Suppression of HIV replication in the resting CD4$^+$ T cell reservoir by autologous CD8$^+$ T cells: Implications for the development of therapeutic strategies

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CD8$^+$ T cell-mediated antiviral activity against HIV has been described consistently in infected individuals; however, the role of this activity in controlling replication of HIV in the latently infected, resting CD4$^+$ T cell reservoir is unclear. By using an *ex vivo* system, we show that replication of HIV in this viral reservoir is effectively suppressed in coculture by autologous CD8$^+$ T cells in long-term nonprogressors (LTNPs) and in patients whose viremia was controlled by highly active antiretroviral therapy (HAART), but not in therapy-naive patients who had substantial levels of plasma viremia. This antiviral activity was largely independent of cytotoxic CD8$^+$ T lymphocytes (CTL). When the role of soluble CD8$^+$ T cell-derived factors was examined, we found that CC-chemokines played a major role in inhibition of viral replication in the latent viral reservoir in some LTNPs and patients receiving HAART, but not in chronically infected patients who were not receiving antiretroviral therapy. Potent antiviral activity, independent of CC-chemokines, was found mainly in patients in whom HAART was initiated shortly after the acute phase of HIV infection. These results indicate that CD8$^+$ T cells provide potent suppressive activity against HIV replication in the latent viral reservoir via direct cellular contact in patients who are naturally LTNPs or in those who are treated with HAART. Furthermore, the profound antiviral activity exerted by non-CC-chemokine soluble factors in infected patients who began HAART early in HIV infection suggests that preservation of this HIV-suppressive mechanism by early initiation of therapy may play an important role in the containment of viral replication in infected patients following interruption of therapy.

HIV-1 | CD8$^+$ T cells | soluble factors | latency | HAART

Cell-mediated immune responses play an important role in host defenses against viral infections. In particular, it has been demonstrated that CD8$^+$ T cells are a critical part of this host defense mechanism. Studies of the immune responses generated in HIV-infected individuals suggest that CD8$^+$ T cells play an important role in controlling viremia. In this regard, the emergence of HIV-specific CD8$^+$ cytotoxic T lymphocyte (CTL) activity coincides with the clearance of viremia during primary HIV infection (1, 2) and a decline in the CTL response is associated with disease progression in infected individuals (3, 4). At least two types of CD8$^+$ T cell-mediated antiviral activities have been described in HIV infection. The first is a suppressive activity against HIV involving lysis of infected cells in an antigen-specific, HLA-restricted fashion (5, 6), whereas the second mechanism inhibits viral replication via soluble factors in the absence of cell killing (6–10). The noncytolytic antiviral activity was initially described *in vitro* using CD8$^+$ T cells from HIV-infected patients (7). Although these cells possessing noncytolytic antiviral activity show characteristics of activated CD8$^+$ CTL (11–13), they generally do not require HLA-restriction and are mediated by soluble factors (10, 14, 15). Recent studies have demonstrated that these factors include CC-chemokines, such as MIP-1α, MIP-1β, and RANTES (16), which block the entry of HIV that utilizes the CCR5 coreceptor (R5 virus) by competing for binding to the coreceptor (17–21). Whereas these CC-chemokines exert their antiviral activity at the entry of HIV into CD4$^+$ T cells and macrophages, an as yet unidentified soluble factor(s) has been shown to suppress postentry viral replication by down-regulating transcription of HIV in infected cells (10, 22). Of note, despite the fact that HLA compatibility between effector CD8$^+$ T cells and infected CD4$^+$ T cells does not seem to be required in the soluble suppressor system, maximal suppression of viral replication is seen when CD8$^+$ and infected CD4$^+$ T cells are cocultured in conditions where cell-to-cell contacts are maintained (7, 10, 11, 23).

Although several earlier studies used endogenously infected CD4$^+$ T cells from asymptomatic, HIV-infected patients who were not receiving highly active antiretroviral therapy (HAART) to measure the capacity of soluble factors derived from CD8$^+$ T cells to inhibit viral replication, subsequent studies were based predominantly on *in vitro* infections of CD4$^+$ T cells with laboratory adapted strains of HIV. Moreover, the ability of autologous CD8$^+$ T cells to inhibit replication-competent HIV in the pool of latently infected, resting CD4$^+$ T cells in infected patients at different stages of disease, and with different antiviral treatment regimens, has not been extensively studied. In this regard, the persistence of latently infected, resting CD4$^+$ T cells carrying replication-competent HIV has been firmly established in infected individuals receiving HAART (24–28). Subsequently, the feasibility of complete eradication of HIV in infected individuals has been challenged, in part because of the ineffectiveness of HAART in eliminating this latent viral reservoir (24–28).

In the present study, we have examined the role of autologous CD8$^+$ T cell-mediated suppression of viral replication in the pool of latently infected, CD4$^+$ T cells isolated from peripheral blood of a number of infected patients who differ both in stage of disease and in treatment status.

Materials and Methods

Study Subjects. Seventeen HIV-1-infected patients (Table 1) were studied. These patients included: six chronically infected patients with varying levels of plasma viremia who were not receiving antiretroviral therapy at the time of study; three LTNPs who have maintained stable CD4$^+$ T cell counts and relatively low plasma viral loads over extended periods of time in the absence of

Abbreviations: HAART, highly active antiretroviral therapy; CTL, cytotoxic CD8$^+$ T lymphocytes; PBMC, peripheral blood mononuclear cells; LTNP, long-term nonprogressors.

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Table 1. Profiles of HIV-1-infected patients

Patient	Clinical Status	Antiretroviral therapy at the time of study*	CD4 count, per μl	CD8 count, per μl	Viral load, copies per ml plasma†	Phenotypes of cultured Virus‡	HIV Gag specific CD8$^+$ T cells (%)§
1	Chronic	Naive	231	802	102,927	X4	1.60
2	Chronic	Naive	62	813	153,280	X4	0.33
3	Chronic	Naive	349	647	102,645	R5	1.21
4	Chronic	Naive	443	996	11,990	R5	3.87
5	Chronic	Naive	85	438	21,767	R5	0.65
6	Chronic	Naive	303	792	54,801	R5	0.33
7	LTNP	Naive	1,840	1,600	98	R5	ND
8	LTNP	Naive	541	727	7,257	R5	5.06
9	LTNP	Naive	563	746	2,169	R5/X4	0.28
10	Chronic	HAART	344	635	<50	R5/X4	0.46
11	Chronic	HAART	320	506	<50	R5	0.09
12	Chronic	HAART + IL-2	1,265	949	<50	R5	0.46
13	Chronic	HAART	327	886	<50	R5	0.08
14	Acute	HAART	635	589	<50	R5	0.47
15	Acute	HAART	712	751	<50	R5	0.40
16	Acute	HAART	778	451	<50	R5	0.13
17	Acute	HAART + IL-2	3,064	1,328	<50	R5	0.00

*HAART contained at least one protease inhibitor and/or one non-nucleoside reverse transcriptase inhibitor and two reverse transcriptase inhibitors of HIV-1.
†Measured by ultrasensitive bDNA assay with a detection limit of 50 copies per ml plasma.
‡Coreceptor usage of isolated virus was evaluated on U87.CD4 cells expressing the chemokine receptors CCR5 or CXCR4.
§Determined by measuring INF-γ-positive cells on stimulation with overlapping 20-mer Gag peptides.

antiretroviral therapy; four patients who began HAART during their chronic stage of disease and who were aviremic (<50 copies of HIV RNA per ml of plasma) at the time of study; and four patients who initiated HAART within 4 months following the onset of acute HIV symptoms and who were aviremic (<50 copies of HIV RNA per ml plasma) at the time of the study. To obtain peripheral blood mononuclear cells (PBMCs), all infected patients were subjected to an apheresis according to a protocol that was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board.

Isolation of Resting CD4$^+$ T Cells and CD8$^+$ T cells. Resting CD4$^+$ T cells and CD8$^+$ T cells were isolated from peripheral blood of HIV-1-infected individuals by using column-based cell separation techniques (StemCell Technologies, Vancouver) as described in refs. 29 and 30.

Cell Cultures. Following isolation of resting CD4$^+$ T cells and CD8$^+$ T cells from HIV-1 infected individuals, each population of cells (3 to 5 $\times$ 10^6) was incubated in 12-well plates with complete medium consisting of RPMI medium 1640 supplemented with 10% FCS, penicillin-streptomycin, L-glutamine, IL-2 (20 units/ml, Roche Molecular Biochemicals), and anti-CD3 antibody (OKT3) in the presence of 10 $\times$ 10^6 irradiated allogeneic PBMCs isolated from healthy HIV-seronegative donors in the following settings: resting CD4$^+$ T cells alone, coculture of resting CD4$^+$ T cells and CD8$^+$ T cells (1:1), and resting CD4$^+$ and CD8$^+$ T cells (1:1) in transwells in the presence and absence of monoclonal anti-CC-chemokine antibodies (20 μg/ml of each anti-MIP-1 α, MIP-1β, and RANTES antibody; R&D Systems). In the transwell studies, CD8$^+$ T cells were placed in the upper chamber and were separated from resting CD4$^+$ T cells, which were placed in the bottom chamber, by semipermeable membranes (Costar) so that only soluble factors were allowed to be exchanged in the medium. Cultures were incubated in a 37°C CO_2 incubator for 12 days and supernatants from each culture were removed and replaced with fresh medium and anti-CC-chemokine antibodies, where indicated, on days 3, 6, 9, and 12 for determination of HIV-1 p24 levels by ELISA (Coulter) and MIP-1 α and RANTES levels by ELISA (R&D Systems).

Phenotyping of Cultured HIV. Virus-containing supernatants collected at the peak of viral replication in stimulated resting CD4$^+$ T cells were phenotyped for coreceptor usage by exposure to the human glioma cell lines U87/CD4 expressing the chemokine receptors CCR5 or CXCR4 (kindly provided by D. R. Littman, New York University, New York).

Flow Cytometric Analysis of HIV-Specific CD8$^+$ T Cells. The frequency of CD8$^+$ T cells specific for HIV was determined by analysis of intracellular IFN-γ-positive cells on stimulation with HIV-1$_{HXB2}$ Gag peptides. A pool of 49 overlapping 20-mer peptides (1 μg of each; National Institutes of Health AIDS Reagent Program) spanning the entire HIV-1$_{HXB2}$ gag sequence was initially incubated with 3 $\times$ 10^6 PBMCs for 10 min in a round-bottom, 5-ml tube (Becton Dickinson) in 0.1 ml of complete medium in a 37°C CO_2 incubator. Subsequently, 0.4 ml of complete medium was added to the tube followed by a 2 h incubation. After the 2 h incubation, Brefeldin A (Sigma) was added to the medium at a final concentration of 10 μg/ml to inhibit secretion of IFN-γ. At 6 h of incubation, the cells were washed twice and fixed with 1$\times$ Fixing solution (Becton Dickinson) for 10 min at room temperature followed by another washing. Cells were permeabilized with 1$\times$ Permeabilization Solution 2 (Becton Dickinson) and further incubated at room temperature for 10 min. After washing, cells were stained with the following antibodies: FITC-conjugated anti-CD8, PE-conjugated anti-IFN-γ, PerCP-conjugated anti-CD69, and APC-conjugated anti-CD3 antibodies (Becton Dickinson). With a gate on CD3$^+$CD8$^+$ lymphocytes, approximately 100,000 events were collected by using FACSCalibur (Becton Dickinson) and the frequency of IFN-γ^+CD69$^+$ cells was analyzed by using CELLQUEST software (Becton Dickinson).

Statistical Analysis. Spearman Rank Correlations method was used as a measure of correlation between the suppressive capacity of CD8$^+$ T cells against viral replication in CD4$^+$ T cells in various culture conditions and immunologic and virologic parameters of infected patients. The Bonferroni method was used to adjust P values for multiple testing.

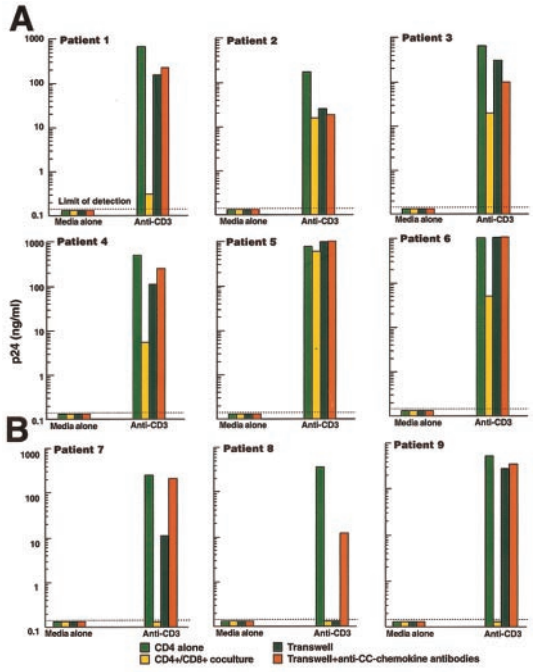

Fig. 1. Role of autologous $CD8^+$ T cells in suppression of viral replication in antiretroviral therapy naive infected patients. Highly enriched resting $CD4^+$ T cells and $CD8^+$ T cells from chronically infected patients who were not receiving any form of antiretroviral therapy (*A*) and infected LTNPs (*B*) were stimulated with irradiated PBMCs from HIV-seronegative donors and anti-CD3 antibodies in four different culture conditions as described in *Materials and Methods*. The cultures were maintained for 12 days with supernatants being removed every 3 days and replaced with fresh complete medium and anti-CC-chemokine antibodies where indicated. Supernatants were assayed for p24 on days 3, 6, 9, and 12. Results of p24 ELISA on day 12 are shown. p24 values below 10pg/ml were plotted under the dotted line.

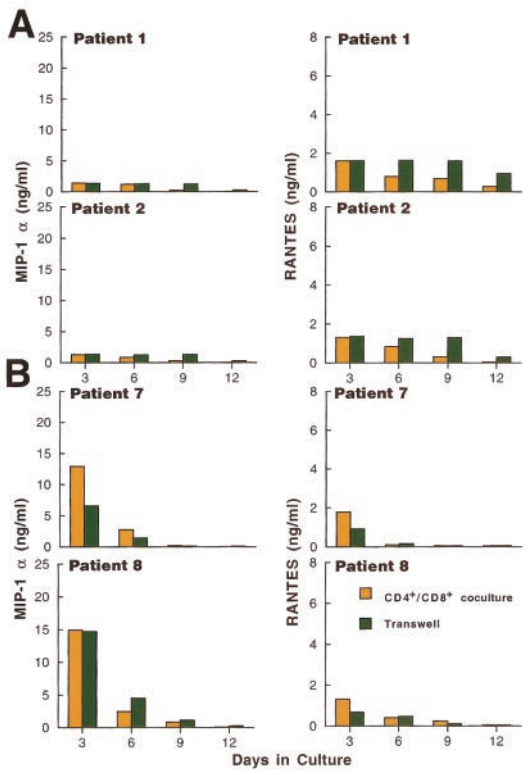

Fig. 2. Levels of CC-chemokine production by the cultures from antiretroviral therapy naive infected patients. Supernatants from both $CD4^+/CD8^+$ coculture and transwell culture conditions derived from chronically infected patients who were not receiving antiretroviral therapy (*A*) and infected LTNPs (*B*) were collected every 3 days and levels of MIP-1 α and RANTES were measured by ELISA.

Results

Role of Autologous $CD8^+$ T Cells in Suppression of Viral Replication in Antiretroviral Therapy Naive, Infected Patients. To investigate the role of autologous $CD8^+$ T cells in suppressing viral replication in a stimulated pool of latently infected, resting $CD4^+$ T cells from antiretroviral therapy naive patients, we measured the degrees of inhibition of HIV replication by autologous $CD8^+$ T cells in the following culture conditions: $CD4^+$ T cells alone, $CD4^+/CD8^+$ T cell cocultures, transwell, and transwell plus anti-CC-chemokine antibodies. We first examined cells from chronically infected patients who were not receiving antiretroviral therapy at the time of the study (Table 1). In the absence of cellular activation, viral replication was absent in all culture conditions (Fig. 1*A*). On stimulation of resting $CD4^+$ T cells with irradiated allogeneic PBMCs from HIV-seronegative donors and anti-CD3 antibodies, viral replication was detected in cultures of all six individuals, in each culture condition, and at all time points examined (days 3, 6, 9, and 12—only peak p24 values on day 12 are shown throughout the paper; Fig. 1*A*). In the $CD4^+/CD8^+$ coculture setting in which physical contact between $CD4^+$ and $CD8^+$ T cells was allowed, relatively weak, but varying degrees of viral suppression was observed ranging 0.09–3.33 log (median 1.50 log reduction) when compared with viral replication in the $CD4^+$ T cell culture (Figs. 1*A* and 5). However, in the transwell condition, in which soluble $CD8^+$ T cell-derived factors were allowed to circulate freely, relatively low levels of suppression of viral replication in $CD4^+$ T cells were observed (median 0.48 log reduction, ranging from 0–0.84 log when compared with viral replication in the $CD4^+$ T cell culture; Figs. 1*A* and 5). The presence of excess anti-CC-chemokine antibodies in the transwell cultures did not affect the levels of viral suppression described above (median 0.39 log reduction, ranging from 0–0.97 log with respect to viral replication in the $CD4^+$ T cell culture; Figs. 1*A* and 5). Taken together, these data demonstrate that only weak antiviral activity by $CD8^+$ T cells is mediated via cellular contacts and that soluble $CD8^+$ T cell-derived factors, including CC-chemokines, do not play an important role in suppression of viral replication in $CD4^+$ T cells from chronically infected patients who were not receiving antiretroviral therapy. When levels of MIP-1 α and RANTES in the $CD4^+/CD8^+$ coculture and transwell settings were measured from this group of patients by ELISA, relatively low levels (less than 2 ng/ml) of each CC-chemokine were detected (Fig. 2*A*; representative data from two patients from each group are shown throughout the paper).

Cells from a small number of LTNPs were subjected to the same culture conditions described above. These individuals have maintained relatively stable $CD4^+$ T cell counts and low plasma viral loads for extended periods of time (6–14 years), in the absence of any form of antiretroviral therapy (Table 1). In the transwell condition, varying degrees of suppression of viral replication in $CD4^+$ T cells were observed (median 1.36 log reduction, ranging 0.34–4.59 logs; Figs. 1*B* and 5). In one patient (patient 8), no detectable viral replication was observed in the transwell condition (Fig. 1*B*). When neutralizing anti-CC-chemokine antibodies were added to the transwell culture, antiviral effects exerted by soluble factors were abolished in one patient (patient 7) and particularly inhibited in another patient (a 1.49 log suppression was still maintained in patient 8). Patient 9 showed no initial suppression originally with CC-chemokines (Figs. 1*B* and 5). In the $CD4^+/CD8^+$ coculture condition, no evidence of viral replication could be detected in any of the three patients, strongly suggesting that the cellular contact-mediated antiviral activity of $CD8^+$ T cells

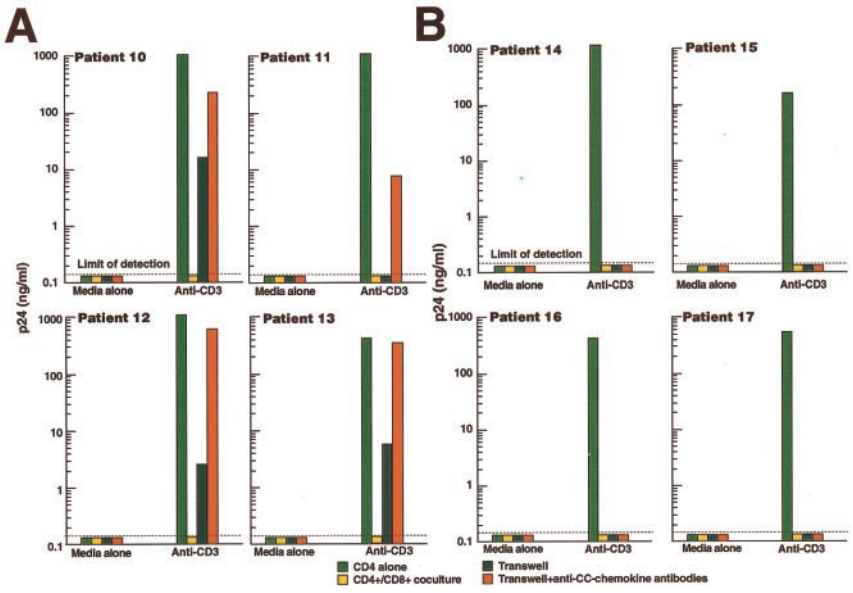

Fig. 3. Role of autologous CD8$^+$ T cells in suppression of viral replication in infected individuals receiving HAART. Highly enriched resting CD4$^+$ T cells and CD8$^+$ T cells from chronically infected patients who were receiving HAART (*A*) and those who initiated HAART during primary infection (*B*) were stimulated with irradiated PBMCs from seronegative donors and anti-CD3 antibodies in four different culture conditions as described in *Materials and Methods*. The cultures were maintained for 12 days with supernatants being removed every 3 days and replaced with fresh complete medium and anti-β-chemokine antibodies where indicated. Supernatants were assayed for p24 on days 3, 6, 9, and 12. Results of p24 ELISA on day 12 are shown. p24 values below 10pg/ml were plotted under the dotted line.

plays a central role in inhibition of viral replication in the CD4$^+$ T cell reservoir in LTNPs (Figs. 1*B* and 5). The levels of MIP-1 α in the CD4$^+$/CD8$^+$ cocultures and transwell settings were considerably higher than in those of chronically infected patients receiving no antiretroviral therapy (Fig. 2*B*). The amounts of CC-chemokines in both cultures peaked at the earliest time point (day 3) sampled and dramatically decreased thereafter (Fig. 2*B*).

Role of Autologous CD8$^+$ T Cells in Suppression of Viral Replication in Infected Individual Receiving HAART. It has been demonstrated that the CD8$^+$ T cell-mediated suppressive activity against HIV declines with disease progression (31, 32). However, studies addressing the role of these suppressive factors in inhibition of endogenous viral replication in CD4$^+$ T cells from infected patients receiving HAART have been lacking. To further examine this issue, cells from infected patients receiving HAART in whom treatment was initiated during the chronic phase of HIV infection, and in whom maximal suppression of plasma viremia had been achieved, were subjected to the same culture conditions as described above. Patients 10–13 had received various HAART regimens for over 6 months during chronic HIV infection. In the transwell culture condition, substantial, but incomplete suppression of viral replication was observed with a median of 2.32 log (ranging from 1.74–4.32 log) suppression when compared with viral replication in the CD4$^+$ T cell culture alone (Figs. 3*A* and 5). In the presence of excess amounts of anti-CC-chemokines in the transwell setting, the effect of soluble CD8$^+$ T cell-derived factors against HIV was reduced to a median of 0.44 log (ranging from 0.08–2.13 log) when compared with viral replication in the CD4$^+$ T cell culture alone (Figs. 3*A* and 5), strongly suggesting that CC-chemokines played an important, although not necessarily exclusive, role in suppressing HIV replication in infected CD4$^+$ T cells. However, when CD4$^+$ T cells were cocultured with CD8$^+$ T cells, viral replication was not detected. Of note, the addition of anti-CC-chemokines in this culture condition did not abrogate the capacity of CD8$^+$ T cells to suppress viral replication via cell-to-cell contact (data not shown). These findings indicate that in infected patients who began HAART during chronic HIV infection (although CC-chemokines substantially suppressed viral replication) the most potent mode of viral suppression by CD8$^+$ T cells is mediated via cell-to-cell contact with infected CD4$^+$ T cells. Furthermore, given that CD8$^+$ T cell contact was relatively inefficient in suppressing virus replication in the CD4$^+$ T cell reservoir of chronically infected, viremic patients who were not receiving therapy (Figs. 1*A* and 5), our results suggest that, in chronically infected patients, HAART can restore the antiviral activity mediated by CD8$^+$ T cell contact.

Finally, resting CD4$^+$ T cells from four infected patients (patients 14–17), in whom HAART was initiated within 4 months following

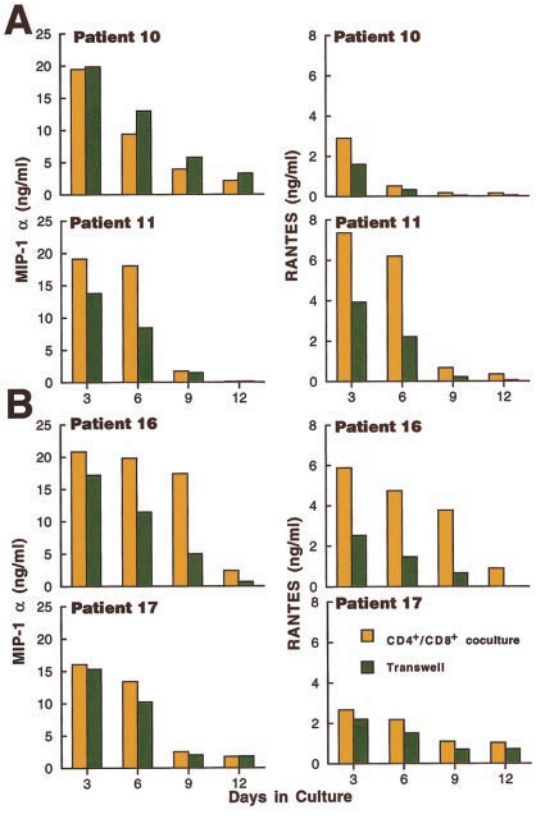

Fig. 4. Levels of CC-chemokine production by the cultures from infected individuals receiving HAART. Supernatants from both CD4$^+$/CD8$^+$ coculture and transwell culture conditions derived from chronically infected patients who were receiving HAART (*A*) and those who initiated HAART during primary infection (*B*) were collected every 3 days and levels of MIP-1 α and RANTES were measured by ELISA.

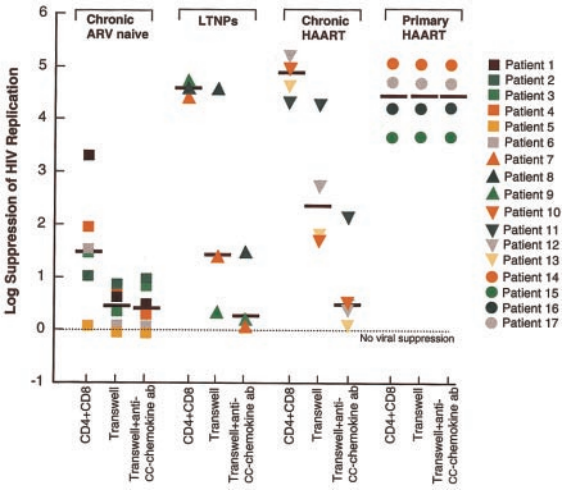

Fig. 5. Levels of viral suppression in the stimulated pool of latently infected, resting $CD4^+$ T cells by $CD8^+$ T cells in various culture conditions. The log p24 value in each culture condition containing $CD8^+$ T cells was subtracted from that of the $CD4^+$ T cell culture and the difference was plotted. The dotted line indicates no suppression of viral replication in the cultures containing $CD8^+$ T cells. Solid bars indicate the median values.

the acute phase of HIV infection, were subjected to the culture conditions described above. In all four patients, viral replication was completely suppressed in the $CD4^+/CD8^+$ coculture, the transwell, and the transwell plus anti-CC-chemokines conditions (Figs. 3*B* and 5). HIV replication was detected only when $CD4^+$ T cells were cultured in the absence of $CD8^+$ T cells (Fig. 3*B*). Given that replication of HIV in $CD4^+$ T cells was completely suppressed in the transwell setting, even in the presence of anti-CC-chemokine antibodies, these findings indicate that soluble $CD8^+$ T cell antiviral factors other than CC-chemokines were secreted from the $CD8^+$ T cells of these individuals and played an important role in inhibition of HIV replication. The levels of CC-chemokines in the $CD4^+/CD8^+$ coculture and transwell settings from all infected patients receiving HAART were relatively higher than in those of chronically infected patients who were not receiving any form of antiretroviral therapy (Fig. 4). The amounts of CC-chemokines in both culture conditions peaked at the earliest time point (day 3) we sampled and dramatically decreased at the later time points (Fig. 4). Given that the maximal suppressive effects on viral replication were detected on day 12 in the majority of cultures and the amounts of CC-chemokines had decreased to the minimal levels by day 12, the profound suppression of viral replication in the transwell culture in the presence of excess anti-CC-chemokines was most likely due to soluble $CD8^+$ T cell factors other than CC-chemokines in patients who began HAART shortly after being infected with HIV.

Relationship Between Suppression of Viral Replication by $CD8^+$ T Cells and Immunologic and Virologic Parameters of Infected Patients. To address the relationship between the capacity of $CD8^+$ T cells to suppress viral replication in latently infected, resting $CD4^+$ T cells in both $CD4^+/CD8^+$ coculture and transwell settings and various immunologic and virologic parameters of the infected patients, we performed statistical analyses by using Spearman Rank Correlations method. First, we sought to determine the relationship between the degree of suppression of viral replication by $CD8^+$ T cells in both culture conditions and the frequency of HIV-1-specific $CD8^+$ T cells measured by expression of intracellular IFN-γ on stimulation with $HIV\text{-}1_{HXB2}$ gag peptides (Table 1). No correlation was found between the frequency of HIV-specific $CD8^+$ T cells and the log reduction of viral replication in the $CD4^+/CD8^+$ coculture and the transwell culture relative to HIV replication in the $CD4^+$ T cell culture ($P > 0.5$ and $P > 0.5$, respectively). When the log reduction of viral replication in both culture conditions relative to the $CD4^+$ T cell culture was compared with other immunologic parameters, such as $CD4^+$ and $CD8^+$ T cells counts, a statistically significant correlation was found only with $CD4^+$ T cell counts of infected patients ($P = 0.04$ in the $CD4^+/CD8^+$ coculture and $P = 0.04$ in the transwell culture), but not with $CD8^+$ T cell counts ($P > 0.5$ in both culture conditions). Finally when the log reductions of viral replication in the $CD4^+/CD8^+$ coculture and the transwell culture conditions, relative to the $CD4^+$ T cell culture, were compared with plasma viral loads, a strong relationship was observed ($P = 0.002$ and $P = 0.002$, respectively). These data strongly suggest that HIV-specific cytotoxic $CD8^+$ T cells were not responsible for suppression of HIV replication by $CD8^+$ T cells in both culture conditions. Furthermore, autologous $CD8^+$ T cells in infected patients with higher $CD4^+$ T cell counts and lower plasma viral loads exhibited enhanced degrees of cell- and soluble-factor-mediated inhibition of viral replication in infected $CD4^+$ T cells.

Discussion

In the present study, we have examined the role of autologous $CD8^+$ T cells in inhibition of viral replication in latently infected, resting $CD4^+$ T cells from patients who were not receiving antiretroviral therapy as well as from patients in whom HAART was initiated at various times following initial infection and in whom plasma viremia was markedly suppressed. It has been firmly demonstrated that latently infected, resting $CD4^+$ T cells carrying replication-competent HIV are present in infected patients (33, 34) and they are felt to be the major impediment to our ability to withdraw therapy in HIV-infected patients because of the fact that they persist in patients in whom plasma virus has been driven by HAART to below levels of detectability (24–28). In this regard, the importance of immune-mediated containment of viral replication in this reservoir is particularly relevant in light of recent attempts to interrupt the administration of HAART in HIV-infected patients whose viremia is well controlled on treatment (35–38). Given that these cells may serve as the viral reservoir from which HIV replication reemerges following discontinuation of HAART in some infected patients (39, 40), it is of great interest to investigate whether viral replication in this latent HIV reservoir can be contained by the host's immune system, such as autologous $CD8^+$ T cells, in the absence of HAART. In this regard, despite the fact that CD8-mediated cytotoxic and soluble suppressive factors have been shown to inhibit viral replication *in vitro* (6, 10), their role in the containment of replication-competent HIV in the pool of latently infected, resting $CD4^+$ T cells has been unclear. In addition, the suppressive activity exhibited by autologous $CD8^+$ T cells against HIV replication in endogenously infected $CD4^+$ T cells from infected LTNPs and patients receiving HAART has not been extensively studied.

The present study clearly demonstrates that replication of HIV in the latent $CD4^+$ T cell reservoir is profoundly suppressed by autologous $CD8^+$ T cells via cellular contacts in $CD4^+/CD8^+$ cocultures of LTNPs and of infected patients who were receiving HAART regardless of timing of initiation of therapy after initial infection. This cellular contact-mediated suppressive phenomenon by $CD8^+$ T cells was not generally observed in the $CD4^+/CD8^+$ coculture derived from infected patients who were not receiving any form of antiretroviral therapy and in whom relatively high levels of plasma viremia were evident. In this regard, given that there was no correlation between the frequency of HIV-specific $CD8^+$ T cells in the peripheral blood compartment in these infected individuals and the capacity of the $CD8^+$ T cell population to inhibit viral replication in $CD4^+/CD8^+$ cocultures, it appears that autologous $CD8^+$ T cells delivered the cell-to-cell contact-mediated antiviral effects against infected $CD4^+$ T cells independently of CTL activity.

When the role of soluble $CD8^+$ T cell factors was examined relative to suppression of HIV in the latent $CD4^+$ T cell reservoir,

CC-chemokines played a major role in the containment of viral replication in the transwell cultures derived from some LTNPs and all infected patients who were receiving HAART and in whom treatment was initiated during the chronic phase of HIV infection. A weak soluble $CD8^+$ T cell factor-mediated antiviral activity against HIV was observed in the transwell cultures derived from infected patients who were not receiving therapy, as observed in their $CD4^+/CD8^+$ cocultures. In contrast, the antiviral activity exhibited by autologous $CD8^+$ T cells derived from infected patients who were receiving HAART and in whom therapy was initiated during chronic stage of disease was substantial in both culture conditions. Given that these patients likely experienced high levels of plasma viremia before initiation of HAART, it is possible that active viral replication *in vivo* has a deleterious impact on the capacity of $CD8^+$ T cells to control HIV in infected $CD4^+$ T cells and that this function is at least partially restored on initiation of HAART.

Whereas CC-chemokines played a central role in the suppression of viral replication in the transwell cultures derived from some infected LTNPs and patients receiving HAART in whom therapy was initiated during the chronic stage of disease, an as yet unidentified soluble $CD8^+$ T cell factor(s), other than CC-chemokines, was responsible for the profound antiviral effects in the transwell cultures derived from infected patients in whom HAART was initiated early in HIV infection. It is possible that CC-chemokines produced by $CD8^+$ T cells from these individuals also play an important role in neutralizing HIV produced by the latent $CD4^+$ T cell reservoir. However, given that viral replication in $CD4^+$ T cells was completely abrogated by autologous $CD8^+$ T cells in the transwell cultures, despite the presence of excess amounts of anti-CC-chemokine antibodies, a soluble $CD8^+$ factor(s), other than CC-chemokines, is likely responsible for inhibiting HIV replication in these patients.

Our study has potentially important implications for the pathogenesis of HIV disease and the immune-mediated containment of viral replication, especially in light of recent attempts to interrupt the administration HAART in HIV-infected patients whose viremia is well controlled on treatment. It has been shown that the frequency of HIV-specific CTL is dramatically reduced on initiation of HAART, perhaps because of the lack of available antigen in treated patients (41). However, as demonstrated in our work, HAART dramatically improves the effectiveness of autologous $CD8^+$ T cells in suppressing viral replication in $CD4^+$ T cells through cellular contacts. This suppressive mechanism against HIV may play an important role in lymphoid tissues of infected patients where both populations of cells share the same microenvironment. In addition, our study suggests novel beneficial consequences of early initiation of HAART in HIV infection. In this regard, it has been suggested that $CD4^+$ T cells from infected individuals in whom HAART was initiated during primary HIV infection maintain strong HIV-specific proliferative responses and may play an important role in the persistence of antiviral immunity (42). Recent studies have repeatedly demonstrated that plasma viremia invariably rebounds on discontinuation of HAART in patients in whom prolonged suppression of viral replication had been achieved (35–38, 43). Given that the vast majority of these infected patients initiated HAART during their chronic stage of disease, it is likely that their $CD8^+$ T cells had lost the non-CC-chemokine soluble $CD8^+$ T cell factors described above. Given that noncytolytic $CD8^+$ T cell antiretroviral responses are detected during primary HIV infection (44), preservation of $CD8^+$ T cell-mediated immunity by early institution of HAART may allow $CD8^+$ T cells to produce soluble factors other than CC-chemokines that may, along with HIV-specific CTL, down-regulate viral replication in infected $CD4^+$ T cells even in a setting where HAART is discontinued. The search for the non-CC-chemokine $CD8^+$ T cell soluble factor(s) that suppresses HIV replication has thus far been elusive (10). However, the fact that such a factor(s) may be important in the inhibition of viral rebound from the $CD4^+$ T cell reservoir may have important implications for strategies designed to interrupt HAART in patients whose viremia is well controlled on therapy.

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