denhardt solution–0.1% SDS–0.05 M Tris (pH 7.5)–10% dextran sulfate at 42°C for 18 h. The filter was then washed three times with 1× SSC–0.1% SDS at 65°C and exposed to X-ray film at −70°C.

Depletion of $CD8^+$ lymphocytes from PBL. PBL were stimulated with PHA for 3 days and incubated with 1:10-diluted OKT8 monoclonal antibody (Ortho) at a cell concentration of 10^7/ml for 1 h at 4°C. Cells were washed twice with phosphate-buffered saline and then incubated with rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) for 1 h at 37°C. After being washed three times with phosphate-buffered saline, cells were transferred to interleukin-2-containing medium with or without HIV-1. These cells were 72 to 85% $CD4^+$ and less than 1% $CD8^+$.

RESULTS

HIV-1 replication in PBL from HIV-1 carriers with or without exogenous HIV-1 infection. Preliminary studies were performed to determine how PBL from HIV-1 carriers respond to HIV-1 infection in vitro. PHA-stimulated PBL from HIV-1 carriers, which were exogenously infected with HIV-1, were expanded in the presence of interleukin 2, and RT activities of the culture supernatants were measured. The results are summarized in Table 1, and the kinetics of RT activities of the culture supernatants in representative experiments is shown in Fig. 1. Following exogenous infections, PBL from seronegative donors showed high RT activities in culture supernatants, peaking between 10 and 16 days after infections (Fig. 1A). In contrast, exogenous HIV-1 infection of PBL from asymptomatic carriers resulted in no induction of RT activity during 3 weeks of culture. Without exogenous infection, PBL from these individuals generated no RT activity either (Fig. 1B). Cultures of PBL from patients with ARC and AIDS had significant RT activities with or without exogenous HIV-1 infection (Fig. 1C).

$CD4^+$ cells in cultures of PBL from seronegative individuals significantly decreased in number following exogenous

TABLE 1. HIV-1 replication in PBL from normal and HIV-1-infected individuals, with or without exogenous HIV-1 infection in vitro

Blood donor	No. of subjects tested	Peak RT activity in PBL culture supernatants (10^3 cpm/ml)[a]	
		With exogenous infection	Without exogenous infection
Seronegative individual	5	133.3 ± 38.3	0.6 ± 0.2
Asymptomatic carrier	5	0.8 ± 0.7	0.8 ± 0.5
ARC-AIDS patient	2	96.3 ± 27.7	52.6 ± 5.5

[a] PHA-stimulated PBL from donors were incubated in cell-free HIV-1 or control medium for 2 h at 37°C, washed, and then cultured in interleukin-2-containing medium for 3 weeks. The cell concentration was adjusted to 10^6/ml every 3 to 4 days, and the RT activities in the supernatants were measured before replacement of culture medium. Maximal RT activities in the supernatants were detected between 10 and 16 days after infection. Data are expressed as mean ± standard deviation.

HIV-1 infection. For example, a culture of PBL from a seronegative individual, 10 days after exogenous infection, had 6% $CD4^+$ and 55% $CD8^+$ cells, whereas a simultaneous culture of PBL from the same subject, without exogenous infection, had 62% $CD4^+$ and 24% $CD8^+$ cells. This drastic decrease in $CD4^+$ cell number was accompanied by an emergent elevation of RT activity in the supernatant. The PBL from asymptomatic HIV-1 carriers did not spontaneously produce virus in culture or allow viral replication following exogenous HIV-1 infection. The growth and $CD4^+$ cell number of these PBL were not significantly impaired following exogenous HIV-1 infection. For example, a culture of exogenously infected PBL from an asymptomatic HIV-1 carrier had 45% $CD4^+$ and 49% $CD8^+$ cells after 10 days, whereas a simultaneous culture without exogenous infection had 51% $CD4^+$ and 46% $CD8^+$ cells. This indicates that the absence of HIV-1 infection of these PBL was not due to the absence of target cells as the result of a preferential expansion of $CD8^+$ cells in the cultures.

Binding of HIV-1 envelope glycoprotein to the CD4 molecule of PBL from asymptomatic HIV-1 carriers. Since $CD4^+$ cells in PBL from the asymptomatic HIV-1 carriers showed a similar range of mean fluorescence intensity to those in PBL from seronegative controls by cell surface immunofluorescence analysis (data not shown), these cells were presumed to possess enough HIV-1 receptors for infection. The coprecipitation method was used to assess the binding ability of the CD4 molecule of PBL from HIV-1 carriers to the HIV-1 envelope protein. Radiolabeled HIV-1 proteins were mixed with unlabeled lysates of PBL from a normal individual or an asymptomatic HIV-1 carrier and then immunoprecipitated with the OKT4 antibody. OKT4 precipitated viral envelope glycoprotein (gp120) with lysates of PBL from HIV-1 carriers as well as with lysates of normal PBL (Fig. 2). This indicates that the CD4 of HIV-1 carriers normally serves as a receptor of HIV-1, suggesting that the impairment of viral

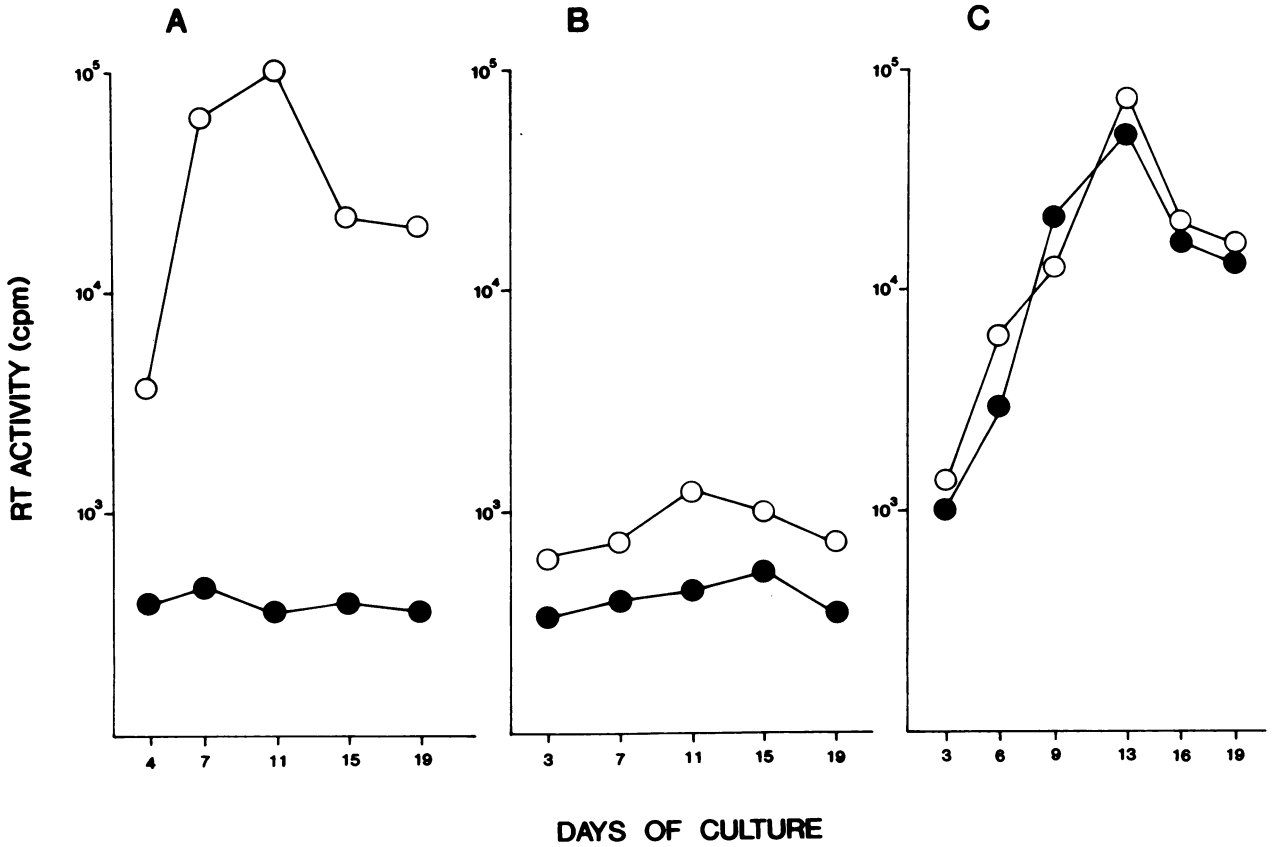

FIG. 1. Kinetics of RT activities in the supernatants of PBL cultures from a seronegative individual (A), an asymptomatic HIV-1 carrier (B), and a patient with AIDS (C), with (○) or without (●) exogenous HIV-1 infection. Experiments were performed in the same manner as noted in Table 1.

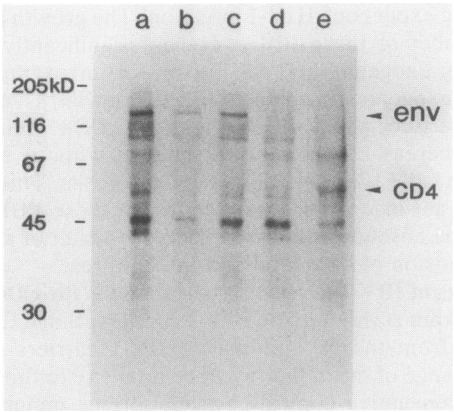

FIG. 2. HIV-1 envelope protein (env) was coprecipitated with CD4 antigen of PBL from an asymptomatic HIV-1 carrier, as well as from a seronegative individual. ^{35}S-labeled MOLT-4/III$_B$ lysate was mixed with cold cell lysates of MOLT-4 cells (lane a), normal PBL (lane b), or HIV carrier PBL (lane c) or left unmixed (lane d), immunoprecipitated with OKT4, and subjected to SDS-polyacrylamide gel electrophoresis. The radiolabeled MOLT-4/III$_B$ cell lysates used were preabsorbed with OKT-4 before use. A ^{35}S-labeled cell lysate of uninfected MOLT-4 was immunoprecipitated with OKT4 to indicate the size of CD4 (lane e). kD, Kilodaltons.

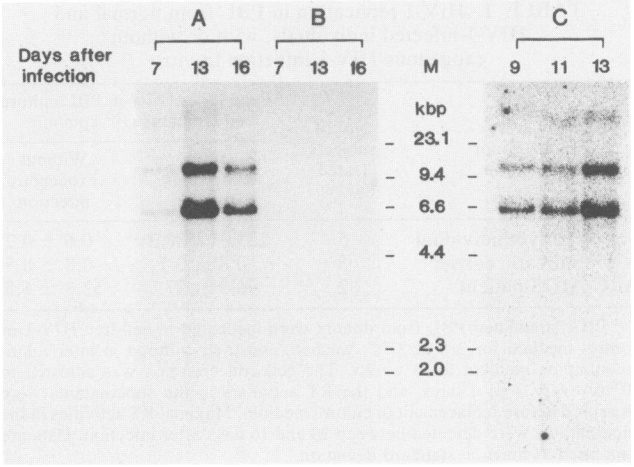

FIG. 3. DNA blot hybridization analysis of unintegrated proviral DNA in HIV-1-infected PBL from a normal individual (A), an asymptomatic HIV-1 carrier (B), and a patient with AIDS (C) after exogenous HIV-1 infection. The Hirt extracts from 10^7 cells were harvested on the indicated days after infection, separated on an agarose gel, and subjected to Southern blot hybridization with ^{32}P-labeled HIV-1-specific DNA probe. The markers (M) are *Hin*dIII-digested λ DNA. kbp, Kilobase pairs.

replication of these cells following exogenous infection of HIV-1 is a postbinding event.

Accumulation of unintegrated proviral DNA in HIV-1-infected PBL. To investigate which step of viral replication is involved in this inhibition, we assessed the intermediate products of the viral life cycle in lymphocytes. It has been reported that exogenous retrovirus infection induces the accumulation of extrachromosomal proviral DNA in target cells (4). Preliminary studies revealed that unintegrated HIV-1 proviral DNA of 5×10^6 normal human PHA-stimulated PBL became detectable 7 days after infection, peaked on days 11 to 13, then gradually decreased in quantity. Southern blot analysis followed by gel electrophoresis demonstrated two bands, one about 10 kilobase pairs and the other 6 kilobase pairs. These band sizes are compatible with those reported by Shaw et al. (35) and are thought to be a double-stranded linear form and a closed-circular form of unintegrated HIV proviral DNA, respectively. The latter band was often accompanied by another faint band 0.6 kilobase pairs higher, which is believed to have an extra set of long terminal repeats.

A significant amount of unintegrated proviral DNA was detected in HIV-infected PBL from a seronegative subject (Fig. 3A), but not in PBL from an asymptomatic HIV-1 carrier within 3 weeks of culture after exogenous HIV-1 infection (Fig. 3B). Exogenous HIV-1-infected PBL from the patient with AIDS had detectable amounts of unintegrated proviral DNA (Fig. 3C). These findings correlate with the RT activities produced by the PBL given in Table 1 and suggest that the inhibition of HIV-1 replication in asymptomatic HIV-1 carriers must occur before accumulation of unintegrated proviral DNA.

Susceptibility of CD8$^+$ lymphocyte-depleted PBL from asymptomatic HIV-1 carriers to exogenous HIV-1 infection. We then sought to evaluate whether the depletion of CD8$^+$ lymphocytes affects the susceptibility of PBL from HIV-1 carriers to exogenous HIV-1 infection, since spontaneous viral release can be induced from such PBL by depleting CD8$^+$ lymphocytes. PBL from asymptomatic HIV-1 carriers or seronegative controls were treated with a CD8-specific monoclonal antibody and complement and then infected exogenously with HIV-1. Table 2 shows RT activities observed in the supernatants of these PBL cultures. Following exogenous HIV-1 infection, CD8$^+$ cell-depleted PBL from all the asymptomatic HIV-1 carriers tested released high levels of RT, although the kinetics of RT activity generation in the culture supernatants differed among donors. Maximal RT activities in exogenously infected cell populations were observed 7 to 14 days after infections. Without exogenous HIV-1 infection, CD8$^+$ cell-depleted PBL from carriers no. 1 and 2 generated maximal amounts of RT activity 3 to 4 days later. It is noteworthy that depletion of CD8$^+$ cells alone from PBL from carrier no. 3 resulted in generation of RT at a borderline level, whereas the same cell population supported viral replication following exogenous HIV-1 in-

TABLE 2. Spontaneous viral replication in CD8$^+$ cell-depleted PBL from asymptomatic HIV-1 carriers with or without exogenous HIV-1 infection in vitro

Blood donor	Exogenous HIV-1 infection	Peak RT activity in culture supernatants (10^3 cpm/ml)		Day of culture[b]
		Unseparated PBL	CD8$^+$ cell-depleted[a] PBL	
Seronegative individual	−	0.6	0.7	14
	+	123.2	101.1	14
HIV carrier no. 1	−	0.6	132.2	14
	+	0.7	137.3	10
HIV carrier no. 2	−	0.5	111.0	10
	+	0.4	115.7	7
HIV carrier no. 3	−	0.3	2.5	16
	+	2.0	105.0	11

[a] PHA-stimulated PBL were treated with OKT8 and complement to deplete CD8$^+$ lymphocytes. These PBL included more than 72% CD4$^+$ and less than 1% CD8$^+$ cells. Cells were then incubated with HIV-1 or control medium in the same manner as noted in Table 1, footnote *a*.

[b] Day of culture when peak RT activities were detected. The day of exogenous HIV infection is counted as day 0.

TABLE 3. Host range of viruses released in culture supernatants of PBL from HIV-1 infected individuals

Source of viruses[a]		Susceptibility to HIV infection of[a]:		
Donor	Culture supernatant	MOLT-4	H9	Normal PBL
Patient with AIDS	Unseparated PBL with exogenous HTLV-III$_B$ infection	+	+	+
	Unseparated PBL alone	−	−	+
Asymptomatic HIV carrier	CD8$^+$ cell-depleted PBL with exogenous HTLV-III$_B$ infection	+	+	+
	CD8$^+$ cell-depleted PBL alone	−	−	+
None	HTLV-III$_B$	+	+	+

[a] Host range of virus in culture supernatants (days 10 to 11) of unseparated PBL from a patient with AIDS listed in Table 1 and CD8$^+$ cell-depleted PBL from HIV-1 carrier no. 1 listed in Table 2. The culture supernatant of H9/III$_B$ (HTLV-III$_B$), the same aliquot utilized for exogenous infection of the PBL populations, was also tested.

[b] MOLT-4 cells, H9 cells, and PHA-stimulated PBL from normal subjects were incubated with each culture supernatant for 2 h at 37°C and washed three times prior to culture. Susceptibility to the viruses was judged by RT activities in culture supernatants 12 days after infection: −, RT ativity less than 0.6×10^3 cpm/ml; +, RT activity more than 7.7×10^3 cpm/ml.

fection. Unseparated PBL from any of the HIV-1 carriers tested did not generate a significant amount of RT with or without exogenous HIV-1 infection, as described above. Exogenous infection on normal PBL as controls (unseparated or CD8$^+$ cell depleted) induced efficient viral replication. Thus, PBL from asymptomatic carriers became susceptible to exogenous HIV infection by depletion of CD8$^+$ cells, as did unseparated PBL from patients with ARC and AIDS.

Characterization of HIV-1 strains replicating in PBL from HIV-1 carriers following exogenous HIV-1 infection. Unseparated PBL from patients with ARC and AIDS and CD8$^+$ cell-depleted PBL from asymptomatic carriers supported HIV replication following exogenous infection (Tables 1 and 2). However, most of the uninfected control cultures of these CD8$^+$ cell-depleted PBL also produced HIV spontaneously. To confirm that the exogenously introduced HIV-1 strain (HTLV-III$_B$), as well as the previously integrated HIV-1 strain, truly replicated in these cultures, viruses released in the culture supernatants of PBL from a patient with AIDS and an asymptomatic carrier were characterized for susceptible host range (Table 3). Spontaneously released viruses from both individuals were able to infect normal PBL but not MOLT-4 or H9 cells. On the other hand, the culture supernatant of exogenously infected PBL contained virus which replicated in MOLT-4, H9, and PBL, a host range which is compatible with that of HTLV-III$_B$. This suggests that the unseparated PBL from the patient with AIDS and the CD8$^+$ cell-depleted PBL from the asymptomatic carrier supported replication of exogenously introduced HIV-1.

Induction of unintegrated proviral DNA in CD8$^+$ cell-depleted PBL from HIV-1 carriers. We then examined whether unintegrated proviral DNA can be induced in CD8$^+$ cell-depleted PBL from HIV-1 carriers following exogenous HIV-1 infection. Following exogenous HIV-1 infection, CD8$^+$ cell-depleted PBL from an HIV-1 carrier rapidly accumulated unintegrated HIV-1 proviral DNA as early as 5 days after infection. Interestingly, CD8$^+$ cell-depleted PBL obtained from the same subject but not exogenously infected, which spontaneously produce HIV, also showed faint bands of unintegrated DNA (Fig. 4). Since unintegrated DNA is generated mainly from exogenously infecting virus, the existence of unintegrated DNA in the cell population without exogenous infection suggests that new infections may be occurring in this culture. This suggests that spontaneous viral replication may consist of two phases: active production of virus from integrated provirus, and further infection of other cells in culture by newly produced virus. Importantly, removal of CD8$^+$ lymphocytes resulted in the accumulation of unintegrated HIV-1 proviral DNA in these PBL responding to new infection of HIV-1, exogenously added or spontaneously released. This contrasts with the absence of accumulation of proviral DNA in these PBL in the presence of CD8$^+$ lymphocytes following exogenous HIV-1 infection.

Failure to protect PBL, obtained from HIV-1 carriers, against exogenous HIV-2 infection. Lastly, we sought to determine whether PBL from HIV-1 carriers resist viral replication following HIV-2 infection. Unfractionated PBL from two asymptomatic HIV-1 carriers and a seronegative individual were exogenously infected with HIV-1 or HIV-2. Figure 5 shows maximal RT activities detected in the supernatants of these PBL cultures. Two different HIV-1 isolates (HTLV-III$_B$ and HIV-ysm) and one HIV-2 isolate (LAV-2) were used for infections, and all of these isolates replicated well in PBL from a seronegative subject. Peak RT activities were detected between 7 and 16 days after infection. Interestingly, PBL from both HIV-1 carriers tested also supported HIV-2 replication. The kinetics of RT activities in these culture supernatants were similar to those of activities in culture supernatants of cells from seronegative individuals. In contrast, HTLV-III$_B$ did not replicate in PBL from

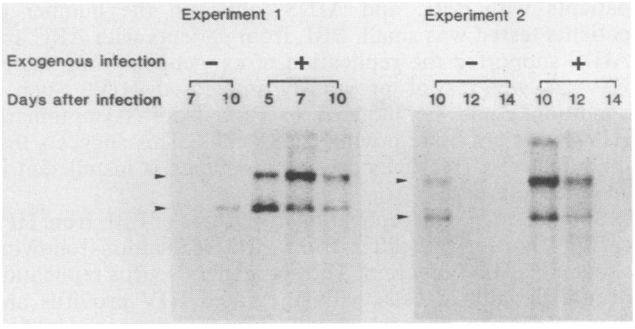

FIG. 4. Kinetics of accumulation of unintegrated HIV-1 proviral DNA in CD8$^+$ cell-depleted PBL from an asymptomatic HIV-1 carrier with (+) or without (−) exogenous HIV-1 infection in vitro. The HIV carrier tested was the same subject as carrier no. 2 in Table 2. PHA-stimulated PBL were treated with OKT8 antibody and complement prior to exposure to HIV-1. Experiments 1 and 2 were set up in the same fashion with PBL from the same donor, but were performed 2 weeks apart. Hirt extracts from 10^7 cells were sampled on the indicated days after infection. DNA blot hybridization analysis was performed as described in the legend to Fig. 2.

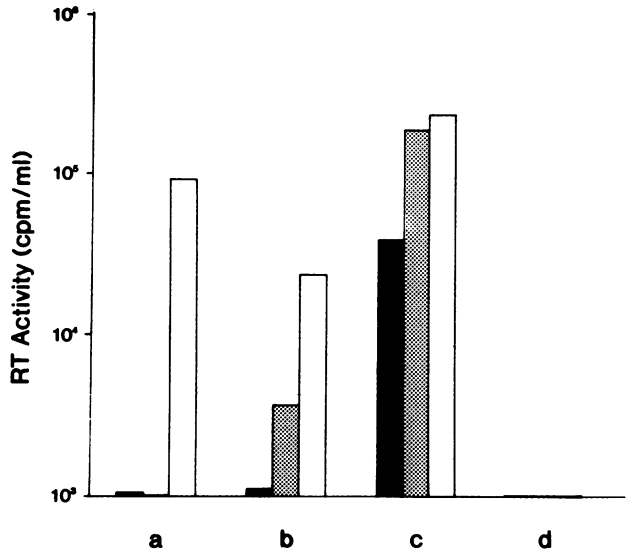

FIG. 5. Maximal RT activities detected in the supernatants of PBL cultures from two asymptomatic carriers (■, ▨) and a seronegative donor (□), following exogenous infection with two different HIV-1 isolates (a and b), an HIV-2 isolate (c), or control medium (d). PHA-stimulated unfractionated PBL from each donor were incubated with culture supernatants of H9/III$_B$ cells (a), H9/ysm cells (b), or CEM/LAV-2 cells (c), containing 2×10^4 cpm of RT activity per ml, and then maintained in the same manner as noted in Table 1, footnote *a*.

any of the asymptomatic HIV-1 carriers. Another HIV-1 isolate, HIV-ysm, also replicated poorly in PBL from an HIV-1 carrier, but a low level of viral replication was observed in PBL from one of the carriers. These observations suggest that the suppression of viral replication in HIV-1 carriers may be specific for HIV-1 strains.

DISCUSSION

In this study, we showed that PBL from asymptomatic HIV-1 carriers were resistant to HIV-1 replication in vitro and that the resistance of these PBL was mediated by CD8$^+$ lymphocytes. This resistance was not observed in PBL from patients with ARC and AIDS, although the number of patients tested was small. PBL from patients with ARC and AIDS supported the replication of exogenously introduced HIV as well as of previously integrated HIV. Such a condition could be induced in PBL from asymptomatic HIV-1 carriers by removing CD8$^+$ cells. This suggests that the effect of CD8$^+$ cells on this resistance is insufficient in advanced disease.

Spontaneous viral replication in cultures of PBL from HIV carriers appears to differ from viral replication following exogenous HIV infection. The spontaneous virus replication occurs initially in cells with integrated HIV provirus and then spreads to other intact cells, whereas exogenous infection involves all the target cells at one time. CD8$^+$ lymphocytes inhibited HIV-1 replication in both cases. If the viral production from integrated provirus alone occurs, but is not detectable, the inhibition of virus spread to the remaining potential target cells by CD8$^+$ lymphocytes would result in the control of spontaneous HIV replication in unseparated PBL. However, the nature of HIV replication differs among individuals. CD8$^+$ cell-depleted PBL from one of the asymptomatic carriers (Table 3, carrier no. 3) showed very little spontaneous virus replication, and yet the same cell population supported the replication of HIV-1 introduced exogenously. This indicates that latently infected cells from this patient could not produce infectious virus in the absence of CD8$^+$ lymphocytes. The absence of spontaneous virus replication in CD8$^+$ cell-depleted PBL from some HIV carriers has also been reported by Walker et al. (43). The absence of spontaneous viral replication in this patient might be result of defective provirus, or different cell tropism of the virus. These observations suggest that the asymptomatic phase of HIV-1 infection may be divided into subgroups according to the state of spontaneous HIV-1 replication in CD8$^+$ cell-depleted PBL.

Intact HIV-1 binding, the absence of cytopathic effect, and the absence of unintegrated HIV-1 proviral DNA in the PBL of asymptomatic HIV-1 carriers following HIV-1 infection in vitro suggest that an exogenous HIV-1 infection may be blocked at an early phase of the virus life cycle in these PBL. Interference at an early stage of infection has been found in other retrovirus infections (38). Some endogenous retroviral gene products restrict murine leukemia virus infections (21). The latently HIV-infected cells might be resistant to an exogenously introduced HIV in the same manner. However, the estimated proportion of latently infected cells is about 1% of CD4$^+$ cells in HIV carriers (34), which would not account for the absence of HIV replication in the remaining PBL following exogenous infection. A kind of virus interference was found in T cell lines expressing HIV glycoprotein; these lines were resistant to cytolysis following HIV infection because of a reduction in the number of CD4 molecules on the cell surface (37). However, the resistance of PBL from HIV-1 carriers described here is not due to reduction in the number of CD4 molecules expressed by lymphocytes. PBL from asymptomatic carriers expressed normal quantities of CD4, and binding to HIV-1 was intact. The expression of CD4 alone is not sufficient to facilitate productive infection of cells by HIV or SIV (18, 25); a second cellular receptor for viral entrance may be necessary (13, 20). This step might be involved in CD8$^+$ lymphocyte-mediated interference with HIV replication. Another possibility is that the interference occurs at a late stage of the virus life cycle, because the accumulation of unintegrated DNA may reflect mainly the propagation of the virus following the initial cycle of virus replication (19). The activation of HIV regulatory genes has been reported to be important for effective virus production (12). The suppression of expression of these genes at the initial cycle may result in the limited viral replication. Recently, resistance to HIV infection by cells producing alpha interferon has been reported (3, 29). It remains to be determined whether cytokines such as interferons are involved in interference in PBL of HIV carriers.

Interestingly, PBL from asymptomatic HIV-1 carries were resistant to replication of HIV-1 but not HIV-2. The specificity for HIV-1 strains suggests that immune mechanisms might be involved. HIV-specific CD8$^+$ lymphocyte-mediated cytotoxicity has been reported to be readily detectable in PBL from HIV carriers (40, 41). Recently, CD8-specific antibodies have been shown to inhibit the suppression of spontaneous HIV and SIV replication by CD8$^+$ lymphocytes, suggesting that virus-specific cytotoxicity may be involved in the mechanisms of suppression (39). In our preliminary experiments, however, we detected no significant cytotoxicity in PBL from asymptomatic carriers against autologous CD8$^+$ cell-depleted PBL exogenously infected with HIV-1 (data not shown). Further studies are necessary

to determine whether HIV-1 specific cytotoxicity is involved in the mechanisms of resistance of PBL to exogenous HIV-1 infection demonstrated in the present studies.

The present studies delineate a new aspect of the suppression of HIV replication in PBL from HIV carriers: interference with virus replication following exogenous HIV infection. Although the precise mechanism(s) of how $CD8^+$ lymphocytes induce such interference has still to be defined, the effect of these cells may contribute to the maintenance of an asymptomatic state in HIV carriers.

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