Most CD4+ T Cells from Human Immunodeficiency Virus-1 Infected Patients Can undergo Prolonged Clonal Expansion

Erik Langhoff, Juliana McElrath, Harry J. Bos, Jill Pruett, Angela Granelli-Piperno, Zanvil A. Cohn, and Ralph M. Steinman
Laboratory of Cellular Physiology and Immunology, The Rockefeller University, and Irvington Institute, New York, New York 10021

Abstract

We have addressed the capacity of HIV-1 infection to alter the growth of primary CD4+ T cells, but at the clonal level. Single T cells were expanded in the presence of PHA, IL-2, and small numbers of accessory dendritic cells. We report two new findings. First, T cells from seropositive individuals, even those with AIDS and markedly reduced CD4+ counts, exhibit a normal cloning efficiency, and proliferative capacity. This result is in contrast to two prior reports of a low cloning efficiency in CD4+ T cells from HIV-1-infected patients. Second, when we added high doses of exogenous HIV-1 to T cell clones from control subjects, we observed infection but not cytotoxicity or loss of CD4+ cells, following addition of virus stocks at days 0, 3, and/or 7 of clonal growth. The same HIV-1 isolates markedly reduced CD4+ T cells in bulk mononuclear cultures. When tested at day 11, HIV-1 mRNA was expressed in some cells of exogenously infected clones by in situ hybridization; when tested at day 18, several clones could transactivate a TAT-sensitive cell line. These findings suggest that the loss of CD4+ T cells in infected individuals is not the inevitable result of the activation of latent infection, or spread of a productive infection, during clonal growth.

Introduction

The immune deficiency associated with HIV-1 infection is characterized by a progressive loss of CD4+ T lymphocytes (1). The lethal opportunistic infections of the disease are to a large extent ascribed to malfunction and depletion of the CD4+ subset, for which HIV-1 virus has a well-established tropism (2–4). However, the frequency of CD4+ cells that are productively infected with HIV-1 is much < 1% (5). To explain the marked depletion of CD4+ lymphocytes, it is possible that many T cells carry latent virus, which becomes activated upon antigen stimulation of lymphocytes leading to cell lysis (1, 6). Indeed, it has been reported that T cells from HIV-1 infected patients cannot be cloned efficiently in vitro (7, 8). One explanation would be an early cell lysis of growing T cell clones by activation of latent virus. Another proposed mechanism for the loss of CD4+ cells is that a small number of productively infected cells may kill normal T cells after syncytium formation (9). We have addressed these possible etiologies for the loss of CD4+ T cells by using a recently described cloning technique. The method allows early visual detection of developing CD4+ T cell clones in response to a mitogenic stimulus (10). Surprisingly, we find little evidence for a cytopathic infection in CD4+ T cells, from endogenous or exogenous HIV-1, when the T cells are grown as clones in the presence of dendritic cells from seronegative individuals. These findings indicate that the proliferative potential of most T cells from HIV-1 infected individuals can be substantial.

Methods

Patients. 50 ml heparinized venous blood was obtained on three occasions by venipuncture of 14 HIV-1 seropositive male homosexuals with no record of intravenous drug abuse. All patients had below normal CD4+/CD8+ ratios, and most had low absolute CD4+ T cell counts (see Table I). All patients were on a previous occasion skin tested for delayed type hypersensitivity responses to a group of antigens: 0.1 ml intermediate strength Candida, Trichophyton (Hollister-Stier Laboratories, Elkhart, IN) and 5TU PPD (purified protein derivative[1]; Connaught Laboratories Ltd., Willowdale, Ontario). Responses were recorded at 48 h and included > 5 mm induration or erythema with Candida and Trichophyton and > 10 mm induration with PPD. Control samples were heparinized venous blood from the concentrated leukocyte fractions (New York Blood Center) from seronegative healthy donors.

Cell isolation. Blood mononuclear cells were isolated on Ficoll-Paque and allowed to form rosettes with neuraminidase-treated sheep erythrocytes. From the E-rosette positive cell fraction, enriched populations of CD4+ T lymphocytes were obtained by panning to exclude OKT8 (CD8), 9.3F10 (class MHC II), and OKM1 (CD11b) positive cells (10). The purity of the CD4+ T cells was determined by double fluorescence FACScan analysis using a combination of FITC-anti-CD4 (leu 3) and PE-anti-CD8 (leu 2) (Becton-Dickinson & Co., Mountain View, CA). In all cases the percentage of CD4+ cells in the starting populations was > 75% and in most cases 85–90%, while the T cell clones were almost all CD4+ (see Results). Dendritic cells enriched from buffy coats were used as accessory cells for T cell cloning (10, 11). Briefly, high density blood mononuclear cells were obtained from Percoll gradients (predominantly T cells, B cells, and dendritic cells). T cells were removed by rosetting with sheep erythrocytes. Cells that did not form rosettes were separated by a second refloatation in dense Percoll after 2 d in culture. The low density population contained most of the dendritic cells, which were further enriched by panning to exclude leukocytes with surface markers for monocytes (Fc receptors), B cells (CD45R; MAb 4G10), NK cells (CD11b; MAb OKM1).

T cell cloning. We used a recently described method for cloning of T cells (10). 1,000 irradiated (3,000 rad) dendritic cells (~ 50% pure) in 50 μl were added as feeders or accessory cells. This dose of dendritic cells corresponds roughly to their incidence in 10^5 bulk mononuclear cells, which is the standard accessory population for optimal cloning efficiency. CD4+ T lymphocytes (50 μl) were plated in 96 well round-bottomed microplates (Linbro, Flow Laboratories, VA) at an average frequency of 1 T cell/well. The cultures were supplemented with 50 μl growth medium which was PHA (20 μg/ml; Difco Laboratories, De-

Address reprint requests to Dr. Langhoff, Laboratory of Cellular Physiology and Immunology, Box 280, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399.

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1. *Abbreviation used in this paper:* PPD, purified protein derivative.

Table I. Clinical Parameters of Examined HIV-1 Infected Individuals

Patient No.	Diagnosis	Drug therapy	CD4/CD8 count cell/mm³*	Skin testing Candida/Trichophyton/PPD
1	Asymptomatic	None	310/890	All negative
2	Asymptomatic	None	380/620	10 mm Candida 6 mm Trichophyton
3	Asymptomatic	AZT‡ disulfiram	240/500	All negative
4	Asymptomatic	disulfiram	550/1630	All negative
5	Asymptomatic	None	510/440	All negative
6	Asymptomatic	AZT DTC§	370/590	All negative
7	Asymptomatic	None	530/840	11 mm Candida
8	ARC	AZT	200/620	All negative
9	ARC	Ampligen	280/580	8 mm Candida
10	ARC	AZT	400/1830	All negative
11	ARC	None	320/1860	All negative
12	ARC	AZT	610/1880	5 mm Candida
13	AIDS/KS	DTC	220/920	All negative
14	AIDS/KS	AZT	420/990	All negative

* The normal range of CD4 and CD8 counts are 550–1,100 and 300–600 cells/mm³. ‡ AZT, azidothymidine. § DTC, diethyldithiocarbamate.

troit, MI) plus 300 U/ml rIL-2 (DuPont, Wilmington, DE), or PHA-conditioned medium (10, 12). The latter was made in our laboratory by stimulation of blood mononuclear cells (2×10^6 cells/ml) with 20 μg/ml PHA for 48 h in RPMI 1640 medium supplemented with 2 mM glutamine, antibiotics, 20 μM 2-mercaptoethanol, and 10% human serum (10). The culture plates were incubated at 37°C in 5% CO_2 in a humidified incubator and clones were enumerated by microscopy at days 7, 10, and 14 and the cloning efficiencies were calculated based on 120–160 observations. A maximum of 67% positive wells is predicted by the Poisson distribution when plating is performed at 1 cell/well. Thus, the cloning efficiency was calculated as 3/2 of the observed percentage of wells with clones. Further expansion of clones beyond 14 d was obtained by transferring the clones from the microwells to 24 well plates (16 mm) precoated with an irradiated (3,000 rad) layer of adherent cells in a total volume of 1.5 ml in 30% (vol/vol) conditioned medium supplemented with 20% purified human IL-2 (Electro-Nucleonics, Inc., Fairfield, NJ).

Endogenous HIV-1 production from CD4 positive patient T cells. The $CD4^+$ T cells from the erythrocyte rosette fraction (1×10^6 cells/ml in 6 ml) were cocultured with PHA stimulated lymphoblasts (0.5×10^6/ml) in 25 cm^2 tissue culture flasks (Costar, Cambridge, MA) and were supplemented with IL-2 (600 U/ml; New England Nuclear Research Products, Boston, MA). Culture supernatants were collected over 3–5-d intervals, concentrated with polyethylene glycol 8000 and analyzed for virus production by p24 antigen ELISA (New England Nuclear). Productive infection in T cell clones was measured similarly by p24 ELISA from 100 μl of the clone supernatants.

Infection of bulk cultures and T cells clones with exogenous HTLV-IIIb. For in vitro propagation of virus in bulk cultures of blood mononuclear cells, the system described by McDougal et al. was used (6). Ficoll-Paque separated mononuclear cells from seronegative donors were stimulated with 20 μg/ml PHA for 3 d at 2×10^6 cells/ml in medium. The PHA-stimulated cells were harvested by centrifugation and resuspended to 2×10^6 cells/ml in medium without PHA but supplemented with 300 U/ml rIL-2. The lymphoblast-rich cell cultures were inoculated with an HIV-1 supernatant containing HTLV-IIIb virus (10,000 cpm reverse transcriptase/ml) or with a fresh patient isolate that had been expanded on PHA stimulated T cell blasts. Every 3 d the culture medium was exchanged with rIL-2 supplemented fresh medium, and the cultures were maintained at 1 to 2×10^6 cells/ml. At each time point, viable cell counts were determined by trypan blue exclusion, and expression of $CD3^+$, $CD4^+$, and $CD8^+$ antigens was measured by flow cytometry. Cytocentrifuged cell preparations for in situ hybridization (below) were also made. For propagation of virus in $CD4^+$ T cell clones, the HTLV-IIIb isolate was in six different experiments added (25 μl) to the cloning wells at days 0, 3, or 7 (1,400, 700, or 70 cpm/well). In two additional experiments a patient HIV-1 isolate was added to the cloning wells at day 3 (700 cpm/well). In control plates, 25 μl of supernatant from an uninfected CEM-4 cell line or from uninfected T cell cultures were used.

In situ hybridization for HIV-1 mRNA. For in situ hybridization studies glass slides were coated for 3 h in Denhardt's medium (13) at 65°C, dipped briefly in distilled water, transferred to ethanol-acetic acid (3:1 vol/vol) for 20 min and air dried. After making the cytocentrifuge preparations (3×10^4 cells per slide; cytocentrifuge; Shandon Southern Instruments, Inc., Sewickley, PA) the slides were air dried and immediately fixed for 20 min at room temperature in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) with 5 mM MgCl. The slides were rinsed for 3 min in 3× PBS, twice for 1 min in 1× PBS, and dehydrated in ethanol. After drying the slides were stored at −20°C until use.

The plasmid pBH10, containing a 9-kb insert with the complete cDNA of HTLV-III (14, 15), was digested with Bam HI and Hind III. The 0.6 kB Hind III fragment, containing a part of the gag gene was isolated and random primer labeled as described (16) with a mixture of ^{3}H-nucleotides ([^{3}H]dTTP, [^{3}H]dCTP, [^{3}H]dATP; New England Nuclear). The cytospin preparations were rehydrated, treated for 20 min at room temperature with 0.2 N HCl, washed in 2× SSC for 20 min at 50°C, followed by an acetylation step for 10 min in 0.1 M 3-ethanolamine and 0.25% acetic anhydride. The slides were washed for 5 min in PBS. On each slide 20 μl of hybridization mixture was loaded, which contained 4×10^5 cpm of ^{3}H-labeled cDNA probe, 50% deionized formamide, 1× Denhardt's solution, 2% Dextran sulphate, 0.3 M NaCl, 80 μg/ml salmon sperm DNA, 5 mg/ml yeast tRNA, and 10 mM Tris-HCl, pH 7.5. Siliconized coverslips were placed on top of the cells and sealed with rubber cement. The slides were incubated at 42°C overnight and then washed at 37°C as follows: 30 min in 4× SSC, 2 × 1 h in 4× SSC, 2 × 1 h in 2× SSC, and at least 30 min in 1× SSC. The washed slides were dehydrated in ethanol and dipped in NTB2 emulsion (Eastman Kodak, Rochester, NY) diluted 1:1 with double distilled water. After 2–4 d exposure, the slides were developed (D19; Kodak) for 5 min, rinsed for 1 min in tap water, and fixed (Rapid Fixer; Kodak) for 5 min. The slides were washed with tap water for 30 min and stained with hematoxylin. Cells were considered positive for

HTLV-IIIb RNA expression when there were more grains than the mean + 3 SD of the grains in control cell preparations. The background grains per cell in uninfected control T cell preparations was 1.7±1.2.

Transactivation of LTR-CAT transfected H938 cells. The $CD4^+$ H938 cell line described by Felber et al. was used (17). This cell line carries an HIV-1 5′ LTR-CAT construct, which is transactivated upon contact with a small number of infected cells (17). Briefly, the total content (150 μl) of the cloning wells (with or without clones) were transferred at day 18 to cultures with 2.5×10^5 H938 cells in 1 ml growth medium and cultured for 48 h before analysis of CAT enzyme activity. A positive CAT assay reflects transactivation of the CAT gene by the TAT product from cells infected with HIV-1 (17).

Statistical analysis. Differences in cloning efficiencies between groups were tested with an analysis of variance (one-way). $P < 0.05$ were considered significant.

Results

Patient sample. 14 seropositive patients were included in this study (Table I). Half had symptoms, and two of these had AIDS and Kaposi's sarcoma. Most patients had a decreased absolute number of $CD4^+$ T cells, and the CD4/CD8 ratio was < 1. Only four patients exhibited delayed type hypersensitivity upon skin testing with Candida, Trichophyton, and PPD antigens.

Cloning of T cells from HIV-1-infected patients. In the cloning assay we used dendritic cells from a seronegative donor as accessory cells for the prolonged growth of single T cells from patients or from control subjects. Because only 1,000 dendritic cells were needed to induce a maximal yield of clones (10), it was possible to identify the growing clones as early as day 5 of culture. Typical clones from two patients and one control at day 7 are shown in Fig. 1. At day 14, the clones had increased further in size, so that the cultures contained a cluster of cells surrounded by a large sheath of lymphoblasts as described (10). In all cases, the sizes of the patient clones and cell viability (trypan blue) were comparable to the controls. There was no evidence of syncytium formation (Fig. 1). No clones were detected in wells that only contained T cells or dendritic cells.

The cloning efficiencies of patient and control T cells were measured at days 7, 10, and 14 by microscopic examination. At each time point, the yield of clones was similar (Table II) in patient groups and controls ($P = 0.23$, $F = 1.62$, analysis of variance). By day 14, the cell yield was 20,000–40,000 cells, corresponding to a minimum of 15 rounds of clonal expansion. The reproducibility of the cloning efficiency was tested on two occasions for one patient (66 and 75%) and one control (64 and 69%).

We also examined the cloning efficiency of single T cells expanded in exogenous rIL-2 rather than bulk T cell conditioned medium (Table III). We were concerned that the latter, because of its high content of IFN-γ (> 280 U/ml), might interfere with the activation of a latent HIV-1 infection. However, the cloning efficiency was comparable with either recombinant or bulk growth factors. We conclude that $CD4^+$ T cells from HIV-1 seropositive donors can undergo prolonged growth with a cloning efficiency that is comparable to controls.

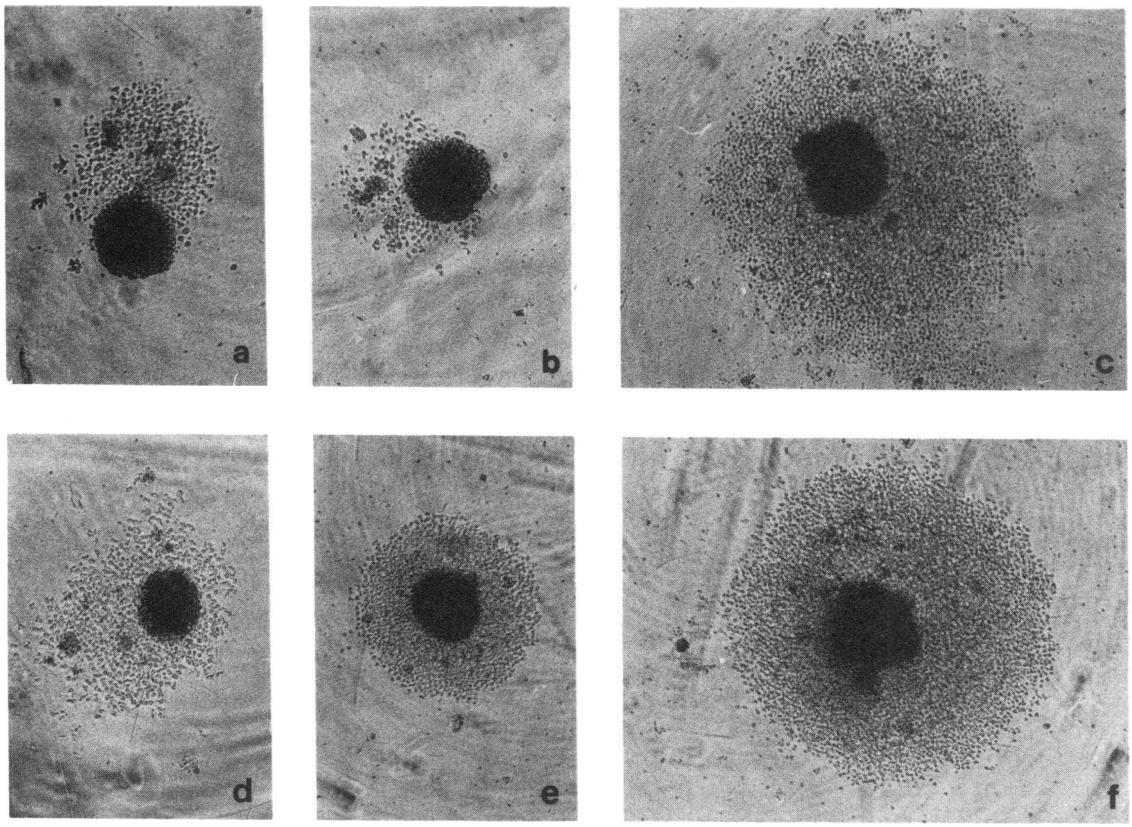

Figure 1. Typical clones from one control subject, two patients, and one control clone cultured with HIV-1 virus. The clones were photographed at day 7 and day 14 with an inverted microscope and shown at a final magnification of 150. The clones consists of a tight cluster of cells surrounded by a sheath of enlarged lymphoblasts (*a;* Control at day 7. *b;* Patient 3 at day 7. *c;* Patient 3 at day 14. *d;* Patient 9 at day 7. *e* and *f;* Control clone grown in the presence of HIV-1 virus at day 7 and 14, respectively).

Table II. Cloning Efficiencies of CD4+ Lymphocytes from 14 HIV Infected Patients and 4 Healthy Control Subjects

Donor	Clinical status	$CD4^+/CD8^+$ ratio	Cloning efficiency (%)* d7/d10/d14
Control‡	—	2.2	63/63/64
Control	—	1.8	39/45/48
Control‡	—	2.4	66/66/69
Control	—	3.8	79/90/91
Control	—	1.6	45/48/54
Group median:		2.0	63/63/64
Patient 1	Asymptomatic	0.4	77/81/83
Patient 2	Asymptomatic	0.6	45/62/67
Patient 3	Asymptomatic	0.5	85/86/86
Patient 4	Asymptomatic	0.3	69/72/69
Patient 5	Asymptomatic	1.2	69/82/82
Patient 6	Asymptomatic	0.6	64/66/69
Patient 7	Asymptomatic	0.6	63/75/75
Group median:		0.6	69/75/75
Patient 8	ARC	0.3	69/72/72
Patient 9	ARC	0.5	46/59/66
Patient 10	ARC	0.2	50/54/59
Patient 11	ARC	0.2	42/46/48
Patient 12	ARC	0.3	86/88/88
Group median:		0.3	50/59/66
Patient 13	AIDS/KS	0.2	61/72/75
Patient 14	AIDS/KS	0.4	43/51/58
Group median:		0.3	53/62/67

* Percent cloning efficiency examined at days 7, 10, 14.
‡ One control subject and patient 9 were tested on two occasions in the cloning assay. The cloning efficiency (%) of patient 9 was in the second experiment 69/75/75. A one-way analysis of variance was used for statistical analysis of cloning efficiencies between patient and control groups at day 14 ($P = 0.23$).

Lack of production of HIV-1 in clonal cultures. Endogenous production of virus was evaluated with an ELISA assay for the HIV-1 p24 antigen. 115 cultures from patients 2, 8, and 12 were negative after 2 wk of growth. In contrast, bulk cell cultures from patients 2 and 8 with lymphoblasts and IL-2 produced p24 (data not shown).

Table III. Comparable Cloning Efficiencies of Patient CD4+ Lymphocytes in the Presence of Bulk Conditioned Medium or rIL-2 and PHA

Donor	Day 7	Day 10	Day 14
Conditioned medium			
Patient 8	58	77	86
Patient 12	86	88	88
Lectin and rIL-2			
Patient 8	54	74	74
Patient 12	64	85	94

Single $CD4^+$ T cells from two patients with low $CD4^+$ T cell counts were cloned using as a source of growth factor either bulk leukocyte conditioned medium (which contained > 280 U/ml of INF-γ) or rIL-2 with PHA. The efficiency of cloning was measured at days 7, 10, and 14 in 100–120 wells.

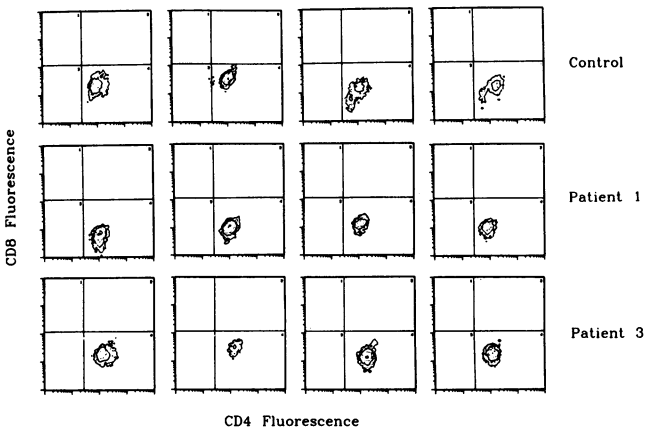

Figure 2. Double fluorescence labeling for CD8 (PE-leu2) and CD4 (FITC-leu3) antigens. Staining was performed directly in the cloning wells at day 13. Note that the expression of CD4 is comparable in the patient and the control clones.

Normal expression of CD4 antigen on patient clones. Expression of CD4 and CD8 was monitored by two-color flow cytometry in 4 control clones and in 12 clones from patients. PE-anti-CD8 and FITC-anti-CD4 were added directly to the cloning wells at 11 d of growth. 11/12 patient clones were $CD4^+CD8^-$, and the level of CD4 was comparable to control clones (Fig. 2).

Cloning of normal T cells in the presence of exogenous HIV-1. A high dose of a standard T lymphotropic virus isolate, HTLV-IIIb, was added to single cell cloning wells of control donors at day 0 and/or day 3. The highest doses of virus in terms of RTase units (1,400 or 700 U/150 μl) in a round-bottomed well with one T cell was roughly comparable to the dose used to establish a productive infection in bulk mononuclear cells (10,000 U/ml). Remarkably, cloning efficiency was not decreased (Table IV), and there was no evidence of cytotoxicity (trypan blue stain) or syncytium formation (Fig. 1, *e* and *f*). In two other experiments, virus was added at days 0 and 7 with the same results, i.e., no observed cytopathic effects. When the same virus stock was added to bulk blood mononuclear cell cultures, the virus caused the expected loss of $CD4^+$

Table IV. Cloning Efficiencies of CD4+ Lymphocytes in the Presence of Exogenous HIV-1

Donor	Day 0 None/700 cpm*	Day 3 None/70 cpm	Day 3 None/700 cpm
Conditioned medium			
Exp. 1	85/69	85/96	96/96
Exp. 2	88/80	96/96	101/96
Lectin and rIL-2			
Exp. 1	53/53	69/61	61/53
Exp. 2	72/80	72/69	88/80

* $CD4^+$ T cells from 2 healthy control subjects were cloned in either PHA-conditioned medium, or PHA and rIL-2. At different time points HIV-1 virus (700 or 70 cpm of reverse transcriptase activity, HTLVIIIb isolate) was added to the cloning wells.

Table V. In Situ Hybridization of HIV-1 mRNA in Cell Cultures Infected with Exogenous HIV-1

	Days after infection with HIV-1 virus							
	0		4	7	11	14		18
(A) Bulk mononuclear cells								
% $CD4^+$ T cells	ND		54	28	7	5		2
% positive cells by in situ hybridization	0		ND	5	9	0		0
(B) $CD4^+$ T cell clones								
Clone number	1	2	3	4	5	6	7	8
% HIV-1 positive cells	0	7	0	0	59	0	1	0

(*A*) In bulk mononuclear cell cultures inoculated with HIV-1 virus, the percentage of $CD4^+$ T cells was followed by immunolabeling (FITC-leu 3 and PE-leu 2). Cytocentrifuged cell preparations from each time point were used for in situ hybridization for HIV-1 mRNA. (*B*) Eight clones were grown in the presence of HIV-1 virus. 11 d after initiation of the experiments cytocentrifuge preparations were made for in situ hybridization for HIV-1 mRNA. Four control clones (not shown) cultured without HIV-1 were negative.

cells that has been reported by others (6, 18). A second HIV-1 isolate, derived from a patient and propagated in short-term culture with PHA-stimulated human T lymphoblasts, was also added to cloning wells at day 3. In two experiments, the cloning efficiencies at day 14 were again not affected by exogenous HIV-1 virus (78 and 84%), even though this isolate also eliminated most CD4 cells from 11 to 14 d PHA-stimulated bulk mononuclear cells.

Since exogenous HIV-1 was lytic for bulk mononuclear cells, but not T cells growing as clones with accessory dendritic cells, we examined the clones for productive infection. Because of the high dose of exogenous HIV, p24 assays gave a high background even in wells without clones. Therefore, we evaluated the clones by in situ hybridization with a ^{3}H-cDNA probe for HIV-1 gag mRNA. Positive cells were seen in bulk mononuclear cells infected with HIV-1 (Table V). Of eight clones examined at day 11 of growth, three contained T cells with HIV-1 mRNA (Table V). Fig. 3 shows the clarity of the in situ approach on bulk mononuclear cells (Fig. 3a), CEM T cell cultures (Fig. 3 *b*), and a T cell clone (Fig. 3 *d*) all infected with the same HTLV-IIIb isolate. The grain counts were similar in the clonal and bulk T cell cultures, suggesting that the levels of gag mRNA were similar. At no time did we observe hybridization signals on uninfected cells (Fig. 3 *c*).

To detect the presence of an active tat gene product in the exogenously infected clones, we cocultured the clones with the $CD4^+$ H938 cell line tranfected with LTR-CAT. Three of seven clones tested at day 18 induced responses by the CAT assay. Fig. 4 shows transactivation by two clones (lanes *1* and *3*) of four shown. In wells without clones, but with exogenous HIV-1, little or no CAT enzyme activity was detected (lanes *6* and *7*). Therefore, clonal expansion of T cells occurs in our culture system in the presence of some HIV-1-infected lymphocytes.

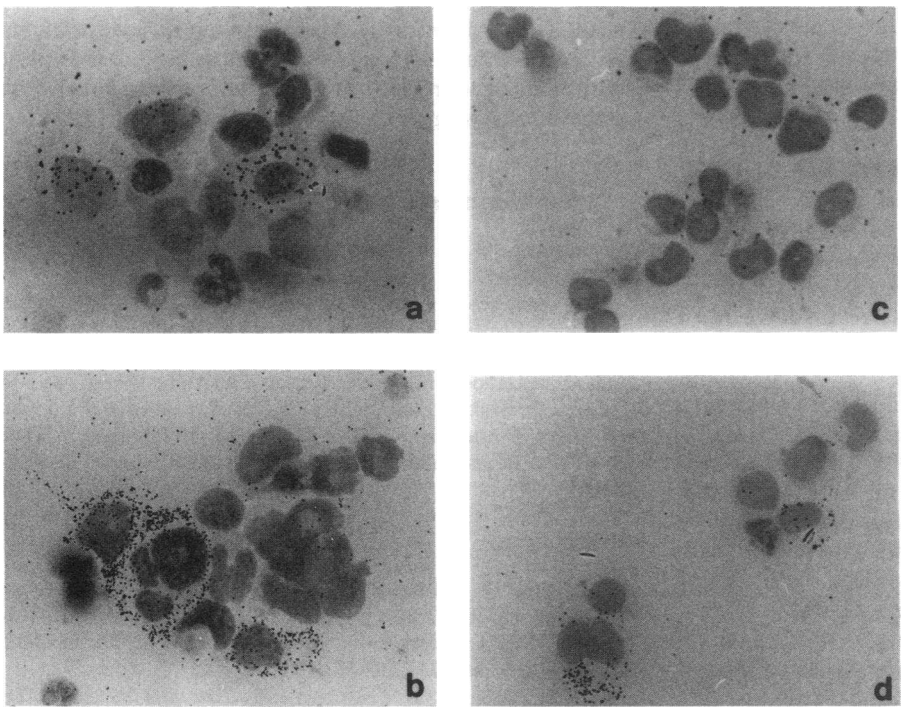

Figure 3. In situ hybridization to detect HIV-1 mRNA. A 0.6 kB ^{3}H-cDNA probe was applied to (*a*) HIV-1 infected PBMC cell cultures at d. 11. (*b*) HIV-1 infected CEM cell lines. (*c*) uninfected control T cell clone. (*d*) T cell clone infected at day 0 with HIV-1.

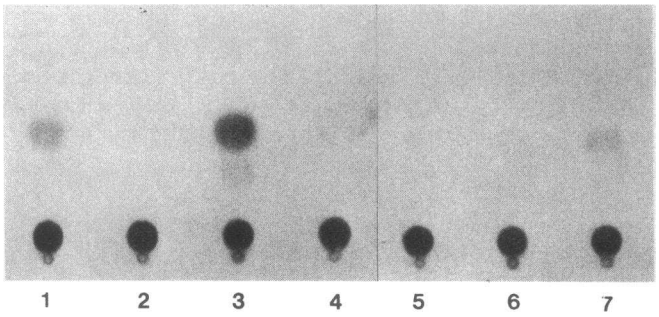

Figure 4. Transactivation of the LTR-CAT construct in H938 cell lines. Lanes *1–4;* wells with T cell clones grown in the presence of HIV-1 (HTLVIIIb). Two of the clones (lanes *1* and *3*) transactivated the H938 cells. Lane *5;* H938 cell alone, lanes *6* and *7;* wells in which a T cell clone did not grow but HIV-1 (700 RTase cpm/well) was added to the well.

Discussion

The malfunction and loss of $CD4^+$ T cells is a critical consequence of HIV-1 infection and likely predisposes to lethal opportunistic infections. Our experiments consider some of the possible mechanisms for the loss of $CD4^+$ T cells, but in contrast to many prior studies we have emphasized freshly isolated $CD4^+$ cells and clonal assays.

Although very few blood cells are productively infected with HIV-1 (5), there is evidence that the infection is much more extensive. First, it has been reported that single $CD4^+$ T cells from patients can only be cloned with a very low efficiency (7, 8). This suggests that many T cells are carrying latent virus, which is activated upon clonal expansion, or that single T cells carry a strong inhibitor of cell growth. Second, in studies with cell lines, a few infected cells transmit by syncytium formation the infection to uninfected cells (9). Our observations, however, provide a very different picture of the growth potential of $CD4^+$ T cells even when taken from patients with extensive loss of $CD4^+$ T cells.

When single $CD4^+$ T cells from HIV-infected individuals were stimulated in culture with PHA, growth factors, and normal accessory dendritic cells, the lymphocytes grew continuously producing large clones with the same efficiency as T cells from controls (Table II). There was no evidence for production of HIV p24, for a loss of CD4 antigen that can occur upon HIV infection (3, 18–20), or for cytotoxicity and syncytium formation. Therefore, the idea that many T cells are carrying latent HIV, which can be activated upon clonal expansion, does not seem to be the case even in a group of patients with low CD4 counts and frank ARC/AIDS (Table I). Further studies, as with DNA amplification, will be required to establish if a significant fraction of the patient's T cells are maintaining virus in a latent state during clonal expansion, but the biology is clear. T cells from these patients can undergo prolonged growth.

Other studies of the cloning capacity of T lymphocytes from HIV infected patients have reported a decreased cloning efficiency with different experimental protocols. In one study, single T cells were cloned in the presence of rIL-2, PHA and bulk mononuclear cells as accessory cells. The cloning efficiency was decreased (range 15–36%) for both $CD4^+$ and $CD8^+$ subsets (7). A second study (8) found more than a five fold decrease in the cloning efficiency, again of both $CD8^+$ and $CD4^+$ cells, using a limiting dilution approach. Both studies used a high number of bulk mononuclear cells as accessory cells, and not dendritic cells. It is conceivable that the use of enriched dendritic cells improves clonal growth. In both prior reports, the cloning efficiency of patient $CD8^+$ cells was also found decreased. Some of the patients in these studies differed from the patients in our study by having $CD4^+$ T cell counts < 100 per mm^3. However, the median cloning efficiency of $CD4^+$ T cells from these patients was not different from that of patients with $CD4^+$ T cell counts $> 100/mm^3$. It is possible that the coculture with dendritic cells provides a protective environment that may not be present when bulk mononuclear cells are used as accessory cells. This possibility is currently under investigation. Of some interest as well will be to test if dendritic cells from patients will support the cloning of normal or patient T cells. Such experiments, however, will require improved methods for enriching dendritic cells from small volumes of patient blood.

It was clear that exogenous HIV-1 dramatically reduced $CD4^+$ cell counts when bulk mononuclear cells were being stimulated with PHA (Table V) as was the experience of others (1, 4, 6, 21). Yet we did not observe this to be the case in our primary clones using a standard HTLVIIIb virus stock, and we had similar results with a fresh patient virus isolate. Exogenous virus (HTLVIIIb) clearly infected some of the cells in the T cell clones, and the levels of gag mRNA by in situ hybridization were comparable to bulk mononuclear cells (Figs. 3 and 4). Nonetheless the clones remained robust, and syncytia were never apparent. It is possible that the few cells that became infected in our cloning cultures died rather than propagated the infection. Alternatively, a persistent but noncytopathic infection could have been established as has been noted in one study of bulk cultures (18). We will examine these possibilities in the future using the polymerase chain reaction to detect HIV-1 DNA.

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