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Inhibition of Human Immunodeficiency Virus Replication in Acutely Infected CD4+ Cells by CD8+ Cells Involves a Noncytotoxic Mechanism

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The mechanism by which CD8+ T cells from human immunodeficiency virus (HIV)-infected individuals suppress HIV replication in acutely infected CD4+ T cells was investigated. Cytotoxicity was not involved, as the antiviral activity of the CD8+ cells did not correlate with the ability to lyse HIV-infected or uninfected CD4+ T cells. In addition, the frequency of HIV-infected CD4+ cells increased during coculture with CD8+ T cells even in the absence of detectable levels of virus replication. Moreover, separation of the CD4+ and CD8+ cells by a 0.4-μm-pore-size filter delayed HIV replication, indicating a role, at least in part, for a soluble factor. However, cell contact was required for optimal antiviral activity. These results extend further the observation on the mechanism of antiviral HIV activity by CD8+ cells from infected individuals. They support the conclusion that CD8+ cells can play a major role in preventing development of disease in HIV-infected individuals.

Peripheral blood from individuals infected with the human immunodeficiency virus (HIV) contains a population of CD8+ T lymphocytes that suppresses replication of the virus. This inhibition of virus production has been detected against naturally infected CD4+ cells enriched from the peripheral blood of infected subjects (8, 24, 28, 29, 31) as well as CD4+ cells infected in vitro with isolates of HIV (9, 30).

The mechanism by which CD8+ cells inhibit HIV replication in infected CD4+ cells is not well defined. Tsubota and coworkers (24) have suggested that cytotoxic cell activity is responsible for control of HIV replication in naturally infected CD4+ cells. They based this conclusion on the observation that contact between the CD8+ cell and virus-infected CD4+ cells was required for inhibition of virus growth and that the CD8+ cells with anti-HIV activity expressed surface antigens commonly detected on cytotoxic but not suppressor CD8+ cell subsets. However, other studies have demonstrated that virus-infected CD4+ cells are not eliminated from culture by CD8+ cells (9, 28, 30, 31) and that fluids from cultures containing CD8+ lymphocytes inhibit HIV replication (1, 27). This latter finding suggests a role for a soluble factor in controlling HIV replication in naturally infected CD4+ cells.

The objective of this study was to characterize the mechanism by which CD8+ cells inhibit viral growth in CD4+ cells acutely infected in vitro with HIV (30). The results indicate that suppression of virus replication does not correlate with cytotoxic activity mediated by anti-HIV CD8+ cells. Moreover, a soluble CD8+ T-cell-derived factor appears to be involved, although the most efficient inhibition of virus growth requires contact between the infected CD4+ cells and effector CD8+ T cells.

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MATERIALS AND METHODS

Subjects. Nine healthy HIV-seropositive homosexual men volunteered for this study. National Institutes of Health guidelines for human experimentation were followed, and the project was approved by the University of California Committee for Human Research. Peripheral blood from HIV-seronegative individuals was obtained from the Irwin Memorial Blood Bank, San Francisco, Calif.

Isolation and culture of PMC. Peripheral blood mononuclear cells (PMC) were isolated from the blood of HIV-seropositive and -seronegative subjects by centrifugation over Ficoll-Hypaque density gradients (Sigma, St. Louis, Mo.) as described previously (28, 29). Cells were cultured at a density of 2×10^6/ml in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated (56°C for 45 min) fetal calf serum (FCS; Hyclone, Logan, Utah), and 5% purified interleukin-2 (Pharmacia, Silver Spring, Md.). This complete growth medium was supplemented for the first 3 days of culture with purified phytohemagglutinin (PHA; Burroughs Wellcome, Research Triangle Park, N.C.) at a concentration of 1 μg/ml.

Monoclonal antibodies. Monoclonal antibodies leu 2a, leu 3a, and leu 4, which recognize the CD8, CD4, and CD3 proteins, respectively, were provided by Becton Dickinson Corp. (Mountain View, Calif.). The natural killer cell-specific monoclonal antibody 3G8, which recognizes the CD16 protein (19), was provided by Sara Clarkson (University of California, San Francisco).

Enrichment of T-cell subsets. T cells expressing the CD4 and CD8 surface antigens were enriched from the 3-day PHA-stimulated PMC populations by using immunomagnetic beads coupled to anti-CD4 or anti-CD8 monoclonal antibodies (25). In brief, 5×10^7 PMC were washed twice with phosphate-buffered saline (PBS) containing 1% FCS (PBS-FCS). Cells were resuspended in 5 ml of PBS-FCS and incubated with 3×10^7 to 5×10^7 Dynabeads directly conjugated to anti-CD4 monoclonal antibodies (Dynal Inc., Great Neck, N.Y.) for 20 min at 4°C on a rotating platform.

Highly enriched populations of CD4+ cells were then prepared by recovery of anti-CD4-coated beads, using the Dynal magnetic concentrator. Purified CD8+ cells were prepared by treating the PHA-stimulated PMC from seropositive or seronegative individuals with anti-CD8 antibody-coated beads in a similar fashion. The magnetic beads generally eluted from the cell surface 48 h later.

In our previous studies (27–31), CD4+ and CD8+ cells were isolated by the panning technique of Wysocki and Sato (33). In comparison with this procedure, the use of the magnetic beads did not alter the kinetics of HIV replication in the CD4+ cells or the ability of CD8+ cells to inhibit virus replication.

In some experiments, CD4+ T cells were enriched by using monoclonal antibodies to the HLA-B27 (Chemicon, El Segundo, Calif.) or HLA-A2 (Atlantic Antibodies, Scarborough, Maine) histocompatibility antigens. Usually, 1×10^7 to 3×10^7 cells were incubated in 1 ml of PBS-FCS containing 10 μg of the appropriate anti-class I HLA antibody for 20 min at room temperature. Cells were washed once and incubated with goat anti-mouse immunoglobulin-coated magnetic beads (Dynal), and adherent cells were isolated as described above. Cell populations recovered by using the Dynabeads were routinely greater than 98% pure by flow cytofluorometric analysis (14).

Virus replication assay. Highly enriched, PHA-stimulated CD4+ T cells (4×10^6 to 6×10^6) from HIV-seronegative subjects were incubated with 1,000 50% tissue culture infective doses (TCID) of HIV-1_{SF33} for 2 h at 37°C and washed two times; 5×10^5 cells were cultured in 24-well tissue culture plates in RPMI 1640 growth medium. This HIV strain replicates rapidly and is very cytopathic for CD4+ cells (23). Infected cells were cultured either alone or in the presence of various numbers of PHA-stimulated CD8+ cells. Culture supernatants were assayed for virus-associated reverse transcriptase (RT) activity at 3-day intervals (7).

Quantitation of HIV-infected cells. The proportion of HIV-infected CD4+ cells was determined immediately before and after coculture with CD8+ T cells by an endpoint dilution analysis as previously described (6). Briefly, various numbers of CD4+ cells (10^4, 10^3, 10^2, and 10^1) were cocultured for up to 21 days in 24-well tissue culture plates with 2×10^6 PHA-stimulated allogeneic PMC from an HIV-seronegative blood donor. Cells were neither added nor removed from the cultures, and half of the medium in each well was replaced every 3 days with fresh RPMI 1640 growth medium, when RT levels in culture supernatants were assessed. The lowest number of CD4+ cells required to give a positive culture was taken as the endpoint. The titer of infected cells was expressed as 1, 10, 100, or 1,000 TCID per 10^4 CD4+ cells.

Cytotoxic cell assay. The ability of CD8+ T cells to lyse uninfected or HIV-infected CD4+ cells was determined in a standard ^{51}Cr release assay (2) according to the scheme outlined in Fig. 1. PHA-stimulated CD4+ cells (10^6) were labeled with 50 μCi (1 μCi = 37 kBq) of $Na_2{}^{51}CrO_4$ (DuPont) for 1 h at 37°C and washed three times. Labeled target cells (2×10^4) were then seeded into duplicate wells of a 96-well flat-bottom tissue culture plate with CD8+ effector cells at various effector/target cell ratios in a volume of 200 μl. Plates were incubated for 4 h at 37°C, and 100 μl of supernatant was removed for counting in a Beckman gamma counter. Minimum and maximum ^{51}Cr release values were determined by incubating target cells in culture medium and 1% Nonidet P-40, respectively. Percent specific ^{51}Cr release was calculated using the formula [(cpm minimum release − cpm test release)/(cpm maximum release − cpm minimum release)] × 100.

RESULTS

HIV suppression does not involve CD8+ cell cytotoxicity. We previously observed that HIV-infected CD4+ cells, when blocked in HIV production by CD8+ T cells, will release virus after the CD8+ cells are removed (28, 29, 31). This finding suggested that CD8+ cells did not reduce virus replication by killing the virus-infected CD4+ cells. To evaluate further whether cytotoxic activity mediated suppression of virus replication in acutely infected CD4+ cells, we monitored CD8+ cell lysis of HIV-infected and uninfected CD4+ cells. Moreover, the relationship between inhibition of virus replication by CD8+ cells and any observed cell killing was assessed by measuring the frequency of HIV-infected CD4+ cells before and after coculture with antiviral CD8+ cells.

In measuring cytotoxic activity, the ability of CD8+ T cells from HIV-seropositive subjects to inhibit replication of HIV-1_{SF33} in CD4+ cells was compared with their lytic activity against these same infected cells in a 4-h ^{51}Cr release assay (Fig. 1). For the virus inhibition studies, the input effector (CD8+)/target (CD4+) cell ratio was 2:1 unless otherwise indicated. As shown in Table 1, CD8+ cells from all nine asymptomatic, HIV-infected subjects reduced the replication of HIV-1_{SF33} by at least 80%. As reported previously (9, 30), PHA-stimulated CD8+ cells from HIV-seronegative subjects had no antiviral activity. Instead, they often enhanced virus production in the culture (Table 1, experiments 1 to 4).

Any cytotoxic activity mediated by these antiviral CD8+ cells against the CD4+ cells was measured at the initiation (day 0) and termination (day 7) of the virus inhibition assays. For the day 0 assays, CD8+ effector cells were assayed immediately after isolation from the PHA-stimulated PMC. Effector CD8+ cells for the day 7 assay were taken from the CD4+/CD8+ coculture used to measure inhibition of virus replication. HIV-infected CD4+ cells that served as targets in this cytotoxic cell assay either were labeled with ^{51}Cr immediately after infection with HIV-1_{SF33} to use at day 0 or were cultured in interleukin-2-containing medium for 7 days before they were labeled and used to measure cytotoxic activity at the termination of the virus inhibition culture. A dose-response relationship between the level of ^{51}Cr released into the culture supernatant and the effector/target cell ratio tested (50:1, 12.5:1, and 3.125:1) was observed in all experiments. Data shown in Table 1 are at a 50:1 effector/target cell ratio.

Among the HIV-infected subjects, substantial variation in CD8+ cell cytotoxic activity directed against the CD4+ target cells was noted. For two of the nine subjects, high levels of lytic activity were detected against both infected and uninfected CD4+ cells on day 0. This strong cytotoxic response was found for at least the first 3 days of culture and frequently persisted for the entire 7-day culture period (unpublished observation). CD8+ cells that lysed the PHA-stimulated CD4+ lymphocytes could be detected in the peripheral blood of these subjects over a period of several weeks to months (data not shown). CD8+ cells from other subjects (experiments 1 to 6 and 8) lysed the target cells at levels equivalent to those for CD8+ cells from HIV-seronegative donors, even though CD8+ cells from these uninfected subjects showed no ability to suppress HIV replication. Taken together, the data demonstrated that CD8+ cells

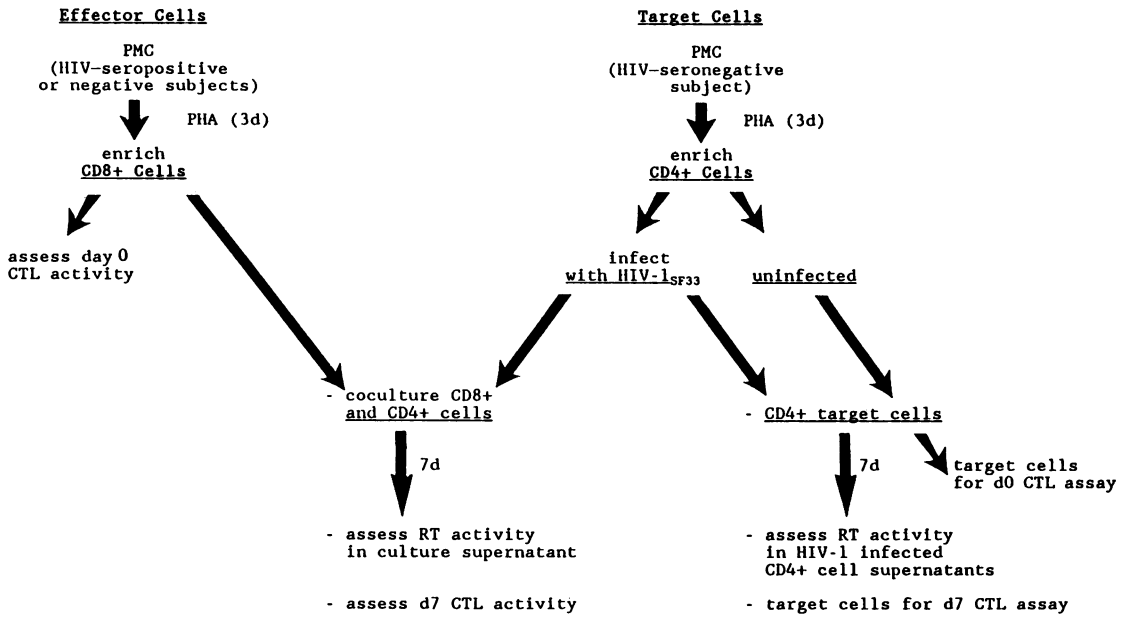

FIG. 1. Analysis of cytotoxic activity and inhibition of HIV growth by CD8+ T cells from HIV-seropositive donors. CD8+ and CD4+ cells were isolated from 3-day (3d) PHA-stimulated PMC from HIV-seropositive and -seronegative subjects, respectively. To measure inhibition of virus replication, a portion of the CD4+ cells were infected with HIV-1_{SF33}, and cocultured with CD8+ cells at a CD8+/CD4+ cell ratio of 2:1. Viral RT activity in the culture supernatants was assayed 7 days later. CTL activity was evaluated at the initiation of the virus inhibition assay (i.e., day 0) by mixing the CD8+ effector cells with ^{51}Cr-labeled, HIV-infected or uninfected CD4+ cells. Populations of the HIV-infected and uninfected CD4+ cells were also maintained for 7 days in interleukin-2-containing RPMI 1640 medium, at which point they were labeled with ^{51}Cr and used as target cells to evaluate cytotoxic activity by CD8+ cells present in virus inhibition assay cultures.

from some HIV-infected subjects could lyse both virus-infected and uninfected CD4+ lymphocytes, but there was no correlation between lytic activity and suppression of HIV replication.

Titration of HIV-1-infected CD4+ cells. Further evidence against a cytotoxic mechanism for HIV suppression in culture was obtained by endpoint dilution analysis of the infected CD4+ cells before and after coculture with CD8+ cells. Representative experiments with CD8+ cells from two different subjects are shown in Table 2.

Supernatants of PHA-stimulated, HIV-1_{SF33}-infected CD4+ cells cultured in the absence of CD8+ cells contained high levels of RT activity on day 7. As expected, replication of the virus was inhibited by the addition of CD8+ cells from HIV-infected individuals at the initiation of culture. Serial 10-fold dilutions of CD4+ cells inoculated onto PHA-stimu-

TABLE 1. Cytotoxic activity of CD8+ cells from HIV-infected and uninfected individuals

Expt	HIV antibody status of CD8+ cell donor	RT activity (10^3 cpm/ml of culture fluid)[a]		% Suppression of RT activity[b]	Cytotoxic activity against CD4+ cells[c]			
					Day 0		Day 7	
		CD4+	CD8+/CD4+		Uninfected	HIV infected	Uninfected	HIV infected
1	+	75.7	6.8	91	ND[d]	ND	9	24
	−		281.4		ND	ND	24	32
2	+	160.0	29.2	82	27	24	19	<1
	−		419.0		12	6	21	18
3	+	214.2	6.1	97	8	9	<1	<1
	−		267.6		13	6	28	10
4	+	105.3	4.9	95	13	8	10	13
	−		290.3		2	3	13	1
5	+	203.1	14.9	93	3	9	13	2
	+		6.6	97	ND	ND	2	12
6	+	1,009.4	3.8	>99	77.5	57	11	12
7	+	277.9	7.1	97	65	89	ND	ND
8	+	207.6	3.4	97	27	39	27	9

[a] CD4+ cells infected with HIV-1_{SF33} were cultured alone or with CD8+ cells with HIV-1-seropositive or -seronegative subjects at a CD8+/CD4+ ratio of 2:1.
[b] Expressed as 100 − {[RT (CD8+/CD4+ coculture)/RT (CD4+ cell culture)] × 100}.
[c] Percent specific ^{51}Cr release from CD4+ target cells at a CD8+/CD4+ cell ratio of 50:1 in a 4-h assay. See text and legend to Fig. 1 for details.
[d] ND, not done.

TABLE 2. Frequency of HIV-infected CD4+ cells before and after coculture with CD8+ lymphocytes from HIV-infected individuals

Subject	RT activity (10^3 cpm/ml of supernatant) on day 7[a]		% Suppression of RT activity[b]	CD4+ cells/well	RT activity (10^3 cpm/ml of supernatant) in cultures of CD4+ cells on[c]:	
	CD4+	CD4+/CD8+			Day 0	Day 7
1	214.2	6.1	97	0	4.7[d]	2.9
				10^1	2.7	2.9
				10^2	2.4	190.1
				10^3	2.5	499.2
				10^4	325.9	736.6
2	472.4	6.7	99	0	2.3	3.8
				10^1	2.5	4.4
				10^2	2.2	6.6
				10^3	2.1	92.4
				10^4	510.3	633.3

[a] For HIV-1_{SF33}-infected CD4+ cells cultured alone or with CD8+ cells from an HIV-1-seropositive individual at a CD8+/CD4+ cell ratio of 2:1. Values for one of four independent experiments are shown.

[b] Calculated as described in Table 1, footnote *b*.

[c] Values are for PMC cultures containing various numbers of HIV-infected CD4+ cells assayed before (day 0) or after (day 7) coculture with CD8+ cells.

[d] Control CD4+ cells cultured in the absence of CD8+ cells had a TCID of ≥1,000/10^4 cells on day 7 (data not shown).

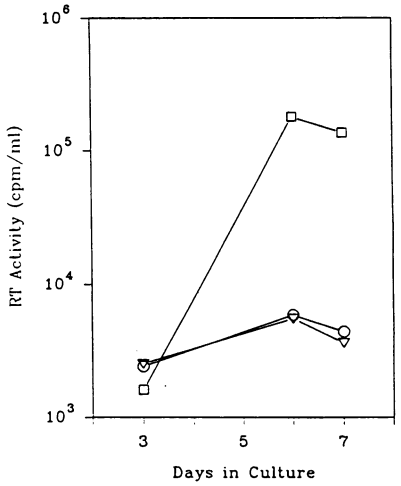

FIG. 2. HIV spread in cultures containing CD4+ cells from two HLA-disparate donors. PHA-stimulated CD4+ cells (5×10^5) from donor 1 (HLA-A2+B27−) were infected with HIV-1_{SF33} and cultured alone (□) or in the presence of CD8+ cells from an HIV-seropositive donor at a CD8+/CD4+ cell ratio of 2:1 (○). A sister culture containing donor 1 CD4+ cells and CD8+ cells from the seropositive donor received 5×10^5 uninfected CD4+ cells from donor 2 (HLA-A2−B27+) 3 days later (▽). CD4+ cells from both donors were recovered from these latter cultures on day 7 by sequential treatment with anti-HLA-A2 and anti-HLA-B27 and magnetic beads, and the TCID of HIV-infected CD4+ cells was determined (see Table 3). Virus replication was measured by viral RT activity in the culture supernatant. A representive of three independent experiments is shown.

lated PMC immediately after HIV infection but before coculture with CD8+ cells revealed a TCID titer of 1/10^4 cells (Table 2). After 7 days of culture, the frequency had increased to at least 1,000 TCID per 10^4 cells (data not shown). CD4+ cells reisolated after 7 days of coculture with CD8+ cells generally had TCID titers of 10 to 100/10^4 cells. The data indicated that coculture of CD8+ cells with CD4+ cells reduced the frequency of infected cells 10- to 100-fold, but the number of infected CD4+ cells increased over the 7-day culture period. Thus, these studies confirmed that the inhibition of virus replication in infected cells by CD8+ cells is not mediated by a cytotoxic cell response. Indeed, it appeared that CD8+ cells could retard but not prevent an increase in HIV-infected cells in culture.

Evaluation of HIV spread in culture. This increase in virus-infected cells could result from either preferential expansion of the infected CD4+ cell population in culture or spread of HIV to uninfected cells over the 7-day culture period. To distinguish between these possibilities, CD4+ cell populations were prepared from two HIV-seronegative donors of defined HLA haplotypes; donor 1 expressed the HLA-A2 antigen but not HLA-B27, whereas donor 2 was HLA-B27 positive and HLA-A2 negative.

CD4+ cells from donor 1 were incubated with HIV-1_{SF33} for 2 h and then treated with 0.25% trypsin for 5 min to inactivate extracellular virus (22a). Endpoint dilution analysis immediately after infection revealed a TCID titer of 1/10^4 CD4+ cells. Duplicate cultures were established that contained CD8+ cells from an HIV-infected individual who was HLA-A2−B27− at an effector/target cell ratio of 2:1. On day 3 after the initiation of culture, uninfected CD4+ cells from donor 2 were added to one of the cocultures. Four days later (1 week after the initiation of the culture), the HIV-infected (donor 1) and uninfected (donor 2) CD4+ cells were reisolated from this culture in a two-step procedure. Goat anti-mouse immunoglobulin-coated Dynabeads were added to the cells after their sequential treatment with monoclonal antibodies to HLA-A2 and HLA-B27, respectively.

As shown in Fig. 2, coculture of the infected CD4+ cells with CD8+ T cells prevented virus production, as detected by RT activity in all cultures. Nevertheless, both the HLA-A2+B27− and HLA-A2−B27+ CD4+ populations had a TCID titer of 10/10^4 cells. Identical TCID titers were obtained in three independent experiments. This number represents a 10-fold increase compared with values for the HLA-A2+B27− cells analyzed immediately after infection (Table 3). These data demonstrated that the increase in the frequency of HIV-infected cells detected during coculture with CD8+ cells resulted from spread of HIV to uninfected cells.

Detection of an antiviral cytokine. The observation that CD8+ cells do not control HIV replication by a cytotoxic response suggested that contact between the CD8+ cells and infected CD4+ cells might not be required for the antiviral activity. To investigate this possibility, PHA-stimulated CD4+ cells from a seronegative donor were infected with HIV-1_{SF2} and cultured either in direct contact with antiviral CD8+ cells or in a transwell culture vessel in which the cell populations were separated by a 0.4 μm-pore-size filter (27). As expected, contact between the two cell types led to complete elimination of detectable virus replication for the duration of the culture period (data not shown). High levels of RT activity were detected when 5×10^5 virus-infected CD4+ cells were cultured in the top well of the transwell culture dish in the absence of CD8+ cells in the bottom well (Fig. 3). The addition of 2×10^6 CD8+ cells to the bottom well of a sister culture resulted in transient interference with virus replication in CD4+ cells contained in the top well. At

TABLE 3. Spread of HIV to CD4+ cells in the presence of antiviral CD8+ lymphocytes from an HIV-infected individual

CD4+ cells/well	RT activity (10^3 cpm/ml of supernatant) in cultures of CD4+ cells from[a]: Donor 1 Day 0	Donor 1 Day 7	Donor 2, day 7
0	ND[b]	5.6	2.8
10^1	8.1	3.2	3.6
10^2	5.0	2.5	3.5
10^3	6.2	302.7	250.7
10^4	516.3	561.3	510.6

[a] CD4+ T cells (5×10^5) from donor 1 (HLA-A2+B27−) were infected with HIV-1_{SF33} and cocultured with 1.5×10^6 CD8+ cells from an HIV-seropositive donor. Three days later, 5×10^5 uninfected CD4+ cells from donor 2 (HLA-A2−B27+) were added (see legend to Fig. 2 for details). On day 7, the viral RT activity in the culture supernatant was determined (see Fig. 2), and both populations of CD4+ cells were reisolated by using monoclonal antibodies to the HLA-A2 and HLA-B27 antigens, respectively. Enriched cells were then serially diluted, and the indicated numbers of cells were added to PHA-stimulated PMC from uninfected donors. Data are from one of three representative experiments.

[b] ND, not done.

day 4, suppression of HIV replication was noted (Fig. 3). By day 7, RT levels increased, but they remained substantially below those in cultures containing CD4+ cells alone (Fig. 3). In an identical culture, the addition of 5×10^5 HIV-infected CD4+ cells in the bottom well to provide viral antigens for stimulation of the CD8+ cells did not enhance the antiviral effect. These results demonstrated the production of a soluble antiviral cytokine by CD8+ cells but indicated that optimal suppression of HIV replication occurred with contact between the two cell types.

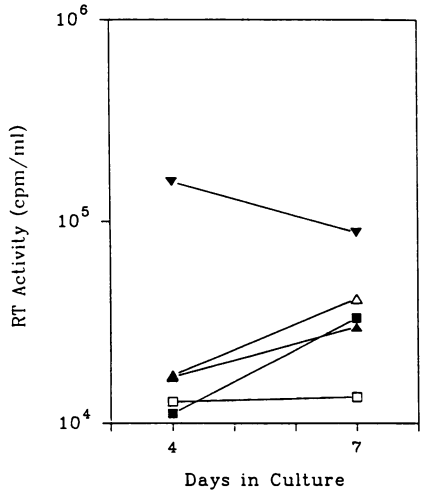

FIG. 3. Production of a soluble antiviral factor by CD8+ cells. The ability of CD8+ cells to produce a diffusible antiviral factor was assessed by using transwell culture plates. The top chamber of each well received 5×10^5 PHA-stimulated CD4+ cells from an HIV-seronegative donor that were infected with HIV-1_{SF2}. The bottom wells either were not reconstituted with CD8+ cells (▼) or received 2×10^6 CD8+ cells from one of two HIV-seropositive subjects (□, △). Sister cultures in which the bottom well contained 5×10^5 HIV-infected CD4+ cells and 2×10^6 CD8+ cells from each of the HIV-infected subjects (■, ▲) were also established. Viral RT activity in the culture medium was assessed 4 and 7 days later. A representative of two independent experiments is shown.

TABLE 4. Inhibition of antiviral activity by monoclonal antibodies to T-cell surface antigens

Cells[a]	Antibody[b]	RT activity (10^3 cpm/ml of culture supernatant) on day 7 Expt 1	Expt 2
−		447.3	256.9
+		2.7	12.5
+	leu 4	222.8	249.5
+	leu 2a	88.8	381.5
+	leu 3a	0.9	8.2
+	3G8	ND[c]	8.1

[a] CD4+ cells infected with HIV-1_{SF33} were cultured alone (−) or in the presence of CD8+ T cells from an HIV-seropositive individual at a CD8+/CD4+ ratio of 1:1 (+).

[b] Monoclonal antibodies to the indicated surface antigens on T (leu 4, leu 2a, and leu 3a) or natural killer (3G8) cells were added to RPMI 1640 growth medium at a concentration of 10 μg/ml at the initiation of culture and 3 days later. Data from two independent experiments are shown.

[c] ND, not done.

Role of cell contact in HIV inhibition. The requirement for cell-cell contact was directly investigated by attempting to block suppression of HIV growth with monoclonal antibodies to CD8+ T-cell surface antigens. PHA-stimulated CD4+ cells were infected with HIV-1_{SF33} and cocultured with antiviral CD8+ cells in the presence and absence of antibodies to CD8 (leu 2a), CD3 (leu 4), CD4 (leu 3a), or CD16 (3G8). As shown in Table 4, when antibodies to CD3 (the T-cell receptor for antigen) and CD8 (an accessory molecule involved in T-cell signaling and adhesion to target cells) were included in the culture medium, high levels of RT activity were detected even in the presence of CD8+ cells. Addition of antibodies to CD4 or CD16 did not interfere with the antiviral activity of the CD8+ T cells.

DISCUSSION

These studies further demonstrate that CD8+ T cells can inhibit HIV replication in CD4+ lymphocytes after in vitro infection with a rapidly replicating, highly cytopathic strain of the virus. They indicated that antiviral activity is similar in several ways to that described previously against naturally infected CD4+ cells (24, 27–29, 31): the response was not HLA restricted, cytotoxicity was not involved, a soluble factor could mediate the response, and cell contact was required for the most efficient antiviral activity.

HIV-specific cytotoxic T lymphocytes (CTL) are present in many tissues of infected individuals (11, 20, 22, 26). These cytotoxic cells are usually HLA restricted, although CTL directed against histoincompatible target cells expressing the HIV envelope protein have been described (11). Previous studies demonstrating that infected CD4+ cells were not eliminated during coculture with CD8+ cells suggested that cytotoxic cell activity was not responsible for suppression of HIV replication (27–31). However, we could not exclude the possibility that infected cells had not expressed viral proteins and thus were not recognized by virus-specific CTL. Spread of HIV to uninfected cells in the culture (Fig. 2; Table 3) indicated that viral antigens were expressed and argues against a role for virus-specific CTL in the control of HIV replication in this system. In addition, CD8+ cell-mediated lysis of uninfected CD4+ cells originally described by Zarling et al. (35) varied markedly from subject to subject (Table 1) and did not correlate with suppression of HIV replication in the cultures.

An increase in the number of infected CD4+ cells in the presence of an antiviral CD8+ cell response was an unexpected observation. It is possible that low levels of cell-free virus not detectable by the RT assay could infect other CD4+ cells. Alternatively, infection of CD4+ cells in culture could be the result of direct cell-to-cell spread of the virus. In support of this hypothesis, macrophage cell lines have been shown to contain infectious intracellular HIV-1 particles that can be transmitted to CD4+ cells in the absence of virus release into the culture supernatant (17, 21). Nevertheless, whether HIV spread to CD4+ cells in the cultures indicates (i) an inability of CD8+ cells to completely suppress HIV replication or (ii) a requirement for higher numbers of these cells to block HIV release needs to be further evaluated.

The observation that virus production is reduced when CD8+ and CD4+ cells are separated by a 0.4-μm-pore-size filter (Fig. 3) indicated that the antiviral activity is mediated at least in part by a soluble factor. A similar finding has been made with use of naturally infected CD4+ cells (1, 27), and similar results were recently reported for studies using acutely infected CD4+ cells (12, 13). The identity of this CD8+ cell-derived factor is not known, but it appears to be distinct from previously characterized lymphokines (13). Activated CD8+ T cells produce gamma interferon and tumor necrosis factor, which have been reported to act synergistically to interfere with HIV replication (32). However, CD8+ cells from uninfected individuals also produce these cytokines (4, 5, 18, 34). This observation and other recent studies (1, 13) suggest that these well-characterized lymphokines are not responsible for the antiviral activity described here.

The findings that optimal suppression required contact between the CD4+ and CD8+ cells and that the antiviral activity can be blocked by monoclonal antibodies to CD3 and CD8 (Table 4) suggest that cell-cell interaction permits better production or delivery of the factor(s). This latter possibility would be consistent with expression of membrane-bound forms of cytokines on human T lymphocytes (10).

It is noteworthy that CD8+ cells from HIV-seronegative donors suppress HIV replication in naturally infected CD4+ cells (1; unpublished observation) but not in acutely infected cells (9, 30) (Table 1). This observation may reflect different replicative states of the virus within the cells. Naturally infected CD4+ cells appear to harbor HIV in a state of latency or restricted replication, and virus production in vitro requires activation with antigen (15) or mitogens (3, 16). Conceivably, HIV in naturally infected CD4+ cells is blocked from reactivation by a soluble factor produced by the CD8+ cells from both HIV-seropositive and -seronegative donors. Suppression of replicating virus, however, would require CD8+ cells from HIV-infected individuals that produce either higher levels of this factor or a second factor that acts at a different stage in the viral replication cycle.

In summary, these studies extend further the observations on the anti-HIV response of CD8+ cells of infected individuals. They indicate that in addition to the CTL activity described by others (11, 20, 22, 26), CD8+ cells may control HIV replication in the host by noncytotoxic mechanisms.

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