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Immunology

Highly purified CD25$^-$ resting T cells cannot be infected *de novo* with HIV-1

CHIN-SHENG CHOU, OCTAVIO RAMILO, AND ELLEN S. VITETTA

The Cancer Immunobiology Center, and the Departments of Microbiology and Pediatrics, The University of Texas Southwestern Medical Center, Dallas, TX 75235

Contributed by Ellen S. Vitetta, December 9, 1996

ABSTRACT **Previous studies have demonstrated that the expression of CD25 can distinguish CD25$^-$ latently infected cells from CD25$^+$ cells actively producing virus. Our studies were designed to characterize the nature and stability of the viral genome in CD25$^-$ quiescent HIV-1-infected cells and to determine whether these cells could be infected *de novo* with HIV-1. Our results show that: (*i*) When unfractionated peripheral blood mononuclear cells are first infected with HIV-1 and the CD25$^-$ cells then isolated, the latter contain only incomplete DNA transcripts and no full-length DNA or 2-LTR circles. Phytohemagglutinin activation of these CD25$^-$ cells results in the generation of full-length viral DNA and p24 production. (*ii*) When CD25$^-$ CD4$^+$ cells are first purified from peripheral blood mononuclear cells and then incubated with HIV-1, viral DNA cannot be detected, suggesting that these purified cells cannot be infected. Furthermore, CD25$^-$ adherent cells do not facilitate the infection of CD4$^+$ CD25$^-$ T cells when they were present at the time of incubation with HIV-1. Taken together, these studies suggest either that (*i*) the CD25$^-$ cells containing incomplete DNA transcripts are derived from infected-activated CD25$^+$ cells, which subsequently become CD25$^-$ or (*ii*) the presence of CD25$^+$ cells is required for the infection of CD25$^-$ cells *in vitro*.**

Using a model of acute *in vitro* infection, we have previously demonstrated that the expression of CD25 can distinguish latently infected cells (CD25$^-$) from cells actively producing virus (CD25$^+$) (1) and that these two populations display significant differences with regard to the nature of the proviral genome. Thus, by combining the use of a potent anti-CD25 immunotoxin and sensitive immunofluorescence staining we have obtained highly purified resting cells. This is important because interpretations of viral latency have been confounded, in part, by the degree of purity of the resting cells. Indeed, partially reverse-transcribed DNAs (2, 3), full-length extrachromosomal DNA (4, 5), and stable integrated viral genomes (6) have been reported to occur in resting peripheral blood mononuclear cells (PBMCs). Although these differences can be explained in part by the different methodologies employed, they could also suggest the existence of several subsets of latently infected cells and possibly the contamination of resting cells with activated ones.

Using our *in vitro* model of acute HIV-1 infection, the present studies were designed to characterize the nature and stability of the viral genome in quiescent HIV-1-infected CD25$^-$ cells and to determine whether CD25$^-$ cells can be infected *de novo*. The results of these analyses have implications regarding the origin of latently infected cells.

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MATERIALS AND METHODS

Virus Strain and Standard Inoculum. HIV-1$_{JR\text{-}CSF}$ is a clinical strain provided by the National Institutes of Health AIDS research and reference reagent program (7). The virus was cultured with phytohemagglutinin (PHA)-activated PBMCs for 6 days. The culture supernatant was purified on an Amicon concentrator (Centriprep 100) by centrifugation at 500 × *g* at 4°C for 1 hr to remove cytokines and mitogens (M_r <100 kD). This standard inoculum was adjusted to 10 times its original concentration, aliquoted, and stored at −80°C.

Purification of CD4$^+$ CD25$^-$ Human Quiescent T Lymphocytes. Fresh PBMCs were obtained from normal healthy donors by centrifugation over Ficoll/Hypaque and were cultured in RPMI 1640 supplemented with 5% autologous plasma [complete-medium (CM)] for 18 hr to remove peripheral blood macrophages by plastic adhesion. Adherent cells were used for additional experiments. The nonadherent cells were further cultured under the same conditions for 2 hr, and were treated with 5 mM leucine methyl ester in RPMI 1640 for 1 hr at 25°C to further deplete the macrophages and natural killer cells (8). CD4$^+$ T cells were purified by positive selection on anti-CD4-coated magnetic beads (Dynal, Great Neck, NY) according to the instructions of the manufacturer. By trypan blue staining the viability of purified CD4$^+$ T cells was >99%. A highly purified quiescent CD4$^+$ CD25$^-$ T-cell population was obtained by culturing CD4$^+$ cells for 6 days in CM containing 100 nM RFT5-dgA, a potent immunotoxin that kills CD25$^+$ cells (9).

Infection of CD4$^+$ T Cells with HIV-1. Unfractionated CD4$^+$ T cells and purified CD4$^+$ CD25$^-$ quiescent T cells were exposed separately to a standard inoculum of HIV-1 for 2 hr and then thoroughly washed and treated for 7 min with trypsin (25 μg/ml) at 37°C to remove attached virus. After washing with PBS, cells were cultured for 6 days either in CM or in CM containing 100 nM RFT5-dgA. On day 7 cells and supernatants were harvested for polymerase chain reaction (PCR) and for p24 antigen assays.

P24 Antigen Assay. The production of p24 antigen in cell-free supernatants was measured using a commercially available immunoassay kit (DuPont).

Intact Cell PCR. Approximately 10^6 cells were washed and pelleted in a 0.5-ml thin-walled thermal cycle tube and then frozen at −20°C for 2 hr. Cells were shocked by adding 15 μl of DNase-free water, heated at 103°C for 4 min, and chilled at 0°C for 3 min. The PCR mix (34.5 μl) containing 5 μl of 10 × *Taq* buffer (500 mM KCl/100 mM Tris·HCl, pH 8.3/1% Triton X-100); 3 μl of 25 mM $MgCl_2$; 2 μl of acetylated BSA (2 μg/μl); 5 μl of dNTP mixture with 2.5 mM each of dATP, dTTP, dGTP, and dCTP; 9.5 μl of DNase-free water; and 5 μl of each primer (5 μM) was added. The mixture was covered with 50 μl of mineral oil and preheated at 95°C for 1 min and maintained at 80°C in a Ericomp thermal cycler. *Taq* (0.5 μl) (*Thermus aquaticus*) DNA polymerase (5 units per μl) was

Abbreviations: PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; CM, complete medium.

added to the bottom of each tube and each sample was heated to 95°C for 1 min and then amplified according to the specific PCR program designed for each individual DNA template listed below. At the end of the PCR cycle, polymerization was completed at 72°C for 10 min. PCR products were analyzed on 3.5% agarose gels. With this technique we can consistently detect 2 copies of HIV-1 proviral DNA in 20,000 cells.

Primer Pairs and PCR Programs. The sequences of most primer pairs and PCR programs have been described (1). Other primer pairs used in our studies included: (*i*) Nef (217 bp): sense, 5′-TGACTTACAAGGCAGCTATAGATC; antisense, 5′-CTCTGGATCAACTGGTACTAG; (*ii*) 2-LTR (536 bp): sense, 5′-CCTTTTAGTCAGTGTGGAAAATCTCTAGCA; antisense, 5′-CAGTGGGTTCCCTAGTTAGC. The programs used with the primer pairs were identical to those used previously (1), with the exception of an annealing temperature of 63°C for the 2-LTR.

RESULTS

Characterization of Quiescent HIV-1-Infected CD25⁻ PBMCs. We have previously shown that highly purified HIV-1-infected quiescent cells can be isolated following infection of PBMCs and elimination of productively infected cells with a potent anti-CD25 immunotoxin (RFT5-dgA) (1, 9). Thus, when PBMCs containing both CD25⁺ and CD25⁻ cells were infected with HIV-1 and then treated with RFT5-dgA for 6 days to eliminate the CD25⁺ cells, we obtained a population of resting cells in which viral production was undetectable by p24 assays (<5 pg/ml). As shown in Fig. 1*A*, unfractionated PBMCs (lanes 1 and 6) contained both incomplete DNA transcripts (RU5) and full-length viral DNA. CD25⁻ PBMCs contained incomplete DNA transcripts (lane 5) but no full-length viral DNA (lane 10). When an increasing number of the originally infected, unfractionated PBMCs were added to the RFT5-dgA-treated, CD25⁻ cells, increasing amounts of full-length viral DNA were detected by PCR (lanes 9, 8, and 7); the addition of only 0.01% of the originally infected PBMCs to the CD25⁻ cells was sufficient to demonstrate the presence of full-length viral DNA (lane 9). These results show that CD25⁻ PBMCs contain partially reverse-transcribed viral DNA and suggest that the viral replication cycle is incomplete in CD25⁻ resting cells.

The presence of 2-LTR circles generally defines nuclear localization of reverse-transcribed double-stranded DNA of a replicating retrovirus (10, 11). Recent reports have described 2-LTR circles in quiescent PBMCs (4). Hence, we also determined whether CD25⁻ cells contained 2-LTR species. As shown in Fig. 1*B*, we could not detect 2-LTR circles in purified CD25⁻ PBMCs (lane 10). However, 2-LTR circles were demonstrated after the addition of 0.1% of the originally infected PBMCs (lane 8). These results confirm our previous reports that the CD25⁺ cells are the cells actively producing virus (1, 9), and indicate that the CD25⁻ PBMCs contain incomplete DNA transcripts, but not full-length viral DNA. These findings further underscore the importance of obtaining a highly purified resting cell population to study the viral genome.

Stability of the Viral Genome in CD25⁻ PBMCs. We next determined whether the incomplete viral DNA transcripts present in CD25⁻ PBMCs were functional and stable. To this end, RFT5-dgA was removed from the cultures and the CD25⁻ cells were activated with PHA (5 μg/ml) for 6 days. As shown in Fig. 2, significant amounts of HIV-1 proviral DNA were detected in these cells, including early DNA transcripts (RU5), intermediate species (Nef, tat/rev, gag, RU5-pbs, and RU5-pbs-gag), and full-length viral DNA. Virus production was restored from undetectable levels (<5 pg/ml) in quiescent cells to 40–51 pg/ml after activation. These results indicate that although only incomplete viral transcripts are present in quiescent CD25⁻ PBMCs, these cells can make complete virions after activation. Hence, in this *in vitro* experimental system, the CD25⁻ cells are a functional latent viral reservoir.

The Ability of HIV-1 to Infect CD4⁺ CD25⁻ Quiescent T Lymphocytes. Having characterized quiescent HIV-1-infected CD25⁻ PBMCs, we next determined whether CD25⁻ cells could be infected *de novo*. If not, it would suggest that HIV-1-infected CD25⁻ cells might be derived from infected, activated CD4⁺ CD25⁺ cells, which subsequently became CD25⁻. In these experiments, CD4⁺ CD25⁻ quiescent T lymphocytes were isolated from fresh PBMCs by first removing macrophages and natural killer cells, and then by positively selecting CD4⁺ cells. CD25⁺ cells were then eliminated by treatment with RFT5-dgA. In three experiments, the highly purified CD4⁺ CD25⁻ quiescent T-cell population contained 98.5–99.4% CD3⁺ cells, 97.8–98.3% CD4⁺ cells, <0.2% CD14⁺ cells, and <1.1% CD8⁺ cells (data not shown). These

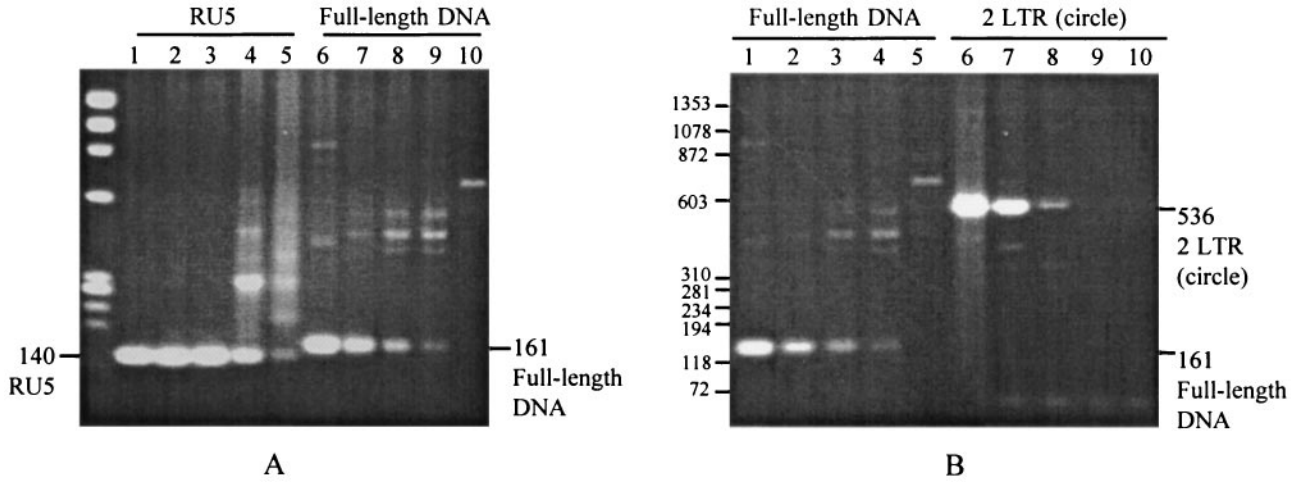


FIG. 1. Analysis of HIV-1-infected unfractionated and CD25⁻ resting PBMCs. (*A*) The proviral genome. PBMCs were infected on day 1 and then incubated with either CM or CM plus RFT5-dgA for 6 days. Following treatment, 10^6 viable cells were collected from unfractionated (A: lanes 1 and 6) or CD25⁻ (B: lanes 5 and 10) PBMCs. From 10^2 to 10^4 unfractionated PBMCs (A) were added to 10^6 CD25⁻ PBMCs (B) as follows: B + 0.01% A in lanes 4 and 9; B + 0.1% A in lanes 3 and 8; B + 1% A in lanes 2 and 7. The proviral genome in cells from each group was studied by intact-cell PCR as described. Lanes 1–5 depict HIV-1 early DNA transcripts (RU5) and lanes 6–10 show HIV-1 full-length DNA. (*B*) HIV-1 full-length DNA and 2-LTR circles. PBMCs were prepared as described in *A*. Lanes 1–5 show full-length DNA and lanes 6–10 show 2-LTR circles. The cell-free culture supernatant was collected from each group of cells and assayed for p24 antigen. p24 concentrations were as follows: lane 1, 36,680 pg/ml; lane 2, 153 pg/ml; lane 3, 22 pg/ml; lane 4, <5 pg/ml; lane 5, <5 pg/ml.

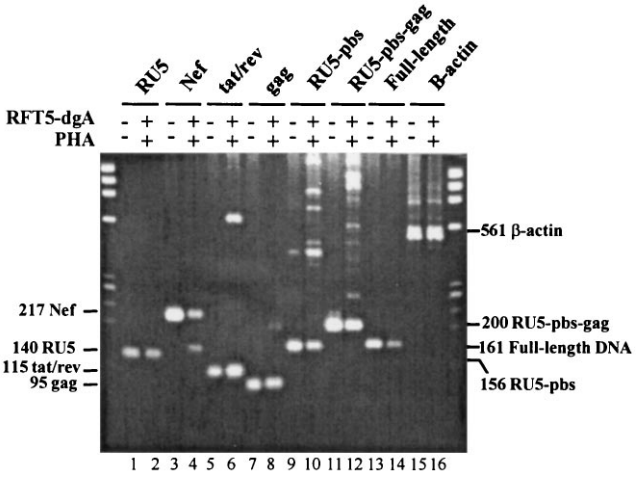


FIG. 2. PHA activation of CD25$^-$ PBMCs. CD25$^-$ quiescent PBMCs were purified by treatment with RFT5-dgA for 6 days. Cells were then thoroughly washed to remove RFT5-dgA and cultured in CM containing 5 μg/ml PHA. Intact cells were harvested 6 days after PHA stimulation and subjected to intact-cell PCR as described. HIV-1-infected unfractionated PBMCs were used as controls and β-actin served as the internal control. PHA-stimulated HIV-1-infected CD25$^-$ quiescent cells are shown in lanes 2, 4, 6, 8, 10, 12, 14, and 16. Unfractionated HIV-1 infected control cells are shown in lanes 1, 3, 5, 7, 9, 11, 13, and 15.

cells were exposed to HIV-1 for 2 hr, washed thoroughly to remove excess virus, treated with trypsin to remove the virions attached to the cell surface, and then cultured for an additional 6 days. As shown in Fig. 3, CD4$^+$ CD25$^-$ cells contained neither incomplete viral DNA transcripts nor full-length viral DNA. In contrast, HIV-1-infected, unfractionated CD4$^+$ T cells contained both incomplete viral DNA transcripts and full-length viral DNA. These results suggest that *in the absence of other cells*, the purified CD4$^+$ CD25$^-$ T lymphocytes cannot be infected with HIV-1.

The Role of Adherent Cells in HIV-1 Infection. Having demonstrated that HIV-1 did not infect purified quiescent CD4$^+$ CD25$^-$ cells, we next determined whether the addition of adherent cells would facilitate infection. Thus, the adherent cell population present in PBMCs might be critical in facilitating the transmission of HIV-1 to CD4$^+$ or other cells either by acting as accessory cells, by becoming infected and then transmitting the virus, or by producing cytokines that facilitate infection. To this end, four different samples were prepared: (*i*) unfractionated CD4$^+$ T cells, (*ii*) quiescent CD25$^-$ CD4$^+$ T cells, (*iii*) unfractionated adherent cells, and (*iv*) CD25$^-$ adherent cells. Cells were exposed to HIV-1, washed thoroughly, treated with trypsin, and then cultured for an additional 6 days as shown in Fig. 4*A*. PCR analysis showed that unfractionated CD4$^+$ cells contained significant amounts of HIV-1 provirus, including both incomplete DNA transcripts and full-length DNA (Fig. 4*A*, lanes 1 and 1′), and that CD4$^+$ CD25$^-$ cells (lanes 2 and 2′) did not contain any viral transcripts. Likewise, we could not detect any proviral genomic species in the CD25$^-$ adherent cells (lanes 4 and 4′), even after these cells were activated with PHA following exposure to HIV-1. Importantly, the CD25$^-$ adherent cells did not facilitate the transmission of HIV-1 to CD4$^+$ CD25$^-$ T cells (lanes 3 and 3′). Because it was difficult to thoroughly deplete adherent cell-bound CD4$^+$ T cells from unfractionated adherent cells, unfractionated adherent cells that were subsequently stimulated with PHA were also included in this set of experiments. As shown in Fig. 4*A*, significant amounts of proviral genome could be revealed in the unfractionated adherent cells after PHA stimulation, suggesting the presence of CD4$^+$ T cells in the unfractionated adherent cell population (lanes 5 and 5′). These results indicate that under the experimental conditions employed, highly purified quiescent CD25$^-$ adherent cells do not become infected with HIV-1, and are unable to transmit HIV-1 to CD25$^-$ CD4$^+$ T cells.

We next determined whether the presence of adherent cells at the time of incubation with HIV-1 could facilitate the infection of the CD4$^+$ CD25$^-$ cells. To this end, fresh PBMCs were treated with RFT5-dgA for 6 days to generate quiescent CD25$^-$ PBMCs (containing both quiescent adherent and quiescent CD4$^+$ T cells) washed thoroughly and then exposed to HIV-1. As shown in Fig. 4*B*, unfractionated PBMCs contain both RU5 transcripts and full-length viral DNA as a result of a successful infection. In contrast, we could not demonstrate the presence of viral transcripts in the HIV-1-exposed, quiescent CD25$^-$ PBMCs. Hence, CD25$^-$ adherent cells did not facilitate the infection of CD4$^+$ CD25$^-$ T cells even when they were present at the time of incubation with HIV-1.

Taken together, these experiments suggest that CD25$^-$ adherent cells do not play a major role in transmitting the virus or facilitating the infection of purified CD4$^+$ CD25$^-$ nonadherent T cells. No proviral genome was detected in CD25$^-$ adherent cells even after PHA activation, even though unfractionated adherent cells (containing both CD25$^+$ and CD25$^-$ cells) were infected by HIV-1 and produced virus following PHA activation. It is therefore possible that the CD25$^+$, PHA-inducible cells that become infected by HIV-1 are

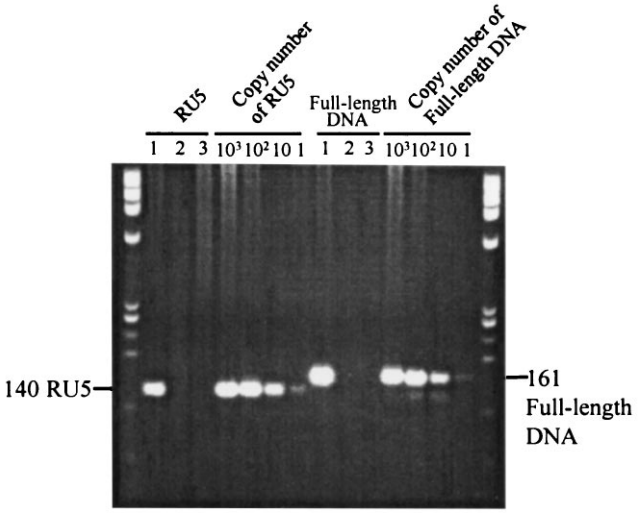
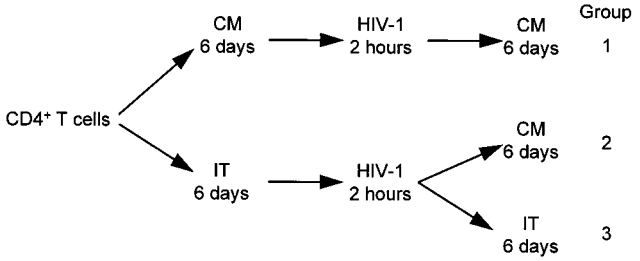


FIG. 3. Infection of CD4$^+$ CD25$^-$ quiescent T lymphocytes with HIV-1. CD4$^+$ CD25$^-$ quiescent T lymphocytes were isolated from fresh PBMCs by removing blood macrophages and natural killer cells, followed by positive selection of CD4$^+$ cells, and elimination of CD25$^+$ cells by treatment with RFT5-dgA as described. Purified quiescent CD4$^+$ CD25$^-$ T cells were exposed to HIV-1 for 2 hr, washed thoroughly to remove excess virus, treated with trypsin to remove virions attached to the cell surface, and cultured in either CM (lane 2) or CM + RFT5-dgA (lane 3) and HIV-1-infected unfractionated CD4$^+$ cells were included as controls. Six days later, cells were harvested for intact-cell PCR and cell-free culture supernatants were assayed for p24 production. (1: 17,740 ± 2, 470 pg/ml; both 2 and 3 were <5 pg/ml.) The protocol schematic was as follows:



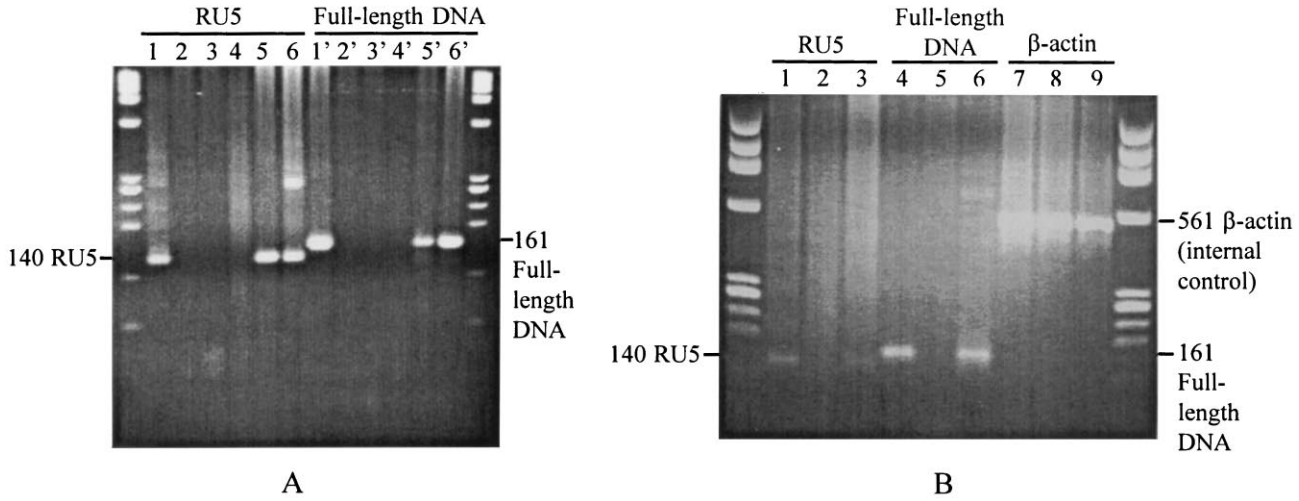
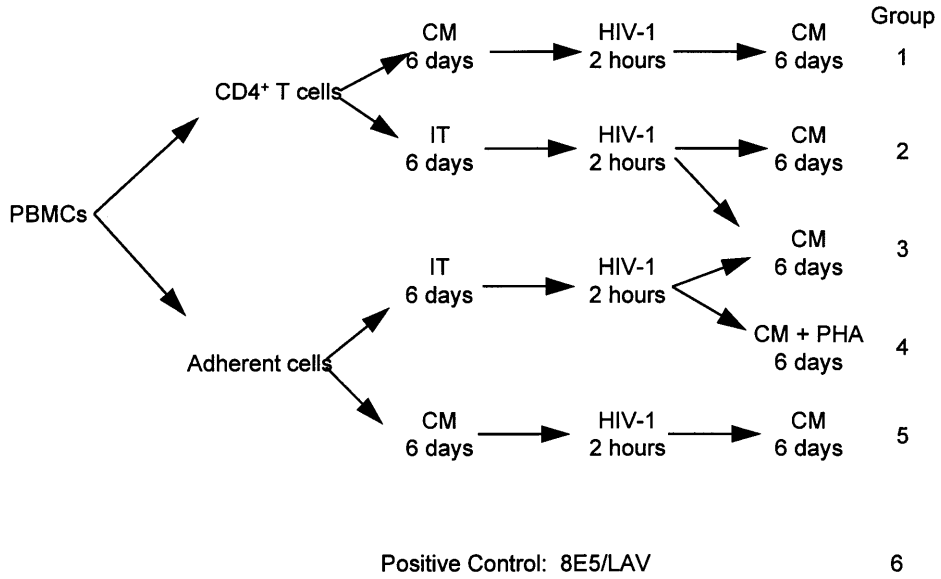


FIG. 4. (*A*) The role of adherent cells in acute HIV-1 infection. To determine whether the adherent cells present in PBMCs could facilitate the infection of $CD4^+$ cells, different groups of cells were prepared and incubated with HIV-1 as described in the protocol schematic: (1) unfractionated $CD4^+$ T cells (lanes 1 and 1′); (2) quiescent $CD25^-$ $CD4^+$ T cells (lanes 2 and 2′); (3) $CD25^-$ adherent cells plus quiescent $CD25^-$ $CD4^+$ T cells (lanes 3 and 3′); (4) $CD25^-$ adherent cells (lanes 4 and 4′); (5) unfractionated adherent cells (lanes 5 and 5′). Cells from each group were subjected to intact-cell PCR and lanes 1–6 show HIV-1 early transcripts (RU5) and lanes 1′–6′ show HIV-1 full-length DNA. The protocol schematic was as follows:



(*B*) The role of adherent cells in the infection of $CD25^-$ cells with HIV-1. Fresh PBMCs were prepared as described. PBMCs were cultured either in CM (lanes 1, 4, and 7) or in CM + RFT5-dgA (lanes 2, 5, and 8) for 6 days, and then exposed to HIV-1 for 2 hr and cultured in CM for 6 additional days, and then harvested and analyzed by intact-cell PCR. The 8E5/LAV cell line was used as positive control (lanes 3, 6, and 9). Lanes: 1–3, early transcripts (RU5); 4–6, full-length DNA; 7–9, β-actin as internal control.

adherent cell-bound $CD4^+$ T cells. Thus, the activation status of the target $CD4^+$ T cell (e.g., $CD25^+$ vs. $CD25^-$) is significantly more important than the presence of adherent cells in determining whether HIV-1 can infect a target cell.

DISCUSSION

Previous studies have demonstrated that the expression of CD25 can distinguish $CD25^-$ latently infected cells from $CD25^+$ cells actively producing virus (1, 9). Our studies were designed to characterize the nature and stability of the viral genome in quiescent HIV-1-infected $CD25^-$ cells and to determine whether these cells could have been derived from either uninfected resting, $CD4^+$ $CD25^-$ cells, which became infected by HIV-1, or from infected activated $CD4^+$ $CD25^+$ cells, which subsequently became $CD25^-$.

The major findings to emerge from our studies are: (*i*) If PBMCs were first infected with HIV-1 and the $CD25^-$ cells were then isolated, the latter contained only early viral transcripts and no full-length DNA or 2-LTR circles. PHA activation of these $CD25^-$ cells results in the generation of full-length viral DNA, and the restoration of p24 production. (*ii*) If $CD25^-$ $CD4^+$ cells were first purified and then incubated with HIV-1, viral transcripts could not be detected, suggesting that these purified cells could not be infected. (*iii*) The presence of $CD25^-$ adherent cells did not facilitate the infection of $CD4^+$ $CD25^-$ T cells even when they were present at the time of incubation with HIV-1. Taken together, our studies suggest either that some other cell (or its product) in the PBMC population is required for the infection of $CD25^-$ cells and/or that $CD25^-$ cells containing early viral transcripts are derived from infected $CD25^+$ cells. With regard to the first possibility, the presence of adherent $CD25^-$ cells did *not* result

in infection of $CD4^+$ $CD25^-$ cells even when these cells were present at the time of incubation with the virus. However, a successful HIV-1 infection could be demonstrated in cultures of unfractionated adherent cells, suggesting that the infection occurred because of the presence of cell-bound $CD25^+$, PHA-inducible T cells, and/or cytokines secreted by these cells.

There has been a great deal of controversy concerning the ability of HIV-1 to infect resting T cells. Earlier reports (12–15) indicated that HIV-1 could not infect resting T lymphocytes, but more recent studies (2–5) using highly sensitive PCR analysis have suggested that HIV-1 can infect resting cells. Thus, Montagnier *et al.* (12) and McDougal *et al.* (13) reported that HIV-1 could bind to resting $CD4^+$ cells, and Zack *et al.* (2, 3), Stevenson *et al.* (4), and Spina *et al.* (5) detected the entry of HIV-1 into resting lymphocytes. Zack *et al.* (2, 3) detected labile partially reverse-transcribed proviral DNA intermediates, whereas Stevenson *et al.* (4), and Spina *et al.* (5) reported the presence of relatively stable full-length unintegrated forms of DNA. In contrast, Zagury *et al*. (14), and Montagnier *et al.* (12) demonstrated that T-cell activation was required for viral gene expression and production of infectious viruses. Gowda *et al.* (15) could not detect the presence of HIV-1 DNA in fresh, unstimulated $CD4^+$ T cells that had been exposed to the virus for periods as long as 48 hr.

None of these investigators addressed the potential facilitating role of adherent cells, the contribution of attached virus, or the presence of activated cells in the PBMCs. Thus, viral particles, containing intravirion DNA (16–19), can be attached to the surface of the target cells, and DNA from attached virions might generate a positive signal in these highly sensitive PCR assays. Indeed, in our preliminary PCR experiments we could consistently detect RU5 DNA, both in cell-free virus supernatants, and in virus-containing supernatant-exposed cells until we used a trypsinization protocol to remove attached virions from the latter. In addition, the unfractionated and unstimulated PBMCs used in earlier studies (2, 4) contained up to 25–30% $CD25^+$ cells and cannot be considered truly resting (1). More recently, Tang *et al.* (20) used a PCR assay and a trypsinization step to demonstrate that virus can enter highly purified nonadherent DR^- $CD4^+$ T cells, but no virus production could be induced after activation of these cells. They initially suggested that monocyte-macrophages played a role in permitting efficient virus infection of resting $CD4^+$ T cells, although they later concluded that this effect probably occurred because of the presence of small numbers of activated $CD4^+$ T cells. Taken together with these results, our results underscore the importance of the activation status of the target cell, and not the presence of adherent cells, in determining whether HIV-1 can infect a target cell.

We conclude that highly purified $CD25^-$ cells cannot be infected *in vitro.* Hence, the origin of the $CD25^-$ cells containing incomplete viral transcripts remains puzzling, particularly in view of the fact that when unfractionated PBMCs were infected with HIV-1 the $CD25^-$ cells contained incomplete DNA transcripts and hence had been infected. These cells may be derived from infected-activated $CD4^+$ $CD25^+$ cells, which subsequently become $CD25^-$ or may only become infected in the presence of other T cells. The first interpretation is consistent with the results of Chun *et al.* (6), who demonstrated the presence of integrated provirus in purified resting $CD4^+$ T cells obtained from HIV-1-infected individuals, thus, suggesting that a productively-infected cell can revert to a resting state. With regard to the second interpretation, it is possible that $CD25^+$ cells must be present for $CD25^-$ cells to become infected. For example, $CD25^+$ cells might secrete cytokines or chemokines that facilitate infection of $CD25^-$ cells by upregulating viral coreceptors such as fusin or CC-CKR5. If this were the case, some $CD25^-$ coreceptor$^+$ cells might become infected. In this regard, it has recently been reported that a viral coreceptor is upregulated only in the presence of interleukin 2 produced by activated T cells (21). Whatever the explanation, these infected $CD25^-$ cells are a source of virus and, in our experimental system, an important viral reservoir. Future studies will be required to establish their origin.

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