Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2308–2312, March 1995
Immunology

$CD8^+$ T cells suppress human immunodeficiency virus replication by inhibiting viral transcription

CARL E. MACKEWICZ, DAVID J. BLACKBOURN, AND JAY A. LEVY

Cancer Research Institute and Department of Medicine, University of California, School of Medicine, San Francisco, CA 94143-0128

Communicated by Maurice Hilleman, Merck Research Laboratories, West Point, PA, December 14, 1994

ABSTRACT **$CD8^+$ cells from human immunodeficiency virus (HIV)-infected individuals suppress HIV replication in cultured $CD4^+$ cells by a noncytolytic mechanism that involves a secreted $CD8^+$-cell antiviral factor (CAF). The results of this study suggest that $CD8^+$ cells, as well as CAF, arrest HIV replication at the level of viral transcription. Culturing naturally infected $CD4^+$ cells actively producing HIV with autologous $CD8^+$ cells or a 50% dilution of culture fluids from these cells results in a >80% reduction in the number of cells expressing HIV antigens and RNA. This effect was observed within 2 days after exposure to $CD8^+$ cells but required 6 days in the presence of CAF-containing culture fluids to reach the same extent of HIV suppression. Northern blot analysis of $CD4^+$ cell extracts revealed that all viral RNA species (unspliced and single and double spliced) were reduced in quantity to a similar extent. CAF-containing culture fluids also had a direct inhibitory effect on HIV long terminal repeat (LTR)-driven transcription in HIV-infected 1G5 cells carrying an LTR–luciferase construct. Suppression of basal levels of LTR-driven transcription was not detected. Thus, the results suggest that the noncytolytic $CD8^+$ cell antiviral activity observed in HIV infection exerts its effects, at least in part, by specifically interrupting HIV transcription. These findings could help in developing therapies for HIV infection.**

Two types of $CD8^+$-cell-mediated antiviral functions have been described in human immunodeficiency virus (HIV) infection. One involves the classical HLA-restricted cytolysis of infected cells (1) and the other results from the inhibition of HIV replication in the absence of cell killing (2). The noncytolytic $CD8^+$-cell antiviral response is characterized by the suppression of HIV replication in cultured $CD4^+$ cells by activated $CD8^+$ cells. It is detected by a reduction in HIV p24 antigen and reverse transcriptase (RT) levels in the culture fluids and can be demonstrated with all strains of HIV-1, HIV-2, and simian immunodeficiency virus tested (2, 3). The suppressive effect involves the activity of a $CD8^+$-cell antiviral factor (CAF) (4–6) and does not require HLA compatibility at the effector phase. The clinical relevance of this type of antiviral activity is suggested by its occurrence in all asymptomatic HIV-infected individuals tested and the reduction in this response with advancement to disease (7, 8).

Many of the details of the $CD8^+$-cell cytolytic response have been elucidated (1, 9). In contrast, very little is known about the mechanism responsible for noncytolytic $CD8^+$-cell suppression of HIV replication. The antiviral effect is not attributed to direct inactivation of RT nor an alteration in the infectivity of virus particles (unpublished data). Moreover, mitogen-induced proliferation and expression of the activation antigens CD25, CD38, CD69, and HLA-DR by the target $CD4^+$ cells is not adversely affected by exposure to $CD8^+$ cells or CAF (2, 5, 6). The objective of the present study was to determine which step(s) in the replication cycle of HIV are affected by the noncytolytic $CD8^+$-cell antiviral activity. The results suggest that viral transcription is specifically suppressed by this immune response.

MATERIALS AND METHODS

Subjects. Heparinized peripheral blood samples were obtained by phlebotomy from HIV-1-seropositive donors. The HIV-infected individuals were clinically healthy with $CD4^+$ T-cell counts >400 cells per μl. Blood samples from HIV-seronegative donors were provided by Irwin Memorial Blood Centers (San Francisco). The study received the approval of the Committee on Human Research, University of California, San Francisco (UCSF).

Flow Cytometric Analysis. T-cell subset purity was analyzed by a double-staining procedure (10) on a Becton Dickinson FACscan by using either fluorescein isothiocyanate (FITC)-conjugated anti-Leu-3a or phycoerythrin (PE)-conjugated anti-Leu-2a with either FITC- or PE-conjugated anti-Leu-4, kindly provided by Becton Dickinson.

CAF-Containing Culture Fluids. Culture fluids containing CAF were obtained by stimulating purified $CD8^+$ T cells (>95% $CD3^+/CD8^+$) [recovered from peripheral blood mononuclear cells (PBMCs) of HIV-infected subjects] with anti-CD3-coated immunomagnetic beads for 3 days as described (6). Culture fluids collected every 2 days thereafter were passed through a 0.45-μm (pore size) filter and stored frozen at −70°C. CAF content in the $CD8^+$-cell culture fluids was assayed by testing for anti-HIV activity in an acute infection microtiter assay (6). The amount of CAF activity present in the culture fluid is reflected by the extent of reduction in RT activity in the culture fluid from wells treated with $CD8^+$-cell culture fluid compared with that from infected $CD4^+$ cells receiving the control medium. For the studies described in this report, only those $CD8^+$-cell culture fluids that inhibited HIV replication by 50% or more were used (termed CAF-containing fluid). Previous studies have indicated that this HIV-suppressing activity in the $CD8^+$-cell culture fluids is not mediated by any known cytokine (6). In some experiments, $CD8^+$-cell culture fluids that suppressed HIV replication by <10% were used as negative control fluids.

Assay for the Effect of $CD8^+$-Cell Antiviral Activity on HIV Protein and RNA Expression. The effect of $CD8^+$ cells, or CAF fluids, on the production of HIV was assessed by using naturally infected $CD4^+$ cells as the targets. In brief, $CD4^+$ cells were isolated from the PBMCs of HIV-infected individuals by using anti-CD4 immunomagnetic beads (Dynal, Great Neck, NY) as described (7). In each case, the purity of the $CD4^+$-cell populations was 95% or greater and >95% were $CD3^+$ as assessed by flow cytometric analysis (10). The $CD4^+$ cells and the $CD4^+$ cell-depleted population were then cultured separately for 3 days with phytohemagglutinin (3 μg/ml)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAF, $CD8^+$-cell antiviral factor; HIV, human immunodeficiency virus; LTR, long terminal repeat; RT, reverse transcriptase; PBMC, peripheral blood mononuclear cell; IFA, immunofluorescence antibody.

(Sigma) in RPMI 1640 medium containing 2 mM glutamine, 1% antibiotics [penicillin (100 units/ml)/streptomycin (100 μg/ml)], 10% (vol/vol) heat-inactivated (56°C, 30 min) fetal calf serum, and human recombinant interleukin 2 (Collaborative Research) (200 units/ml). Subsequently, the $CD8^+$ cells were isolated from the $CD4^+$-cell-depleted population by using anti-CD8 immunomagnetic beads (7). The $CD4^+$ and $CD8^+$ cells were then cultured separately, changing the culture medium every 3 days until the $CD4^+$ cells were actively replicating HIV (RT activity at >100,000 cpm/ml). The $CD4^+$ cells were washed three times and replated at 2×10^6 cells per well in the absence or presence of an equal number of the autologous $CD8^+$ cells. These cultures were passed at 2-day intervals at which time samples were taken for the quantitation of (*i*) particle-associated RT activity (a measurement of HIV particle production) (11), (*ii*) the number of cells staining positive in an immunofluorescence antibody (IFA) assay with an HIV-infected individual's serum that had previously been shown by Western blot analysis to react with all the major HIV proteins (a measurement of both intracellular and cell surface HIV protein expression) (12), and (*iii*) the number of cells staining positive by *in situ* hybridization (a measurement of HIV RNA expression). *In situ* hybridization was performed by using an alkaline phosphatase-labeled probe specific for HIV gag RNA (DuPont/NEN) and following the manufacturer's manual.

Determination of the percentage of $CD4^+$ cells expressing HIV proteins (IFA positive) or HIV RNA (*in situ* positive) in the wells containing both $CD4^+$ and $CD8^+$ cells was accomplished by taking into consideration the actual percentage of $CD4^+$ cells present at each time point (determined by flow cytometric analysis). HIV replication in $CD8^+$ cells by either of these two methods has not been observed.

In some experiments, the infected $CD4^+$ cells were cultured in a 1:1 dilution (with culture medium) of CAF-containing culture fluid or a negative control culture fluid. Fresh CAF (or control) fluid was added every 2 days.

Infectious Center Assay. Infectious center assays used to enumerate HIV-infected cells were performed by using a standard limiting dilution approach as described (13). In brief, on day 4 or 6 of the above described experiments, cultured $CD4^+$ cells and the mixture of $CD4^+/CD8^+$ cells were washed separately to remove free virus. Serial dilutions of the cells were made to yield from 10^3 to 1 $CD4^+$ cell(s) per well. Each dilution was then added (in triplicate) to 2×10^6 activated seronegative PBMCs (which served as targets for HIV infection and replication). These cultures were then monitored every 3 days for RT activity. The percentage of infected cells was determined from the highest dilution yielding at least one productively infected well by the following formula: (number of productively infected wells/number of $CD4^+$ cells plated per triplicate) × 100.

Measurement of HIV Transcription by Northern Blot Analysis. To measure the relative amount of the various species of HIV RNA transcripts produced in the infected $CD4^+$ cells, experiments analogous to those described above were performed. In this case, however, on day 4 the cells were collected, washed twice, then frozen in a methanol/dry ice bath, and stored at −70°C for subsequent RNA extraction by the method of Chomczynski *et al.* (14). Because of technical difficulties that would lead to loss of infected cells or reduction in the stability of the cellular RNA, $CD8^+$ cells were not removed from the 1:1 $CD4^+/CD8^+$-cell mixtures. Therefore, a compensating amount of exogenous RNA from $CD8^+$ cells (calculated by using flow cytometry data) was added to the RNA extracted from the $CD4^+$-cell controls immediately preceding glyoxal denaturation. Extracted RNA was transferred to nylon membranes for Northern blot hybridization analysis after denaturing by incubation with glyoxal and electrophoresed through 1% agarose gels as described by Williams and Mason (15). HIV transcripts were detected by hybridization with a radiolabeled probe synthesized by a random-primed DNA synthesis reaction (Megaprime DNA labeling system, Amersham). Unincorporated nucleotides were removed by spin column chromatography (MicroSpin Column, Sephacryl S-400HR, Pharmacia). The template for probe synthesis was the HIV long terminal repeat (LTR)–*nef* insert from plasmid pAM3 (16). The template for the β-actin probe was a full-length insert from a plasmid vector provided by B. M. Peterlin (UCSF). Densitometry was performed on autoradiographs with an Ultrascan XL densitometer (LKB).

LTR-Driven Transcription in the 1G5 Jurkat T-Cell Line. The 1G5 cell line was derived from Jurkat cells transfected with a LTR–luciferase gene construct (17). This cell line was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Bethesda, MD). For the studies with HIV, the cells were acutely infected with 10,000 $TCID_{50}$ of HIV-1_{SF33} as described (6) for the infection of primary $CD4^+$ lymphocytes, with the exception that the cells were not pretreated with Polybrene. After a 1-h incubation with virus, the cells were washed three times and plated in 24-well plates in duplicate with complete culture medium

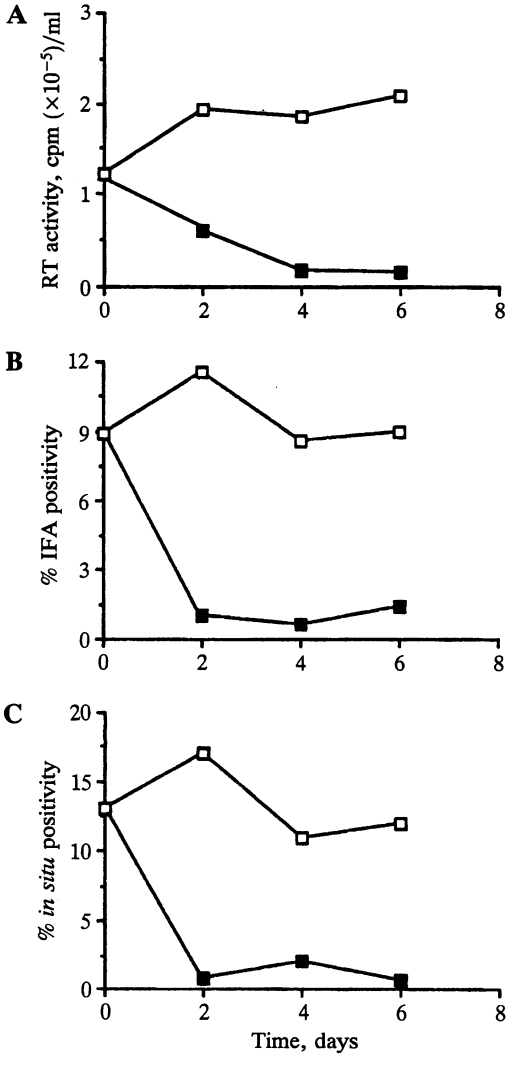

FIG. 1. Effect of antiviral $CD8^+$ cells on HIV production as measured by RT activity in the culture supernatants (*A*), by the number of $CD4^+$ cells staining positive by an IFA assay (*B*), and by the number of cells staining positive by *in situ* hybridization with a *gag*-specific probe (*C*). Day 0 represents the time at which naturally infected $CD4^+$ cells actively replicating HIV were washed and replated alone (□) or with an equal number of autologous $CD8^+$ cells (■). Results are representative of five experiments.

Table 1. Effect of $CD8^+$ cells on HIV replication in $CD4^+$ cells

Exp.	Parameter	Day 0 $CD4^+$	Day 4 (exp. 1) or day 6 (exp. 2) $CD4^+$	$CD4^+/CD8^+$
1	RT activity, cpm ($\times 10^{-3}$)/ml	130	252	39
	% IFA positive	8	35	4
	% *in situ* positive	9	20	1
	% infected cells	—	10	9.5
2	RT activity, cpm ($\times 10^{-3}$)/ml	210	142	23
	% IFA positive	12	21	2
	% *in situ* positive	12	10	3
	% infected cells	—	35	27

Infected $CD4^+$ cells actively producing HIV were cultured alone ($CD4^+$) or in the presence of $CD8^+$ cells ($CD4^+/CD8^+$) as described in Fig. 1. Viral particle production is indicated by RT activity in the culture fluids, the percent of $CD4^+$ cells expressing viral antigens was determined by IFA assay, and the percent of $CD4^+$ cells expressing viral RNA was measured by *in situ* positive hybridization procedures. The percent of infected cells was determined by an infectious center assay using a limiting dilution scheme with cells taken on the indicated day.

lacking recombinant interleukin 2. This method routinely yields >50% syncytium-forming cells by peak HIV replication (6–7 days) (unpublished data). CAF-containing fluids or control culture supernatants were added to the acutely infected 1G5 cells to yield a 1:1 dilution of the fluid. The cultures were passed on day 2, and on day 3 the cells were collected, washed twice, then frozen in a methanol/dry ice bath, and stored at −70°C for subsequent luciferase extraction. The amount of luciferase produced was assayed with a luciferase assay kit from Promega by the manufacturer's instructions. Luminescence was measured with a Lumat LB 9501 luminometer (EG & G, Salem, MA) kindly provided by Wallac (Gaithersburg, MD). Culture fluids from each passage were also monitored for RT activity. LTR-driven luciferase transcription in this cell line has been shown to be extremely sensitive to virus-expressed Tat and to Tat produced as a result of transfection with the *tat* gene (17).

RESULTS

$CD8^+$ Cells and CAF Suppress the Expression of HIV Protein and RNA. The effect of $CD8^+$-cell antiviral activity on HIV protein and RNA expression in naturally infected $CD4^+$ cells was examined (Fig. 1). Autologous $CD8^+$ cells were added at a ratio of 1:1 to $CD4^+$ cells that were actively replicating virus (day 0). In the absence of $CD8^+$ cells, high levels of RT activity were noted on days 2, 4, and 6, while dramatically reduced levels were observed as a consequence of culturing the $CD4^+$ cells with $CD8^+$ cells (as much as 86% lower by day 4 relative to the day 0 control level) (Fig. 1*A*). The amount of $CD4^+$ cells expressing HIV proteins detected by IFA was 9% before $CD8^+$ cells were added. As early as 2 days after addition of $CD8^+$ cells, the percent of IFA-positive cells was reduced to 1% and remained at a low level through day 6 (Fig. 1*B*). *In situ* hybridization measurements yielded analogous results. At day 0, 13% of the $CD4^+$ cells stained positive for HIV RNA, and addition of $CD8^+$ cells to the $CD4^+$ cells caused a large reduction in this level (94% on day 2) (Fig. 1*C*).

Thus, within 2 days, $CD8^+$ cells appear to be inhibiting the production of viral RNA and, consequently, viral proteins in these $CD4^+$ lymphocytes. Despite many previous observations indicating a nonlytic mechanism for this type of antiviral activity, the present data still raised the question of whether the $CD8^+$ cells were eliminating infected cells in the cultures. Therefore, to enumerate the number of infected cells in each culture, we conducted infectious center assays. In the first example, at the initiation of the experiment, the $CD4^+$-cell

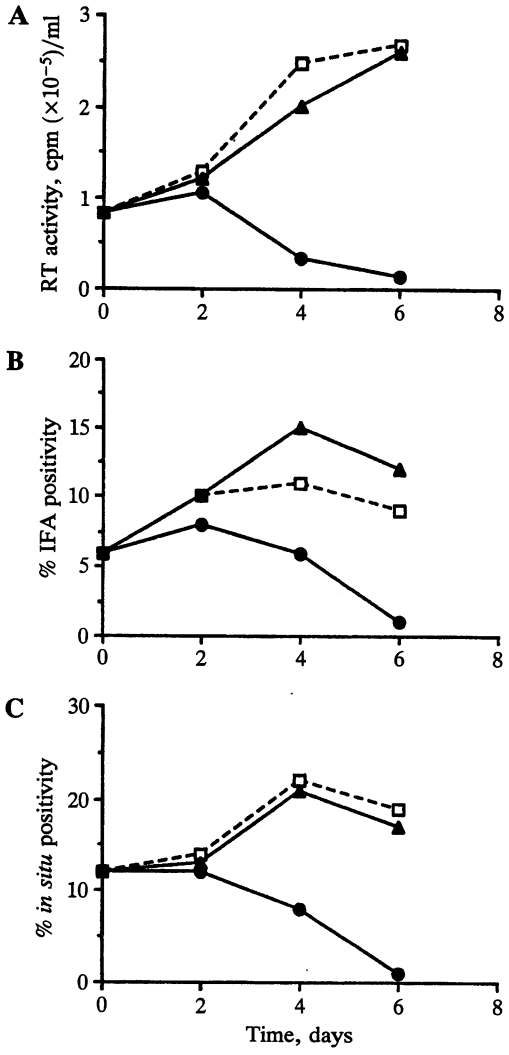

FIG. 2. Regulatory effects of CAF-containing culture fluid on HIV production as measured by RT activity in the culture supernatants (*A*), the number of $CD4^+$ cells staining positive by an IFA assay (*B*), and the number of cells staining positive by *in situ* hybridization with a *gag*-specific probe (*C*). Day 0 represents the time at which naturally infected $CD4^+$ cells actively replicating HIV were washed and replated in culture medium (□), in a 1:1 dilution of a $CD8^+$-cell culture fluid previously shown to have CAF activity (●), or in a 1:1 dilution of a $CD8^+$-cell culture fluid previously shown to lack CAF activity (▲). The cultures were passed and replenished with fresh $CD8^+$-cell culture fluids or control medium every 2 days. Results are representative of two experiments.

culture contained 8% IFA-positive cells and 9% *in situ*-positive cells (Table 1). Four days after culture, the amount of IFA-positive and *in situ*-positive $CD4^+$ cells increased 4- and 2-fold, respectively. In contrast, in $CD4^+$-cell cultures receiving the $CD8^+$ cells, the amount of cells positive by IFA was reduced to 4%, and the percent positive by *in situ* hybridization was reduced to 1%. Despite the reduction of HIV replication in the $CD4^+/CD8^+$ cocultures, the results from the infectious center assay indicated that the amount of infected cells in these cocultures was nearly identical to the amount in the $CD4^+$-cell control culture, 9.5% and 10%, respectively (Table 1). Similar results were seen in experiment 2. Again, $CD8^+$ cells dramatically reduced HIV replication as indicated by all three parameters measured: RT activity, HIV protein synthesis, and HIV RNA expression. Importantly, although the number of infected cells was slightly lower in the $CD4^+/CD8^+$-cell culture, this small difference cannot explain the large reductions in the other parameters of HIV replication.

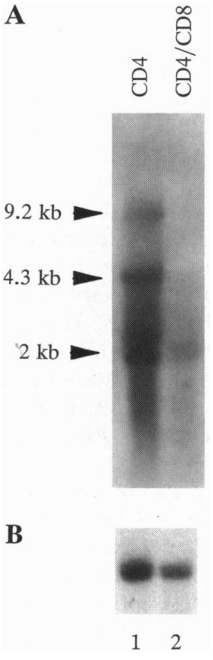

FIG. 3. Northern blot analysis showing the effect of antiviral $CD8^+$ cells on HIV RNA expression (*A*) and β-actin expression (*B*) in naturally infected $CD4^+$ cells. Total RNA was extracted from infected $CD4^+$ cells cultured alone (lane 1) or cultured for 4 days in the presence of an equal number of autologous $CD8^+$ cells (lane 2). (*A*) The hybridization was performed with an HIV LTR–*nef* probe on 70 μg of total RNA in each lane. The distinct bands labeled as 9.2, 4.3, and 2 kb represent unspliced, single-spliced, and double-spliced HIV transcripts, respectively. Levels of RT activity were measured in day 4 supernatants from the cultures and found to be 174×10^3 cpm/ml in $CD4^+$ cells cultured alone (lane 1) and 43×10^3 cpm/ml in $CD4^+$ cells cultured for 4 days with $CD8^+$ cells (lane 2). (*B*) A β-actin probe was used on 7 μg of total RNA in each lane.

Analogous results have been observed when these experiments were carried out by exposing the infected $CD4^+$ cells to a 1:1 dilution of CAF-containing $CD8^+$-cell culture fluid (Fig. 2). While little or no effect was seen on day 2, by day 6 the CAF-containing fluid suppressed RT activity by 84%, the number of cells staining IFA positive by 83%, and the *in situ*-positive cells by 92% compared to results with the control culture medium. A 1:1 dilution of a nonsuppressing negative control $CD8^+$-cell culture fluid did not significantly affect HIV replication in these experiments. Not all CAF-containing fluids are able to suppress these parameters to this extent when ongoing HIV replication is evaluated, even though they can suppress RT levels in the acute infection microtiter assay (data not shown). The reason for this discrepancy is unclear but likely reflects a difference in sensitivity between the two virus assays.

$CD8^+$ Cells Suppress Transcription of Spliced and Full-Length HIV RNA. Because the hybridization experiments described above were performed with a probe specific for HIV gag RNA that only detects full-length viral RNA, we could not assess the effects on transcription of the earlier single- and double-spliced HIV RNA. To directly assess the effects of antiviral activity on the expression of all species of HIV RNA, we used Northern blot analyses on total RNA extracted from infected $CD4^+$ cells exposed to $CD8^+$ cells. A representative experiment is shown (Fig. 3). On day 4 after exposure to $CD8^+$ cells, the level of HIV production, detected by RT activity in the culture fluids, had been reduced by 75% (from 174,000 to 43,000 cpm/ml) compared to the control. In this study, the three types of HIV RNA transcripts were expressed in the control infected $CD4^+$ cells, the 9.2-kb unspliced, the 4.3-kb single-spliced, and the 2-kb double-spliced RNAs (lane 1). Exposure to $CD8^+$ cells caused a marked reduction in the levels of all three HIV RNA species (lane 2). A densitometer scan of this radiograph indicated that the unspliced and single-spliced species were reduced by 80–90% and the double-spliced species was reduced by ≈70%. To determine whether exposure to $CD8^+$ cells suppressed cellular transcription in general, the level of β-actin transcription was also analyzed by Northern blot analysis (Fig. 3). In three experiments, β-actin levels were slightly reduced by exposure of the infected $CD4^+$ cells to the $CD8^+$ cells (average, 11 ± 6%, as detected by densitometry).

CAF Suppresses LTR-Driven Transcription. The ability of CAF to directly inhibit LTR-driven transcription was assessed by using HIV infection of 1G5 cells. The viral Tat protein promotes the transcription of the reporter gene luciferase through the linked LTR. Culturing 1G5 cells in a 1:1 dilution of CAF-containing culture fluids, immediately after infecting them with HIV, caused the suppression of RT levels (Fig. 4*A*) and LTR-driven luciferase transcription (Fig. 4*B*) relative to control levels. Culturing of the uninfected IG5 cells with the same CAF-containing fluid did not affect the basal level of luciferase produced compared to results with the control culture fluid (Fig. 4*C*). Therefore, while CAF does not appear to inhibit basal transcription through HIV LTR, it does suppress the ability of HIV infection to enhance LTR-driven transcription of luciferase.

DISCUSSION

The studies described in this report suggest that the noncytolytic antiviral activity mediated by $CD8^+$ T cells inhibits HIV

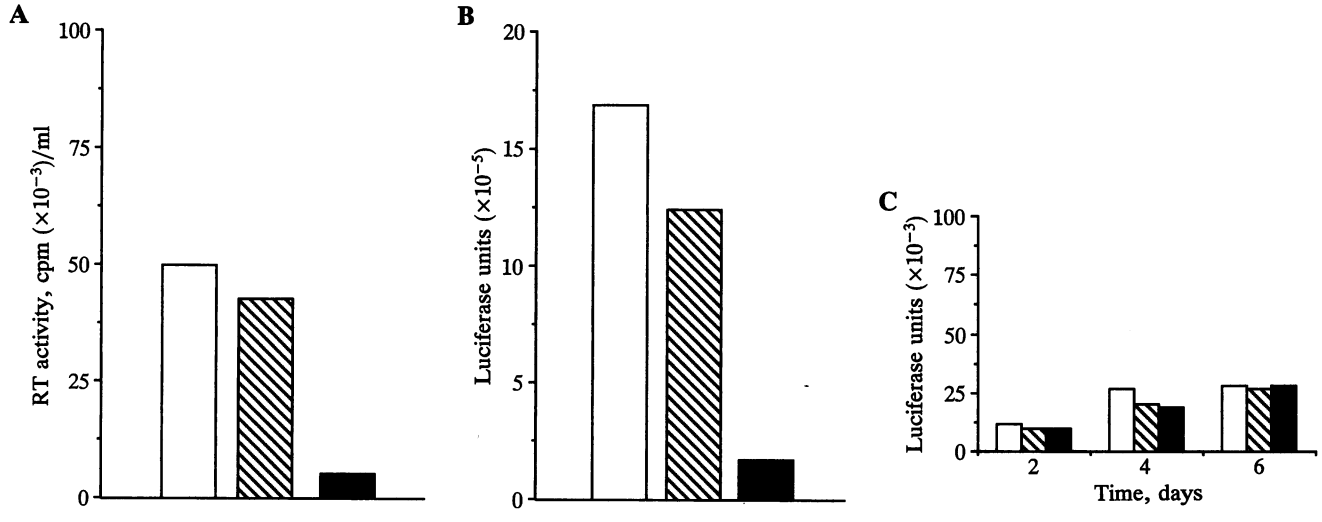

FIG. 4. Effect of CAF on HIV replication and LTR-driven transcription in 1G5 cells. 1G5 cells, containing an LTR–luciferase construct, were acutely infected with HIV-1_{SF33} (*A* and *B*) or left uninfected (*C*) and then cultured in 1:1 dilutions of a control medium (□), a negative control $CD8^+$-cell culture fluid lacking CAF activity (▨), or a CAF-containing $CD8^+$-cell culture fluid (■). The cultures were passed on day 2 and replenished with fresh CAF or control fluids. Day 3 culture fluids were assayed for RT activity (*A*), and cell extracts were assayed for luciferase content (*B*). Luciferase content in the uninfected cells was assessed in extracts of cell samples taken at the indicated time points. The results shown are representative of three experiments.

replication by down-regulating viral RNA transcription. Three lines of evidence support this conclusion. (*i*) Culturing naturally HIV-infected $CD4^+$ cells in the presence of autologous $CD8^+$ T cells resulted in an 80–90% reduction in the number of $CD4^+$ cells producing viral proteins and the number of cells expressing viral RNA (Table 1 and Fig. 1). The lack of virus production cannot be attributed to a decreased number of infected cells as indicated by the results from the infectious center assays (Table 1). Similarly, CAF-containing $CD8^+$-cell culture fluids were also able to reduce expression of HIV protein and RNA in $CD4^+$ cells but to a lesser extent (Fig. 2). Thus, the decreased amount of mature viral particles produced and HIV proteins expressed is likely the consequence of the HIV replication cycle being halted at the stage of RNA synthesis.

(*ii*) Northern blot analyses demonstrated that the transcription of all species of HIV RNA was down-regulated upon exposure of the infected $CD4^+$ cells to autologous $CD8^+$ T cells (Fig. 3). The extent of reduction of unspliced, single-spliced, and double-spliced RNAs was nearly equal although double-spliced RNA may have been slightly less affected. The limited reduction in levels of β-actin expression in the $CD4^+$ cells mixed with $CD8^+$ cells compared to $CD4^+$ cells cultured alone suggests that the regulatory effects of this antiviral activity on transcription may be specific to viral transcription. This conclusion is also supported by previous observations that exposure of infected $CD4^+$ cells to antiviral $CD8^+$ cells while suppressing HIV replication does not affect the proliferation or expression of activation antigens on the $CD4^+$ cells (2).

(*iii*) CAF-containing $CD8^+$-cell culture fluids suppressed the ability of HIV to enhance LTR-driven transcription of the luciferase reporter gene in 1G5 cells (Fig. 4). Yet, CAF does not appear to affect basal levels of LTR transcription in this T-cell line (Fig. 4*C*). While these experiments also support an antiviral mechanism that inhibits HIV replication at the transcriptional level, they do not discern whether this suppressive activity is directly affecting Tat function or, alternatively, the function of cellular factors involved in the transcription of Tat through the LTR (18). These experiments also do not address the possible effects of $CD8^+$-cell antiviral activity on other steps in the HIV replication cycle.

By similar *in situ* hybridization techniques but a different approach than was used in the present studies, Bagasra and Pomerantz (19) also demonstrated that the antiviral activity of $CD8^+$ cells can modulate the expression of HIV RNA. They found that depletion of $CD8^+$ cells from acutely infected PBMCs greatly increased the number of cells exhibiting active transcription of HIV RNA (from <1% to an average of 34%). Without data on the number of infected cells, these results may represent the inhibition of virus spread and not actual effects on viral transcription.

More recently, Powell *et al.* (20) studying a simian immunodeficiency virus model and Chen *et al.* (21) examining human cellular immune responses described a direct inhibitory effect on viral LTR-driven transcription mediated by $CD8^+$ lymphocytes. Both studies demonstrated a suppressive effect by $CD8^+$ cells and by $CD8^+$-cell culture fluids on LTR-driven chloramphenicol acetyltransferase (CAT) transcription. In one study, this inhibitory effect was observed in primary $CD4^+$ cells (21) in both Tat-mediated enhancement of LTR-driven CAT transcription and basal levels of LTR transcription. In the other study using an Epstein–Barr virus-transformed B-cell line (20), an inhibitory effect was observed in the basal level but not in Tat-mediated enhancement of transcription. Because they used a B-cell line their findings may not be relevant to the regulatory events that occur in $CD4^+$ lymphocytes. The difference between our results in the Jurkat T cells showing a lack of effect of CAF on basal levels of LTR driven transfer and those of Chen *et al.* (21) may again be due to specific cellular transcriptional factors present, or their relative abundance, in the target cells studied.

It is unclear whether this type of antiviral activity represents a response of the host that has evolved to maintain homeostasis in a virus-laden environment or simply reflects a beneficial effect of a normal immunoregulatory mechanism. If, for instance, HIV replication is halted by this $CD8^+$-cell activity, presumably in a quiescent proviral state, and the infected cells are not eliminated, then the cellular viral burden would not decline concomitant with this response. Work by Graziosi *et al.* (22) that measured viral burdens during primary HIV infection is in full agreement with this hypothesis. They demonstrated in a limited number of patients that while virus levels in the plasma and the degree of virus replication in PBMCs (detected by RNA production) were down-regulated shortly after infection with HIV, the viral burden in PBMCs did not decline.

In summary, HIV-infected individuals possess $CD8^+$ cells that can suppress active HIV replication in $CD4^+$ lymphocytes without eliminating infected cells. The decrease in virus production can be explained by a reduction in the level of viral RNA, probably due to inhibition of transcription. It remains to be determined specifically how the $CD8^+$ cells (and CAF) are affecting HIV RNA at the molecular level. A better understanding of the mechanism by which this antiviral activity operates could lead to the development of additional antiretroviral therapies.

We thank Jason Wu, Hank Ortega, and Harold Legg for their technical assistance, and Christine Beglinger and Ann Murai for help in preparation of the manuscript. These studies were supported by National Institutes of Health Grant RO1 AI30350; C.E.M. was supported in part by the UCSF AIDS Clinical Research Center (funded by the University of California, Universitywide AIDS Research Program); and D.J.B. was supported in part by the University of California, Universitywide AIDS Research Program (F94-SF-008).

1. Walker, B. D. & Plata, F. (1990) *AIDS* **4,** 177–184.
2. Mackewicz, C. & Levy, J. A. (1992) *AIDS Res. Hum. Retroviruses* **8,** 629–640.
3. Walker, C. M., Thomson-Honnebier, G. A., Hsueh, F. C., Erickson, A. L., Pan, L.-Z. & Levy, J. A. (1991) *Cell. Immunol.* **137,** 420–428.
4. Walker, C. M. & Levy, J. A. (1989) *Immunology* **66,** 628–630.
5. Brinchmann, J. E., Gaudernack, G. & Vartdal, F. (1990) *J. Immunol.* **144,** 2961–2966.
6. Mackewicz, C. E., Ortega, H. & Levy, J. A. (1994) *Cell. Immunol.* **153,** 329–343.
7. Mackewicz, C. E., Ortega, H. W. & Levy, J. A. (1991) *J. Clin. Invest.* **87,** 1462–1466.
8. Landay, A. L., Mackewicz, C. E. & Levy, J. A. (1993) *Clin. Immunol. Immunopathol* **69,** 106–116.
9. Berke, G. (1994) *Annu. Rev. Immunol.* **12,** 735–773.
10. Levy, J. A., Tobler, L. H., McHugh, T. M., Casavant, C. H. & Stites, D. P. (1985) *Clin. Immunol. Immunopathol.* **35,** 328–336.
11. Hoffman, A. D., Banapour, B. & Levy, J. A. (1985) *Virology* **147,** 326–335.
12. Kaminsky, L. S., McHugh, T., Stites, D., Volberding, P., Henle, G., Henle, W. & Levy, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5535–5539.
13. Levy, J. A. & Rowe, W. P. (1971) *Virology* **45,** 844–847.
14. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
15. Williams, J. G. & Mason, P. J. (1985) in *Nucleic Acid Hybridization: A Practical Approach*, eds. Hames, B. D. & Higgin, S. J. (IRL, Oxford), pp. 139–160.
16. Cheng-Mayer, C., Iannello, P., Shaw, K., Luciw, P. A. & Levy, J. A. (1989) *Science* **246,** 1629–1632.
17. Aguilar-Cordova, E., Chinen, J., Donehower, L., Lewis, D. E. & Belmont, J. W. (1994) *AIDS Res. Hum. Retroviruses* **10,** 295–301.
18. Gaynor, R. (1992) *AIDS* **6,** 347–363.
19. Bagasra, O. & Pomerantz, R. J. (1993) *Immunol. Lett.* **35,** 83–92.
20. Powell, J. D., Bednarik, D. P., Folks, T. M., Jehuda-Cohen, T., Villinger, F., Sell, K. W. & Ansari, A. A. (1993) *Clin. Exp. Immunol.* **91,** 473–481.
21. Chen, C. H., Weinhold, K. J., Bartlett, J. A., Bolognesi, D. P. & Greenberg, M. L. (1993) *AIDS Res. Hum. Retroviruses* **9,** 1079–1086.
22. Graziosi, C., Pantaleo, G., Butini, L., Demarest, J. F., Saag, M. S., Shaw, G. M. & Fauci, A. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6405–6409.