

Activation of Infectious Virus from Latent Human Immunodeficiency Virus Infection of Monocytes In Vivo

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Abstract

Individuals infected with HIV may be asymptomatic for years before progressing to overt AIDS. Since HIV can latently infect monocytoid cell lines, we examined whether HIV latency occurs in monocytes in vivo. Freshly isolated monocytes from asymptomatic seropositive individuals examined before and after culture were positive for HIV DNA, but not RNA, as measured by polymerase chain reaction, showing that HIV latency occurs in monocytes in vivo. Coculture of these latently infected monocytes with Con A-activated T cells from HIV-negative normal donors stimulated 90% of the patients' samples and latently infected THP-1 to produce infectious virus. Neither Con A, resting T cells, nor T cell supernatants induced virus. Plasma membranes from activated T cells stimulated HIV production, suggesting cell contact induces factor(s) in monocytes to overcome latency. Thus, monocytes in AIDS patients harbor latent HIV inducible during an immune response, leading to T cell infection and viral-induced pathology. (*J. Clin. Invest.* 1992; 90:1486-1491.) Key words: • human immunodeficiency virus 1 • latency • monocytes • immune activation

Introduction

The two major cell types known to harbor HIV, the etiologic agent of AIDS, are the CD4⁺ T lymphocyte (1-3) and the monocyte/macrophage (4-6). The recently described ability of both cell types to harbor microbiologically latent HIV (7-9) (no viral expression but remaining inducible to produce infectious virus) suggests a mechanism for viral persistence during the long asymptomatic period seen in AIDS (10, 11). Although the selective depletion of the CD4⁺ T cells in advanced AIDS results in increased susceptibility to opportunistic infections, neoplasia, and eventual death, the role of the HIV infected monocyte in the pathogenesis of AIDS has not been fully elucidated. It is clear that the monocyte/macrophage serves as a viral reservoir, as HIV has been detected in tissue macrophages of the brain and other organs (12, 13) as well as blood monocytes (4-6). Increasing evidence suggests that the monocyte/macrophage is mainly responsible for the neurological effects seen in AIDS (14-16).

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Using the cell line THP-1 as a tissue culture model system, we have recently shown that HIV-1 can exist in a latent state for many months in monocytoid cells (9). This latent provirus could be activated to infectious virus by treatment with 5-azacytidine. However, a variety of cytokines and other agents that increase viral expression in chronically infected cells (17-20) did not induce latent virus. These studies were extended to determine if physiological stimuli could induce expression of virus from latently infected THP-1 cells.

Recently, it was shown by Schrier et al. (21) that Con A-activated T cells could upregulate HIV expression in monocytes and provide a permissive host for HIV transmission during an immune response. We found that coculture of latently infected THP-1 cells with normal Con A-activated T cells also induced HIV expression from these latently infected cells. These results afforded us the opportunity to ask if latency occurs in monocytes in vivo as well as to test the physiological relevance of immune activation of latent HIV expression in monocytes of infected individuals and subsequent infection of T cells.

Methods

Cell lines. THP-1, L-THP-1 (THP-1 latently infected with HIV), 8E5 (a cell line containing one copy of HIV-1 DNA) (22), HUT-78, and HUT-102 cell lines were maintained in RPMI with 10% FCS, penicillin (100 µg/ml), streptomycin (100 µg/ml), and glutamine (300 µg/ml). Cells were subcultured 1:5 every 4-5 d.

Isolation of normal peripheral blood cells. Buffy coats from normal healthy donors were used as previously described (23). Briefly, mononuclear cells separated by Ficoll-Hypaque gradient centrifugation were depleted of monocytes by centrifugal elutriation. Highly purified T cells were then isolated by nylon wool columns followed by discontinuous Percoll gradients as described (24). T cells were either activated with Con A (5 µg/ml) or PHA (1 µg/ml) for 48 h, grown in IL-2-containing media or further separated into CD4⁺ and CD8⁺ subsets using magnetic bead technology as previously described (25).

Isolation of monocytes from blood of HIV-infected individuals. HIV-1 seropositive asymptomatic blood donors have CD4⁺ T cell counts and immunologic profiles within normal ranges and are either virus culture positive or negative (26, 27). PBMC were obtained from 30-50 ml of peripheral blood of these donors, as described above. T cells were removed by complement depletion using OKT3 antibody; resuspended in RPMI supplemented with 5% human AB serum (Irvine Scientific, tested HIV negative, Irvine, CA) at $2-4 \times 10^6$ /ml and adhered to six-well plates. At 24 h, nonadherent cells were removed, saved for coculture and HIV analysis by polymerase chain reaction (PCR)¹ and P24 antigen. The adherent monolayers were washed thrice with PBS to remove loosely adherent cells. The cells in the monolayers consisted of >95% esterase and Leu M3-positive, and <1% CD3- and CD8-positive cells.

1. Abbreviations used in this paper: L THP-1, THP-1 cells latently infected with HIV-1; PCR, polymerase chain reaction.

Phenotypic analysis of monocyte monolayers. Cytofluorometric analysis was performed as previously described (9). Leu 1 (CD3), Leu-3A (CD4), LEU-2 (CD8), LEU-M3 (CD16) antibodies were obtained from Becton Dickinson & Co. (Sunnyvale, CA).

Infections. 10^7 cells in log phase growth were infected with 100 μ l of cell-free supernatants as previously described (9, 28). An isolate of HIV-1 obtained from activated T cells (28) was used to infect both monocytoic cells (THP-1; reference 9) and T cells (Hut 78 and Hut 102; reference 28). Viral p24 antigen, as a measure of infectivity, was determined on tissue culture supernatants or on cell pellets lysed with 1% Triton X-100 by ELISA (Cellular Products, Buffalo, NY) according to the manufacturer's specifications with a sensitivity limit of 10 pg/ml.

PCR analysis of RNA/DNA products. Nucleic acids were isolated by the guanidine thiocyanate method previously described (29). Amplification reactions of 1–5 μ g of either RNA (30) or DNA (31, 32) contained 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM of each four deoxynucleotides (Perkin Elmer Cetus, Emeryville, CA), 100 U of M-MLV Reverse Transcriptase (Bethesda Research Laboratories, Bethesda, MD) (for RNA), 2.5 U AmpliTaq DNA polymerase, 1 U RNase Inhibitor for RNA (Promega Corp., Madison, WI), 0.01 M DTT, and 1 μ M of each primer. Primer pairs used were as follows: SK38/38 HIV gag (1543–1570, 1630–1657) (31); Co11/12 HIV TAT splice product (8395–8414, 5955–5974) (32); and Gap 371/546 GAPDH (371–388, 546–565) (33). Amplification was carried out in a thermocycler (Perkin-Elmer Cetus), as described (30–34). Probes used were SK19 HIV gag (1587–1627) Co11/12 HIV TAT (5975–5999); GAP514 GAPDH (514–532). Analysis of hybridized products was seen on a 10% nondenaturing acrylamide/1 \times Tris-acetate-EDTA gel by exposure to Kodak XAR-5 film at -70°C for 2–48 h (with intensifying screens).

Plasma membrane isolation. 48–60 h. Con A-activated normal T cells were washed twice in lysis buffer (10 mM Tris/HCl, pH 7.4, containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$) and broken by dounce homogenization in lysis buffer equal to 1% the original volume. The cell-free lysate was centrifuged at 700 g for 10 min. The pellet was washed once in lysis buffer and the two supernatants combined were then centrifuged at 5,000 g for 10 min. The plasma membranes remain in the supernatant fluid, which was applied to a discontinuous sucrose gradient of 0.25, 1.37, 1.48, 1.60 M, and centrifuged at 25,000 rpm in a rotor (Beckman SW; Beckman Instruments, Fullerton, CA) for 4 h. The plasma membranes, found at the 0.25–1.37 M interface, are removed, diluted 1:4 with TK buffer (50 mM Tris/HCl, pH 7.4; 5 mM $MgCl_2$; 5 mM $CaCl_2$; 25 mM KCl), and centrifuged at 70,000 g for 90 min. Membranes were used in the activation studies at a final concentration of 100 μ g/ml.

Results

Immune activation of THP-1 with latent HIV-1 infection. Since direct, HLA-DR-restricted interactions between T cells and monocytes are involved in many aspects of the immune response, we determined if T cells could induce HIV expression from latently infected THP-1 cells (LTHP-1). THP-1 cells have accessory cell function (35, 36) in that they can replace the requirement for monocytes in stimulating the proliferation of Con A-activated T cells, a response that is not HLA-DR restricted. Thus, LTHP-1 cells were cocultured for 7 d with purified normal (HIV-negative) donor T cells activated with Con A 48 h before coculture. Viral expression was measured by gene amplification using PCR technology (30–34). Normal Con A-activated T cells induced HIV RNA expression in LTHP-1 cells (Fig. 1, lane 3) using primers to both gag (Fig. 1) and spliced tat products of HIV-1 (data not shown). In contrast, coculture of LTHP-1 with a T cell line (Hut-102) or supernatants from Con A-activated T cells did not activate

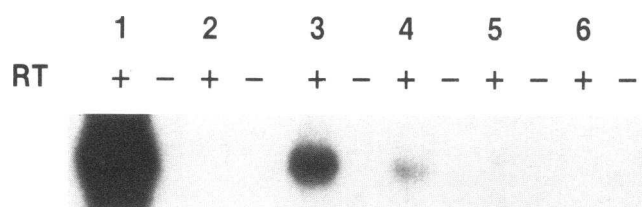


Figure 1. Activation of HIV-1 RNA expression in latently infected THP-1 cells. RNA from LTHP-1 (1×10^5 /ml) cocultured 7 d with equivalent numbers of Con A-activated T cells (conA/T) was isolated as described. 1 and 5 μ g (equivalent to 150,000–750,000 cells) (32) were amplified as described in Methods. The oligomer hybridization autoradiography demonstrates the presence of amplified HIV-1 gag RNA (4-h exposure). Lane 1, 8E5 (1 μ g); lane 2, LTHP-1 cells (5 μ g); lane 3, LTHP-1 cocultured with normal Con A/T (1 μ g); lane 4, LTHP-1 treated with LPS/5-azacytidine (1 μ g); lane 5, LTHP-1 cocultured with Hut-102 (5 μ g); lane 6, LTHP-1 cultured with Con A/T cell supernatant (5 μ g).

viral expression (Fig. 1, lanes 5 and 6). Also, resting T cells, CEM, and Hut-78 did not activate HIV expression. Using separated T cell subsets, CD4+ but not CD8+ T cells activated latent HIV in LTHP-1 to produce infectious virus. T cells remaining in suspension after 7 d were removed, grown in IL-2, and found to be infected. Viral activation and spread to T cells was seen by PCR-RNA by day 3, reaching a peak by day 7 (data not shown).

HIV-1 RNA expression in monocytes from asymptomatic seropositive individuals. To test the physiological relevance of this latent model, we determined whether latency occurred naturally in monocytes from HIV-1 infected individuals. Adherent monolayers of monocytes obtained from blood of HIV-seropositive asymptomatic individuals by CD3+ depletion and overnight adherence consisted of >95% esterase- and Leu M3 positive- and <1% CD3- and CD8-positive cells. Also, as previously shown (37), these adherent monocytes rapidly lose their CD4 positivity. The monolayers were cocultured with and without normal Con A-activated T cells for 7–10 d. After 10 d, the adherent cells incubated in media alone remained CD3 and CD4 negative and Leu M3 positive with a differentiated monocytoic appearance, but now only 50–60% of the cells stained weakly for esterase as previously described (21, 37). The cocultured T cells in suspension were removed from the adherent layer and supernatants assayed for p24 antigen. Monolayers were washed with PBS and nucleic acids were isolated as described in Methods.

The cells were then tested for HIV RNA expression. After reverse transcription of total RNA, cDNA was amplified by PCR (Fig. 2). Amplified products specific for HIV were detected only in the reverse-transcribed samples from monolayers incubated in the presence of Con A-activated T cells both for spliced tat (Fig. 2A, lanes 5, 9, and 11; Fig. 2B, lanes 5, 7, and 9) or gag (data not shown). In contrast, monocyte monolayers incubated in media alone showed no HIV-specific amplified products (Fig. 2A, lanes 4, 8, and 10; Fig. 2B lanes 4, 6, and 8) with the exception of patient 1 (Fig. 2B, lane 2). That RNA from monocytes incubated in media alone was amplified as efficiently as the other RNAs was shown in 18/19 cases by amplification of glyceraldehyde-3-phosphate dehydrogenase (Fig. 2C). In 14/18 cases where no viral expression was seen in monocytes cultured alone, RNA specific for tat and gag as well

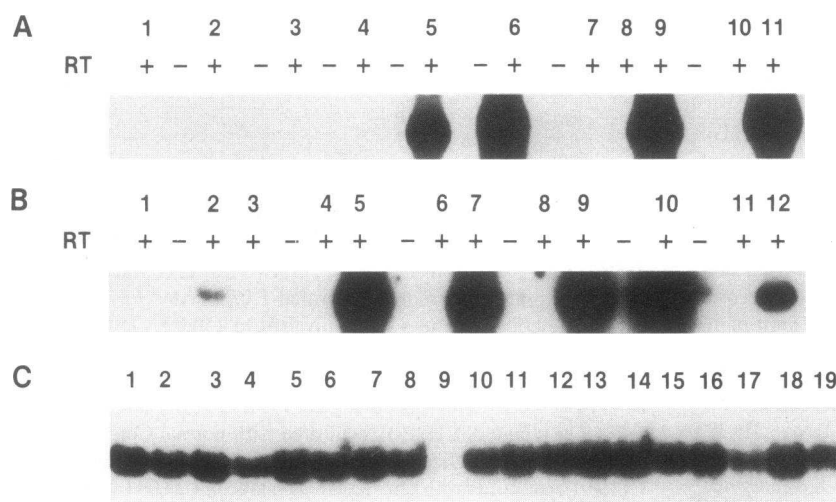


Figure 2. Detection of HIV RNA expression in adherent monocytes from HIV seropositive asymptomatic individuals. RNA from monocytes ($1-5 \times 10^6$ cells) cultured 7 d as adherent monolayers was prepared as described. 1 and 5 μ g of total cellular RNA were subjected to RT-PCR as described. The oligomer hybridization autoradiography detecting amplified products (4-h exposure) is shown. (A) Detection of HIV-spliced tat RNA. Lane 1, normal monocytes (5 μ g); lane 2, patient 5 control (5 μ g); lane 3, patient 5 with con A/T (5 μ g); lane 4, patient 6 control (5 μ g); lane 5, patient 6 with con A/T (1 μ g); lane 6, HIV-1-infected Hut 78 (1 μ g); lane 7, Hut 78 (5 μ g); lane 8, patient 7 control (5 μ g); lane 9, patient 7 with Con A/T (1 μ g); lane 10, patient 8 control (5 μ g); lane 11, patient 8 with Con A/T cells (1 μ g). Longer exposures had the same results. (B) Detection of HIV-spliced tat RNA. Lane 1, normal monocytes (5 μ g); lane 2, patient 1 control (5

μ g); lane 3, patient 9 control (5 μ g); lane 4, patient 2 control (5 μ g); lane 5, patient 2 with Con A/T (1 μ g); lane 6, patient 3 control (5 μ g); lane 7, patient 3 with Con A/T cells; lane 8, patient 4 control (5 μ g); lane 9, patient 4 with Con A/T cells (1 μ g); lane 10, infected Hut 78 (1 μ g); lane 11, DNA from infected Hut 78 (1 μ g); lane 12, RNA and DNA from infected Hut 78 (1 μ g). Longer exposures yielded the same results. (C) Detection of the cellular enzyme glyceraldehyde-3-phosphate-dehydrogenase. RNA from the first 19 samples of monocytes cultured alone is shown as an internal control of equivalent levels of amplifiable RNA added.

as extracellular p24 (30–250 pg/ml) (Table I) was seen after coculture with normal Con A-activated T cells. In addition, the normal T cells cultured in IL-2 subsequent to the coculture were virus positive showing that the activated virus was infectious (Table I).

HIV-DNA in monocytes from asymptomatic seropositive donors. Since these results suggest that HIV can exist in a latent state in monocytes of HIV-infected individuals, we next examined the HIV DNA status in these cells. Interestingly, PCR analysis of the DNA isolated from these monocytes revealed HIV-specific sequences for gag and tat in only 9 of 21 individuals (Fig. 3 A lanes 1, 6, and 8; Table II). However, DNA as well as RNA could be detected in the peripheral monocytes of 17 of 21 individuals after coculture with normal Con A T cells (Table I). The same four individuals were negative for both

DNA and RNA. Primers for GAPDH show that the amplification of the DNA samples was of similar magnitude (data not shown). The limit of detection in our system as determined by PCR on serial dilutions of the 8E5 cell line was 1 infected cell/100,000 cells (Fig. 3 B). This sensitivity is the same as that previously reported (34, 38). Diluting the test DNA sample with 1 μ g of uninfected DNA did not affect the sensitivity of the assay (data not shown). These results indicate that the HIV-1

Table I. Activation of HIV-1 Expression in Primary Monocytes

Sample totals	Culture treatment	DNA*	RNA*	p24 Antigen	Infectivity
				pg/ml	
3	None	3	3	100–250	ND
6	None	6	0	0	0/3
6	Con A/T cells	6	6	30–250	3/3
12	None	0	0	0	0/6
12	Con A/T cells	8	8	30–250	6/6

RNA/DNA from monocytes ($1-5 \times 10^6$ cells) cultured 7 d as adherent monolayers in the presence and absence of equivalent numbers of Con A-activated T cells was isolated as described. 1 and 5 μ g of total cellular RNA/DNA was added to the reaction mixture and subjected to 30 cycles PCR amplification. 2×10^6 cells were detergent disrupted for HIV p24 antigen by ELISA, which was performed as described in Methods. Viral transmission to Hut-102 was used as a measure of infectivity. * Number of positive occurrences.

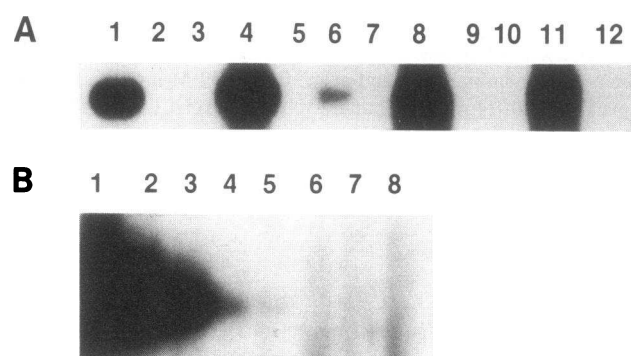


Figure 3. Detection of HIV-1 DNA in adherent patient monocytes by gene amplification. DNA was isolated from patient monocytes ($1-5 \times 10^6$). 1 and 5 μ g total cellular DNA (equivalent to 150,000 and 750,000 cells) (32) was subjected to PCR as described in the methods. The oligomer hybridization autoradiography shows the presence of amplified HIV-1 gag DNA (4-h exposure). (A) lane 1, patient 2 (1 μ g); lane 2, patient 3 (5 μ g); lane 3, patient 4 (5 μ g); lane 4, patient 4 with Con A/T (1 μ g); lane 5, patient 5 (5 μ g); lane 6, patient 6 (5 μ g); lane 7, patient 7 (5 μ g); lane 8, 8E5 (1 μ g); lane 9, patient 8 (5 μ g); lane 10, patient 9 (5 μ g); lane 11, U1 (1 μ g); lane 12, monocytes (5 μ g). (B) Detection of HIV-specific DNA 8E5 cells. DNA from the 8E5 cell line containing one copy of HIV DNA per cell (22) was used as a reference. Lane 1: 1 μ g of 8E5 DNA (equivalent to 150,000 cells) (32). Each subsequent lane represents a 10-fold dilution. Longer times of exposure yielded the same results.

Table II. Comparison of Latent HIV Transmission to T cells and Hut-102

Cells	Primary coculture	Second coculture	
		T cells	Hut-102
Cell lines			
Latent THP-1	None	—	—
Latent THP-1	HUT-102	—	—
Latent THP-1	LPS/5 AZA C	+	+
Latent THP-1	Con A/T cells	+	+
U1	None	+	+
Patients 7, 11, and 12			
Monocytes	None	—	—
Monocytes	Con A/T cells	+	+
Monocytes	HUT-102	—	—
Monocytes	Con A/Hut 102	—	—

$1-5 \times 10^6$ monocytes or LTHP-1 cocultured 7 d with the equivalent number of T cells. Viral transmission to Hut-102 and PHA-activated T cells as a measure of viral production was performed on cell-free supernatants and assessed as described in Methods. Infectivity was determined by the presence of HIV nucleic acids detected by PCR amplification of 1 and 5 μ g of RNA. 2×10^6 detergent-disrupted cells were used for HIV p24 antigen detected by ELISA.

provirus was latent in the peripheral monocytes of these infected individuals but that levels of HIV DNA in eight monocyte cultures inducible for viral expression were below the detectable limits of the PCR analysis. Despite the low levels of latently infected monocytes in these cultures, the cells could still be induced by uninfected Con A-activated T cells to produce infectious virus.

Mechanism of immune activation of HIV-1 from latently infected monocytes. Next, the nature of the immune activation was further studied. For HIV activation from patient monocytes, partially purified plasma membranes from Con A-activated T cells could replace the need for whole cells (Fig. 4 A, lane 11). To determine if proliferating normal T cells were needed, parallel cocultures of patients' monocytes were set up with either Hut-102 or Con A-activated normal T cells. Hut-102 cells were not infected (Fig. 4 A lanes 5 and 8) whereas parallel cultures with activated T cells were virus positive (Fig. 4 A, lanes 6 and 9). In addition, coculture of LTHP-1 with Hut-102 did not result in viral activation even after 6 wk of cocultivation (Fig. 4 A, lane 3). It was possible that Hut-102 cells were neither sensitive for HIV-1 transmission nor permissive to infection by the strain in patients' monocytes. However, we could consistently detect 10 U1 infected cells cocultured for 7 d per 1×10^7 normal monocytes using Hut-102 (Fig. 4 A, lane 13), but not one infected cell (Fig. 4, A, lane 12). In addition, to demonstrate that this viral activation resulted in production of virus that could infect Hut-102, viral transmission experiments using supernatants from these original cocultures were performed using Hut-102 and activated T cells as secondary cocultures (Table II). In every case where activated virus was detected in the supernatants of stimulated monocytes, this virus could be subsequently transmitted cell free to both activated T cells and Hut 102 (Table II). In contrast, none of the initial Hut-102 cocultures activated virus. Thus, these data show that HIV was truly latent in these monocytes and

suggests that activated T cells interact with monocytes differently than the T cell line Hut-102. Furthermore, these results indicate that the detected virus was not passively adsorbed to the surface of these monocytes and infecting the T cells during the coculture period.

The possibility that the HIV expression came from a small percentage of either contaminating T cells or virus-producing cells present in the adherent monocyte monolayers cannot be completely ruled out. This is highly unlikely as Con A alone activated virus from T cells and not from monocytes of these donors (data not shown). Also, in these monocyte cultures, no CD3+ cells could be identified after 10 d of culture, indicating that rare patient T cells were not activated to proliferate by a heterologous mixed lymphocyte reaction. In addition, LPS and cytokines known to increase HIV production (17–20) did not stimulate viral production in these monocytes (Table II). These results provide strong evidence that neither contaminating T cells nor virus-producing cells are responsible for the HIV expression induced in these monocytes.

Next, the conditions necessary for T cells to activate latent virus were studied. First, cell contact was required, as Con A-activated normal T cells seeded into chambers with 4- μ m pore-filtered bottoms placed into culture wells previously seeded with adherent patient monocytes failed to activate virus (Fig. 4 B, lane 8). In addition, supernatants from Con A-activated T cells could not stimulate HIV expression (Fig. 4 B, lanes 4 and

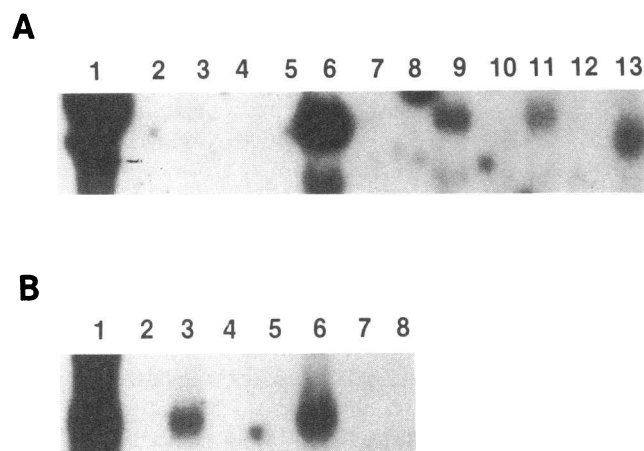


Figure 4. Activation of HIV RNA expression from latently infected monocytes from HIV-seropositive asymptomatic donors. RNA from monocytes ($1-5 \times 10^6$) was prepared as described. 1 and 5 μ g of RNA were subjected RT-PCR amplification as described in the methods. The oligomer hybridization autoradiography of amplified HIV-1 spliced tat RNA product (4-h exposure) is shown. (A) Lane 1, 8E5 (1 μ g); lane 2, normal monocytes (5 μ g); lane 3, LTHP-1 with Hut-102 (5 μ g); lane 4, Hut-102 (5 μ g); lane 5, patient 18 with Hut-102; 5 μ g; lane 6, patient 18 with Con A/T (1 μ g); lane 7, patient 18 control (5 μ g); lane 8, patient 19 with Hut-102 (5 μ g); lane 9, patient 19 with Con A/T (1 μ g); lane 10, patient 19 control (5 μ g); lane 11, patient 19 with membranes from Con A/T (100 μ g/ml) (1 μ g); lane 12, 1 U1 cell/monocytes with 10^6 Hut-102 (1 μ g); lane 13, 10 U1 cells with 10^6 Hut-102 (1 μ g). (B) Lane 1, 8E5 (1 μ g); lane 2, patient 11 control (5 μ g); lane 3, patient 11 with Con A/T (1 μ g); lane 4, patient 11 with supernatant from Con A/T (5 μ g); lane 5, patient 12 control (5 μ g); lane 6, 12 with Con A/T (1 μ g); lane 7, patient 12 with supernatant from Con A/T cells (5 μ g); lane 8, patient 12 with transwell membrane separated Con A/T cells (5 μ g). Longer exposures yielded the same results.

7). Infectious virus was found in media from activated T cell plasma membrane-treated monocytes. However, no virus was detected in the media of patients' monocytes cultured with unactivated T cells, Con A alone, cytokine-rich T cell supernatant, purified cytokines such as Colony-stimulating factor-1, granulocyte macrophage colony-stimulating factor, tumor necrosis factor- α , and IL-6, or media controls. These results suggest that cell contact is required to activate factor(s) in the monocyte responsible for overcoming HIV latency.

Discussion

In agreement with Schnittman et al. (38), we found no HIV-specific DNA by PCR in 12/21 samples of patients' monocytes. However, expression of infectious HIV was induced in 8/12 of those samples after coculture with Con A-activated T cells. The limit of detection in our system as determined by PCR performed on serial dilutions of the 8E5 cell line was found to be 1 infected cell/100,000 cells, similar to previous reports (34, 38). These results indicate that the HIV-1 provirus was latent in the peripheral monocytes of these infected patients but that levels of HIV DNA in some cultures inducible for viral expression were below the detectable limits of the PCR analysis.

Despite the low levels of latently infected monocytes in these cultures, the cells could still be induced by uninfected Con A-activated T cells to produce infectious virus. These and identical results with LTHP-1 indicate that fully competent HIV can be latent in patient monocytes in vivo. However, three alternatives were considered and investigated: that HIV is passively adsorbed to monocytes; that contaminating viral-producing cells, either T cells or monocytes, are responsible for viral spread; and that a very small number of contaminating latent T cells (7, 8) are being activated. The first two possibilities were ruled out conclusively by the sensitivity of the coculture experiments shown herein.

In regard to the presence of latently infected T cells, it has been recently shown that in asymptomatic seropositive donors 1 in every 500 or 1,000 T cells is latently infected (39). Since we plated approximately 100,000 monocytes with >1% CD3 positivity, at most there could only be 1–2 latently infected T cells in each culture. Furthermore, when semipurified membranes were used to activate virus after 7–10 d of incubation, CD3+ positive cells were not detectable in the cultures, ruling out expansion of T cells by heterologous mixed lymphocyte reaction. Also, Con A and PHA, which can activate HIV from latently infected T cells (7, 8), activated virus from T cells but not from monocytes of these donors even when sufficient permissive cells were cocultured to amplify viral production (Table II). These results provide strong evidence that contaminating T cells are not responsible for the HIV induced in these monocytes.

These studies confirm and extend those of Schrier et al. (21), who showed that Con A-activated autologous cells or autologous plus allogeneic cells could stimulate HIV production from macrophages. Similarly, the recent finding of McElrath et al. (40) showing the presence of HIV DNA in monocytes of AIDS patients in samples obtained up to 9 mo apart suggests that continuous infection of monocytes is occurring. However, in these studies, no conclusion can be made about viral latency. Also, recent studies (41, 42) have shown

viral DNA in patients by PCR for up to 5 yr before seroconversion, suggesting long-lived viral latency. We show for the first time that latency occurs in monocytes in vivo and that Con A-activated T cells can activate this latent virus. The intimate relationship between T cells and monocytes suggests that these cells could be infecting each other during the immune response by activating latent virus.

The cellular requirements of activation of latent HIV from monocytes were also studied. In LTHP-1 and latently infected patients' monocytes, activated CD4+ T cells but not CD8+ cells, resting T cells, or Hut-102 could activate latent virus leading to a productive cytopathic infection. Plasma membranes from activated T cells can stimulate monocytes to produce infectious virus, suggesting that cell contact is needed to activate factor(s) in the monocyte responsible for overcoming HIV latency. Although T cell signals involved are not known, activated T cells provide different signals than resting T cells or Hut-102. On activated cells, adhesion molecules like LFA-1 have higher affinity for binding to monocytes. Multiple accessory molecules (CD2, CD28) involved in T cell activation, which bind and signal antigen presenting cells, are candidates (43).

The presence of silently infected monocytes capable of producing virus during an immune response has some therapeutic implications. Since these latently infected monocytes may become long-lived tissue macrophages, it would be difficult to eliminate these cells. Thus, it would be important to determine whether a combination of immune stimulation plus antiviral therapy is capable of eliminating latently infected monocytes. Such an approach could make currently available antiviral agents more effective in vivo by eliminating an important reservoir of virus that may intermittently reactivate and infect uninfected T cells.

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