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Integrated Proviral Human Immunodeficiency Virus Type 1 Is Present in CD4+ Peripheral Blood Lymphocytes in Healthy Seropositive Individuals

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Evidence of a latent human immunodeficiency virus type 1 (HIV-1) infection in healthy, seropositive individuals who do not have viral antigens in their sera and from whom virions cannot be rescued in cocultivation experiments was examined. Proviral DNA was detected by amplification by the polymerase chain reaction procedure. In each of 10 seropositive individuals, the presence of HIV-1 proviral sequences was demonstrated in their peripheral blood mononuclear cells. By using fluorescence-activated cell sorting, we obtained highly enriched subpopulations of peripheral blood mononuclear cells and found that the CD4+ T-cell subset is the cell subset that consistently harbors the HIV-1 proviral sequences. The number of HIV-1-infected CD4+ T cells was variable among the 10 healthy individuals, ranging from 1 in 100 to 1 in 40,000. While in vitro infection of CD4+ T cells causes down regulation and eventual loss of CD4 surface molecules, this is not true in vivo where it is only the CD4+ population that harbors the virus. This disparity may reflect differences between a latent infection in vivo with the lytic response of cells infected in vitro.

Infection of T lymphocytes by human immunodeficiency virus type 1 (HIV-1) involves binding of the virus to the surface CD4 molecule (2, 6, 15, 21), and individuals infected with HIV-1 who develop acquired immunodeficiency syndrome (AIDS) show a profound and selective depletion of CD4+ lymphocytes (13, 19, 27). However, on the basis of in situ hybridization studies, the fraction of CD4+ lymphocytes that actively express HIV-1 is less than 0.01% of the total number of mononuclear cells (10). The evidence that cells of the monocyte/macrophage lineage harbor HIV-1 in AIDS patients suggests that they may serve as a reservoir for the virus in the body and also play a role in AIDS-related dementias (16). Similarly, the presence of HIV-1 in bone marrow myeloid precursors (3) and the ability to infect and propagate virus in normal human bone marrow suggests that myeloid precursor cells can be a virus reservoir during the course of the disease (7).

While cells expressing the CD4 surface molecule harbor HIV-1 in AIDS patients (S. Schnittman, H. C. Lane, M. Baseler, F. Massari, C. Fox, and A. S. Fauci, Abstr. 4th Int. Conf. on AIDS, abstr. no. 1, p. 168, 1988), to understand the etiology of the disease it is important to establish which cells harbor virus at the earlier stages of infection. This information will identify the primary target for effective chemotherapeutic intervention at a time when the total body load of virus may still be small. We have examined 10 healthy individuals who have antibodies against HIV-1 (as determined by enzyme-linked immunosorbent assay (ELISA) and Western blot [immunoblot] assay) but are negative for HIV-1 production by cocultivation and for viral antigen.

In this study, subsets of each of the individuals' peripheral blood mononuclear cells were obtained by fluorescence-activated cell sorting using CD4, CD8, CD14, and CD19 cell surface markers and DNA from each highly purified cell subpopulation was examined by DNA gene amplification by the polymerase chain reaction (PCR) method. We have found that the CD4+ cell subpopulation (T lymphocytes) is the population that consistently harbors HIV proviral sequences and that there are striking differences between individuals in the number of T cells (CD4+) infected with HIV-1. In 2 of the 10 individuals studied, integrated HIV-1 sequences have also been detected in the CD14+ (monocyte/macrophage) subpopulations. The significance of these findings in the pathogenesis of the disease is discussed.

MATERIALS AND METHODS

Cells and cell preparation. Jurkat is an HIV-1-negative T-cell leukemia line (9). ACH2 is a chronically HIV-1-infected T-cell line containing one integrated copy of the HIV-1 genome (5). Whole blood was collected by leukapheresis or in preservative-free sodium heparin syringes from HIV-1-seropositive volunteers (who had provided written consent). Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque density gradient centrifugation of whole blood, washed twice in phosphate-buffered saline (PBS), suspended at 10^7 cells/ml in PBS containing 3% human AB sera, and incubated for 30 min at 4°C to block Fc receptors on monocytes. One half of the unfractionated PBMC were sorted directly to obtain CD4+ and CD8+ T lymphocytes, and the other half of the cells underwent T-cell depletion by rosetting with β-aminoethylisothiuronium bromide-treated sheep erythrocytes. The cells were then centrifuged at 200 × *g* for 5 min and gently suspended in 20 ml of RPMI 1640. Rosetted and nonrosetted cells were separated by Ficoll-Hypaque density gradient centrifugation. The nonrosetted cells enriched for monocytes and B cells were washed two times in PBS and subjected to cell sorting.

Monoclonal antibodies. PBMC were stained with either fluorescein isothiocyanate (FITC)-conjugated anti-Leu 3a (anti-CD4) or FITC-conjugated anti-Leu 2a (anti-CD8) (Bec-

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ton Dickinson Immunocytometry Systems, Mountain View, Calif.). Rosette-negative PBMC were stained with FITC-conjugated anti-Leu M3 (anti-CD14) or FITC-conjugated anti-Leu 12 (anti-CD19; Becton Dickinson Immunocytometry Systems). All cell preparations were stained according to the instructions of the manufacturer. The background controls for each sort consisted of PBMC stained with a nonreactive monoclonal antibody isotype control.

Cell sorting. Fluorescence-labeled PBMC were sorted with an EPICS C flow cytometer (Coulter Electronics, Hialeah, Fla.) equipped with a 2W argon laser with an excitation wavelength of 488 nm. Sorting logic was controlled by the use of forward angle light scatter versus log green fluorescence bit map gating. Cellular debris, erythrocytes, and cell aggregates were eliminated from sorting by forward angle light scatter. Cells that were reactive with each monoclonal antibody were determined by comparing the fluorescence of cells stained with specific antibody and of cells that had been incubated with FITC-labeled isotype controls. Cells were sorted at a rate of approximately 2,000 cells per second with coincidence abort engaged. Both positively and negatively stained cells meeting the appropriate sort criteria were collected into separate tubes containing sterile PBS. Upon completion of cell sorting, both positive and negative cell populations were analyzed for purity by using machine parameters identical to those for sorting.

Sample preparation. For PCR, highly purified subpopulations of sorted PBMC and unfractionated PBMC from the same individual were washed twice in PBS and the cell pellets were stored at −70°C until use. The cells were lysed in freshly prepared lysis buffer containing 10 mM Tris hydrochloride (pH 8.0), 1 mM EDTA, 0.001% Triton X-100, 0.0001% sodium dodecyl sulfate (SDS), and 600 μg of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml. The lysate was digested at 55°C for 1 h, and the enzyme was heat inactivated by boiling for 15 min. Highly enriched sorted subpopulations were lysed at a final concentration of 2×10^6 cells per ml, unfractionated PBMC at 10^7 cells per ml, Jurkat at 10^7 cells per ml, and a mixture of Jurkat and ACH2 at cell concentrations of 10^7 and 2×10^4 cells per ml, respectively. The cell lysates were stored at 4°C for short periods or frozen at −20°C without any appreciable loss of PCR reactivity.

Oligonucleotides and dNTP. The oligodeoxynucleotides used in this study were synthesized with a DNA synthesizer (Applied Biosystems, Foster City, Calif.) and purified on 20% polyacrylamide gels (18). For amplification of HIV-1 DNA sequences, primer pairs representing the long terminal repeat (LTR) (SK 29/30), *gag* (SK 38/39), and envelope (SK 68/69) from conserved regions of the HIV-1 genome were employed (17, 20). For the detection of the amplified products, their corresponding probes (SK 31, SK 19, and SK 70, respectively) were used. The HLA-DQ alpha gene served as an internal control to monitor the efficiency of DNA amplification using oligonucleotide primers (QH26/27) from the second exon (22). Deoxyribonucleotides and T4 polynucleotide kinase were obtained from Boehringer Mannheim Biochemicals, and [γ-^{32}P]ATP (4,500 Ci/mmol) was obtained from ICN Pharmaceuticals, Inc. (Irvine, Calif.). The probes (1 μg) were end labeled with T4 polynucleotide kinase and 50 μCi of [γ-^{32}P]ATP (18). The unincorporated label was removed by passing the kinase reaction mixture through an Elutip column (Schleicher & Schuell, Inc., Keene, N.H.), and labeled oligonucleotides were eluted with 500 μl of 1 M NaCl in TE buffer.

PCR. The PCR was run essentially as described by Saiki et al., with slight modifications (23). Briefly, 50 μl from each cell lysate (~1 μg of genomic DNA) was directly amplified in a 100-μl final volume of the PCR mixture. The PCR mixture contained 10 mM Tris hydrochloride (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 0.2 mM dGTP, 0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dCTP, 50 pmol of each primer, 200 μg of gelatine per ml, and 2 U of Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.). The samples were overlaid with 100 μl of mineral oil and amplified for 30 cycles in a Thermal Cycler apparatus (Perkin Elmer Cetus) that provided 2-min periods of incubation at 94, 55, and 72°C per cycle. At the end of 30 cycles, the apparatus was programmed to add one extra cycle and polymerization at 72°C was extended for 10 min to ensure completion of the amplified products. When shorter primers were used (SK 29/30), two extra cycles were performed initially at annealing temperatures of 37°C instead of 55°C incubation. This allows the shorter primers to anneal to the target DNA more efficiently, and the subsequent run was performed as described above. After amplification, the aqueous phase was extracted with 100 μl of chloroform and amplified products were analyzed for the sequence of interest. TE buffer (10 μl) containing 0.02 pmol of the appropriate ^{32}P-labeled probe and NaCl to give a final concentration of 0.15 M NaCl was added to 30 μl of the PCR mixture. The samples were overlaid with 50 μl of mineral oil and heated at 94°C for 5 min. The probe was allowed to anneal to the amplified DNA sequences at 56°C for 10 min (1, 24). The hybridization mixture was extracted with 50 μl of chloroform, and 10 μl of the aqueous phase was subjected to electrophoresis on 10% polyacrylamide gels (18). Amplified HIV-1 sequences were visualized by autoradiography. Alternatively, 20 μl of the PCR products of the HLA amplification were analyzed after electrophoresis on composite gels containing 3% NuSieve-1% SeaKem agaroses (FMC Corp., Marine Colloids Div., Rockland, Maine). The amplified products were visualized by ethidium bromide staining.

HIV-1 culture. PBMC (5×10^6 cells) or purified lymphocyte subpopulations (10^6 cells) from HIV-1-seropositive individuals were cultured for 3 days in 5 ml of medium containing phytohemagglutinin (PHA) and were then mixed with 5×10^6 PBMC from healthy HIV-1-negative blood donors that were cultured in the same way. The cultures were maintained at 37°C in a 5% CO_2 atmosphere for 28 days. Normal PBMC (5×10^6) were added to the cultures on days 7 and 14. Every second day, cells in the culture were allowed to settle to the bottom of the flask and 5 ml of culture medium was removed and replaced with fresh medium lacking phytohemagglutinin (PHA) (12, 26). Virus growth was monitored by reverse transcriptase and p24 HIV-1 antigen capture assays in cell-free culture supernatants three times a week.

Virologic and immunologic profile. Serum samples were assayed for HIV-1 antibodies by ELISA (Abbott Laboratories, North Chicago, Ill.), and the antibody reactivity was confirmed by Western blot (Dupont, NEN Research Products, Wilmington, Del.). The HIV-1 p24 antigen was assayed in a solid-phase immunoassay (Abbott Laboratories, Chicago, Ill.). All assays were performed following protocols of the manufacturers. The hematology tests were performed by standard laboratory methods.

RESULTS

Clinical status of the participants. All participants in this study are male homosexuals who are positive for HIV-1 antibodies as determined by ELISA and Western blot as-

says. The total CD4+ counts were normal in 7 of 10 individuals (see Table 3), and remaining laboratory tests were normal. Physical examination was normal with no evidence of lymphadenopathy. The clinical and laboratory picture is of stage II HIV infection, as judged by the CDC classification system (4). These individuals were chosen because of these characteristics and were not randomly selected from a population of HIV-1 seropositive patients.

Viral expression. Although these 10 individuals had high titers of antibodies to HIV-1 by ELISA and Western blot assays, no p24 viral antigens were detected in their sera, nor was HIV-1 expressed in cocultivation assays. To address the possibility that autologous CD8+ cells inhibit HIV-1 production in these culture-negative patients (14, 25), we cocultivated either PHA-treated unfractionated PBMC or fractionated CD4+ or CD8+ lymphocytes with PHA-blasted normal PBMC. Portions of culture fluids were removed three times a week and assayed for reverse transcriptase and p24. The cultures were terminated on day 28. Both reverse transcriptase and p24 assays were negative in all of the samples (data not shown). On the basis of the results, we conclude that neither unfractionated nor CD4+ or CD8+ cells were able to express HIV-1 when cultured in the presence of lymphocytes from healthy individuals. Thus, CD8+ cells do not suppress HIV-1 expression by CD4+ infected cells in individuals who are at an early stage of the disease, although in other studies autologous CD8+ cells have been shown to affect the recovery of virus (14, 25).

PCR. To determine if HIV-1 proviral sequences were present in lymphocytes of the 10 individuals, cell lysates of Ficoll-Hypaque-prepared PBMC were subjected to 30 cycles of polymerase chain amplification with HIV-1-specific primers, and the presence of HIV-1 was detected by HIV-1-specific probes. All 10 individuals contained proviral sequences in their PBMC when tested for envelope sequences (Fig. 1A). There are significant differences in the signal intensity between the individuals, even though equal numbers of PBMC were amplified in each case. Samples 1, 7, 8, 9, and 10 gave strong signals, whereas samples 2 through 6 gave weak signals, indicating that a smaller number of cells are harboring the HIV-1 provirus. This may also reflect variability between the primer-probe sequences and differing viral sequence present in these individuals. Presumably, because of this variability, only those sequences that share close homology with the primer-probe sequences will be detected and generate a strong signal. Such an effect can be partially excluded, since similar results were obtained when different primer sets from the LTR (Fig. 1B) and *gag* region of HIV-1 (Fig. 1C) were used. The differences in intensity of the amplified products more likely reflect a true difference in the number of infected cells within the populations that are being compared. This issue is addressed below.

Cell sorting. In order to answer the question of which subpopulation of PBMC harbors the HIV-1 proviral sequences, PBMC from the 10 individuals were sorted by fluorescence-activated cell sorting with monoclonal antibodies to CD4, CD8, CD14, and CD19 cell surface markers. The purity of CD4+, CD8+, CD14+ and CD19+ cell populations obtained by fluorescence-activated cell sorting was 97.9, 98.4, 96.7, and 96.8%, respectively (Table 1). The purity of the reciprocal subpopulations that were unstained by antibody were all greater than 99%. The sorted cells were lysed to give approximately 2×10^6 cells per ml, whereas unfractionated cells were lysed at 10×10^6 cells per ml and contained approximately the same number of CD4+ cells per milliliter as in the CD4+ sorted pool. PCR was performed directly on 50-μl portions of cell lysates with primers from the LTR, and envelope genes. As seen previously, HIV-1 DNA sequences were present in each of the unfractionated cell populations and in every case in the CD4+ T lymphocyte subpopulations. Representative data with the *env* probe are shown in Fig. 2A and B, and the complete results with env and LTR are presented in Table 2. The absence of a positive signal in the CD4− population demonstrates that the CD4+ cell is the subpopulation infected by HIV-1. When cells were sorted for CD8+, it was the negatively stained CD8− population that was positive by PCR; it mainly contained CD4+ T cells. Similarly, no HIV viral sequences were detected in B lymphocytes (CD19+). The CD19− subpopulation was occasionally positive when T cells were not removed efficiently by rosetting (Fig. 2A). In two cases, however, CD14+ (monocyte/macrophage) cells were positive by PCR (a representative sample is shown in Fig. 2B). There was no evidence of any T-cell contamination in the CD14+ subsets when these cells were restained. Most T cells were removed prior to fluorescence-activated cell sorting analysis by sheep erythrocyte rosetting and by excluding T cells during the sorting procedure. Therefore, the positive PCR signal in CD14+ cells is indicative of HIV that

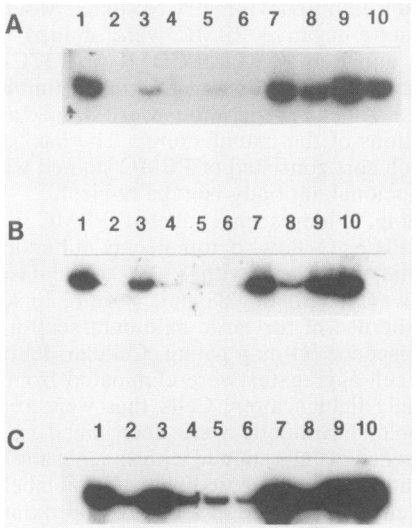

FIG. 1. Analysis of PCR-amplified HIV-1 sequences from healthy, seropositive individuals. Cell lysates corresponding to 5×10^5 PBMC were amplified with HIV-1-specific primers from envelope (SK 68/69) (A), LTR (SK 29/30) (B), and *gag* (SK 38/39) (C). The specific amplified sequences were detected by liquid hybridization with ^{32}P-labeled probes SK 70, SK 31, and SK 19, respectively. The heteroduplex product representing less than 10% of the PCR material was analyzed on 10% polyacrylamide gels, and autoradiography was performed at −70°C for 4 h.

TABLE 1. Purity of sorted PBMC subpopulations

Cell type	Mean % ± SD[a]	
	Positive fraction	Negative fraction
CD4+	97.9 ± 1.0	99.5 ± 0.3
CD8+	98.4 ± 0.7	99.4 ± 0.4
CD14+	96.7 ± 2.5	99.5 ± 0.3
CD19+	96.8 ± 1.5	97.8 ± 1.4

[a] Mean percentage ± standard deviation based on 10,000 cells analyzed per sample for 10 participants.

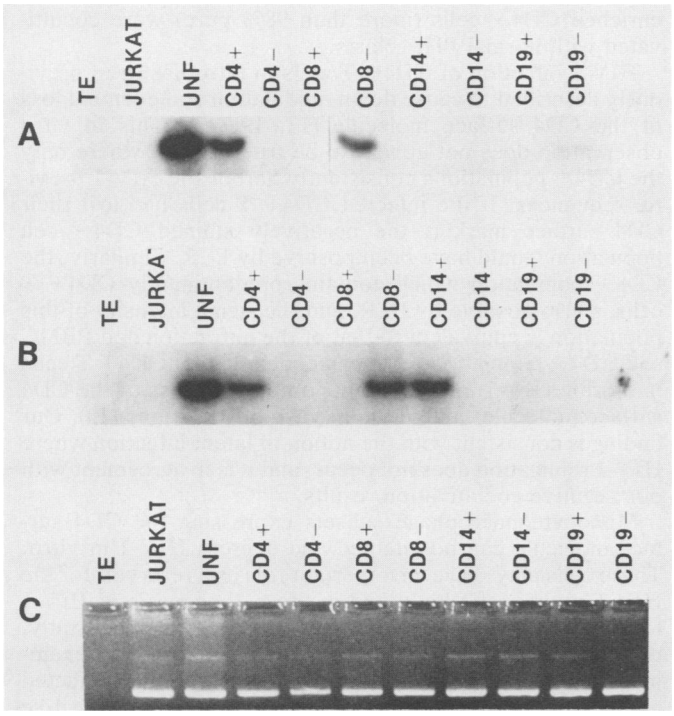

FIG. 2. Amplification of sorted PBMC. Unfractionated PBMC ([UNF] 5×10^5 cells per reaction) and their respective sorted subsets (10^5 cells per reaction) were amplified by using HIV-1 envelope primers (SK 68/69) (A and B) and HLA-DQ alpha primers (QH 26/27) (C). HIV-1-amplified DNA was detected with SK70 ^{32}P-labeled probe by liquid hybridization and analyzed on 10% polyacrylamide gels. Autoradiography was performed at −70°C for 16 (A) and 72 (B) h. All 10 individuals were studied in this way. Gel A represents one individual (subject 8) with only a CD4+ subpopulation positive for HIV-1, and gel B represents one individual (subject 5) with both the CD4+ and CD14+ subsets positive. Gel C represents the analysis of 1 in 10 of the PCR product (for subject 8) amplified for HLA-DQ alpha sequences and fractionated on 3% Nuseave–1% agarose gel stained with ethidium bromide. Jurkat is an HIV-1-negative cell line, and TE represents an amplification containing all reagents except DNA that serves as a negative control.

TABLE 2. PCR results on unfractionated PBMC and sorted subsets tested with primers from three conserved regions of HIV-1

Subject	Positive (+) or negative (−) results for indicated cells								
	Unfractionated			CD4+		CD4−		CD14+[a]	
	env[b]	LTR	*gag*	*env*	LTR	*env*	LTR	*env*	LTR
1	+	+	+	+	+	−	−	+	−
2	+	+	+	+	+	+	−	−	−
3	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	−	−	−	−
5	+	+	+	+	+	−	−	+	−
6	+	+	+	+	+	−	−	−	−
7	+	+	+	+	+	−	−	−	−
8	+	+	+	+	+	−	−	−	−
9	+	+	+	+	+	−	−	−	−
10	+	+	+	+	+	−	−	−	−

[a] The reciprocal CD14− population was occasionally positive because this subset often contains CD4+ cells that remained after the sheep red blood cell procedure.
[b] *env*, Envelope region of HIV-1.

is present in a population of monocyte/macrophage subpopulations of these two individuals. For each sample that was examined, the quality of DNA lysates and the efficiency of PCR amplification was evaluated by measuring the HLA amplification (Fig. 2C).

Viral load. In order to estimate the number of cells infected with HIV-1, crude lysates of PBMC from the 10 individuals were serially diluted in TE buffer and PCR was performed (Fig. 3A). Purified CD4+ T cells were similarly analyzed for four of these individuals (Fig. 3B). The signal intensity from each amplification was compared with serial dilutions of a mixture of Jurkat cells and ACH2 cells (1×10^7:2×10^4 cells per ml). The latter serves as a standard in which each ACH2 cell contains one integrated copy of HIV-1 (5). The results demonstrate that there is indeed a difference in the intensities reflecting the number of infected cells in each individual (Table 3). In the analyses of PBMC, the viral load ranged from 1 infected cell in 500 to 1 in 150,000. Four individuals had a viral burden of 1:500 infected cells, which would correspond to 1 in 100 CD4+ cells (Table 3), a value that is similar to the burden of individuals with AIDS. Values for the number of infected CD4+ cells were

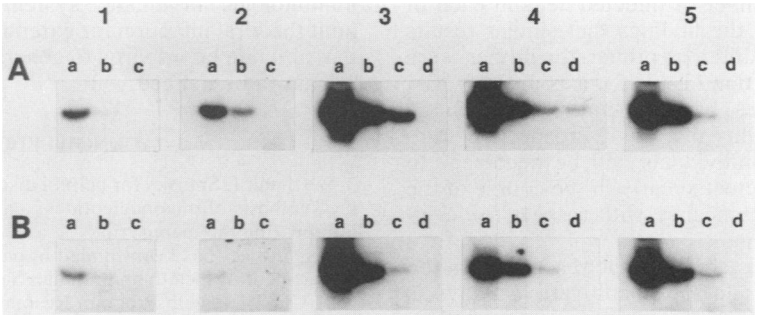

FIG. 3. PCR amplification of envelope sequences of representative 10-fold dilutions of PBMC and their respective CD4+ T cells. (A) Lysates of PBMC (10^7 cells per ml) were serially diluted in TE to represent 5×10^5 (a), 5×10^4 (b), 5×10^3 (c), and 5×10^2 (d) cells per amplification (gels 1 through 4). A mixture of Jurkat:ACH2 (1×10^7:2×10^4 cells per ml) were also included as control and diluted to represent 10^3 (a), 10^2 (b), 10 (c), and 1 (d) ACH2 cells per amplification (gel 5). (B) Lysates from purified CD4+ T cells (2×10^6 cells per ml) diluted in TE to represent 10^5 (a), 10^4 (b), 10^3 (c), and 10^2 (d) cells per amplification (gels 1 through 4). Gel 5 represents dilution of Jurkat:ACH2 as described above. All PCR amplfications and detection of amplified envelope sequences were performed as previously described. The cell lysates were from subjects 5, 6, 7, and 10 (gels 1 through 4, respectively). Autoradiography was performed at −70°C for 18 h.

TABLE 3. Estimated number of latently infected T cells

Subject[a]	No. of T4 cells/μl of blood	% T4[b]	Ratio of infected: uninfected PBMC[c]	% Infected T4 cells[d]
1	637	38	1:500	0.7
2	1,033	34	1:150,000	0.003
3	661	27	1:5,000	0.1
4	222	22	1:5,000	0.1
5	770	28	1:15,000	0.03
6	574	41	1:5,000	0.07
7	646	46	1:500	0.6
8	670	30	1:1,500	0.3
9	333	20	1:500	1.3
10	335	28	1:500	1.0

[a] For each subject, p24 antigen capture, ELISA, and Western blot analyses were carried out. In all subjects, p24 was not detected and strong positive Western blots and ELISAs were observed. Virus could not be rescued from lymphocytes after cocultivation with normal PBMC.

[b] Percentage of T4 cells in the total T lymphocyte count.

[c] Ratio of cells estimated to be infected by PCR from serial dilutions of PBMC cell lysates to control cell line ACH2. Results represent the average of two independent experiments.

[d] The estimated percentage of infected T4 cells, assuming that 75% of PBMC contain T lymphocytes.

similar when PCR was carried out with either PBMC or purified CD4+ cells.

DISCUSSION

We have studied 10 HIV-1-seropositive homosexual males, all of whom are healthy as judged by physical examinations and clinical and laboratory tests. We surmise that these individuals demonstrate an early HIV-1 infection, although the time of infection cannot be determined. On the basis of the lack of HIV-1 viral antigens (p24) and the inability of their PMBC to infect target leukocytes in tissue culture, they are not actively expressing virus or viral expression is below the level detected by cocultivation assays. By PCR, a powerful and sensitive technique, we have demonstrated that each of these individuals has integrated proviral HIV-1 sequences in PBMC. Of the 10 people studied, 5 gave strong PCR signals comparable in intensity to that seen in patients with AIDS. The other five individuals gave weaker signal intensities, and in one case (Fig. 1A, lane 2) the signal was just detectable. Since an equal number of cells were amplified from each person, this represents differences in the relative number of infected cells in each of the individuals. In view of the findings that similar results were obtained when three different primers or probes were used for the PCR amplification (Table 2), it is unlikely that the difference in intensities between individuals can be attributed to genetic variability of HIV-1. Prospective studies on larger numbers of individuals will be necessary to establish if the virus load detected early in the course of the disease is of prognostic significance with respect to development of clinical symptoms.

The observation that virus could not be rescued from the PBMC of these individuals in the cocultivation assays is further evidence that either none or only an extremely low number of infected cells are actively expressing HIV-1. The absence of clinical symptoms may be directly related to what appears to be a quiescent infection. In contrast to other studies (14, 25), our failure to detect virus is not because suppressor cells (CD8+) were inhibiting viral production, since we were also unable to rescue virus when highly enriched CD4+ cells (more than 98% pure) were cocultivated with normal PHA blasts.

HIV-1 infection of CD4+ T cells in vitro has been previously described to cause down modulation and eventual loss of the CD4 surface molecule (13, 19, 27). This in vitro observation does not appear to be true in vivo, where only the CD4+ populations consistently harbor the HIV-1 proviral sequences. If the infected CD4+ T cells had lost their CD4 surface marker, the negatively stained CD4− cell population would have been positive by PCR. Similarly, the CD8− population which contains predominantly CD4+ T cells is also positive by PCR, and the signal intensity of this population is equivalent to that of the unfractionated PBMC and CD4+ populations. We can conclude that CD4+ T cells harboring HIV-1 in vivo show no apparent loss of the CD4 surface molecule, unlike the in vitro observations (13). Our finding is consistent with the notion of latent infection where HIV-1 replication does not occur, and it is in agreement with our negative cocultivation results.

Monocyte/macrophage subsets expressing the CD4 surface molecule can be infected and express HIV-1 in vitro. These cells may serve an important role as a reservoir for the HIV-1 virus in the body and in the pathogenesis of HIV-1 infection (3, 7, 8, 10, 11, 16). However, in this study, utilizing highly enriched CD14+ monocytes that were examined by PCR, only 2 of 10 individuals have HIV-1-infected monocytes, and in both cases there was a weak positive signal. From our data, it is evident that the CD4+ T cell is the predominant cell population that harbors the HIV-1 provirus in the peripheral blood circulation of healthy, seropositive individuals early in the disease.

Comparing PCR results obtained with serial dilutions of PBMC from these healthy seropositive individuals and the control cell line ACH2, a quantitative estimation of the number of infected cells is possible. Assuming that 75% of PBMC are T lymphocytes, we can calculate that there is a range from 0.003 to 1% of the CD4+ T cells that harbor the HIV-1 provirus, compared with results with sera from patients with AIDS, where 1% of the CD4+ T cells is infected (unpublished results). While some healthy HIV-1 seropositive individuals have values comparable to those seen in patients with AIDS, others have a 10- to 500-fold-lower number of infected cells. Low levels of infected CD4+ T cells and low levels of HIV-1 expression may primarily be attributed to interactions of viral regulatory genes with other viral or cellular factors. At an early stage of the disease, with a low frequency of infected cells, the cellular and humoral immunologic surveillance system of the body may be able to limit the viral infection for extended periods of time. Factors that activate latent virus to generate cycles of lytic infection remain to be defined.

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