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Inhibition of SIV/SMM Replication In Vitro by CD8$^+$ Cells From SIV/SMM Infected Seropositive Clinically Asymptomatic Sooty Mangabeys

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Several investigators have demonstrated the ability of CD8$^+$ T cells from HIV-1 infected humans and SIV infected rhesus macaques to inhibit viral replication in vitro. In this report we show that CD8$^+$ cells from naturally SIV infected sooty mangabeys also have the ability to inhibit viral replication in vitro. In addition, initial experiments which seek to elucidate the mechanism and antigen specificity of CD8-mediated suppression are described.

Key words: CD8$^+$ T cells • Viral replication • AIDS

INTRODUCTION

Cell-mediated immunity (CMI) in the form of virus-specific MHC-restricted CD8$^+$ cytotoxic T cells (CTLs) has been shown to play a major role in antiviral immunity [2,25]. In the murine influenza model, adoptive transfer of influenza nucleoprotein (NP) specific CTLs has been shown not only to reduce the replication of the virus in the respiratory tract of mice [8,9,21] but also to protect these mice from lethal influenza infection [16]. In addition to CD8$^+$ T cell clones, natural killer cells (NK) and lymphokine activated killer cells (LAK) also play a role in host defenses against viral infection [5,12,17]. The role of CMI in human infection with HIV-1 is far

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from clear. Some studies have shown the presence of HIV-specific CTLs in the peripheral blood mononuclear cells (PBMC) of patients with AIDS [13,19] and in chimpanzees experimentally immunized with recombinant HIV-1 [23,24]. Other studies have shown the presence of CMI in the form of autoimmunity [10]. Several investigations have demonstrated the ability of $CD8^+$ cells from HIV-1 infected individuals [20] and SIV infected rhesus macaques [6] to regulate HIV-1 and SIV replication in autologous cells in vitro. Currently, it is unclear whether this regulation is due to cytotoxic mechanisms or conventional suppressor T cell mechanisms.

Our laboratory has carried out studies designed to elucidate natural and acquired CMI in both sooty mangabeys (*Cercocebus atys*), a great majority ($> 75\%$) of which are naturally infected with SIV/SMM but remain clinically asymptomatic, and rhesus macaques (*Macaca mulatta*), which, when experimentally infected with SIV/SMM, die of an AIDS-like disease similar to that seen in humans infected with HIV-1 [4,11]. Elucidation of differences in the natural and antigen-specific acquired immunity in these two species may provide important clues to the mechanisms of pathogenesis. Our initial studies demonstrated that PBMC of sooty mangabeys, when compared to rhesus macaques, have a higher frequency of $CD8^+$ T cells which appear to be activated since a high proportion express HLA Class II molecules [1]. Further, in the mangabey it is the $CD8^+$ T cells that mediate NK cell and LAK cell activity [11,14]. In light of the findings that $CD8^+$ cells from SIV infected macaques and HIV-1 infected humans have the ability to inhibit viral replication in vitro, and the prominence of $CD8^+$ cells in the PBMC of naturally infected sooty mangabeys, we wanted to determine whether $CD8^+$ cells from sooty mangabeys have the ability to inhibit viral replication in vitro. It was reasoned that since SIV infection in rhesus and HIV-1 infection in humans usually leads to death, the finding of CD8-mediated viral suppression in the naturally infected yet clinically asymptomatic mangabeys might help in the effort to delineate pathogenic immunity from protective immunity.

MATERIALS AND METHODS

Animals

Adult rhesus macaques and sooty mangabeys housed at the Yerkes Regional Primate Research Center (YRPRC) were the source of blood samples for this study. Rhesus macaques were experimentally injected with 10^4 TCID of SIV/SMM as previously described [1] while the seropositive mangabeys used in this study were all naturally infected with SIV.

Isolation of Lymphocytes and CD8 Depletion

PBMC were isolated using a 60% Percoll gradient as previously described [1]. They were washed twice in media, counted, and adjusted to a concentration of 2×10^6/ml. PHA-P(Gibco, Grand Island, NY) at a final concentration of 0.2% was added to cell cultures which were incubated at 37°C in a 7% CO_2 humidified atmosphere for three days. The cells were then washed twice, and aliquots of the cells were depleted of $CD8^+$ T cells, using a biomagnetic separation system [22]. In brief, 10^7 lymphocytes were incubated with 100 µl of Leu-2a (anti CD8, Becton-Dickinson, Mountain View, CA) on ice for 40 minutes. Excess antibody was removed by washing the cells in media. The media was then aspirated, and the cells were next incubated with 2 ml of goat anti mouse Ig conjugated beads (Collaborative Research, Medford, MA) for 40 minutes on ice. $CD8^+$ cells were depleted by placing the lymphocytes in a magnetic field.

CD8 Suppression of Reverse Transcriptase (RT) Activity

Whole PHA blasts, and CD8 depleted PHA blasts (as prepared above) were adjusted to a concentration of 10^6 cells/ml in a volume of 2 ml of media containing 10 units/ml rIL-2. For some experiments 20 µl of 100 × SIV/SMM grown in the HTC cell line and prepared commercially (Advanced Biotechnologies, Silver Spring, MD) was added to the culture tubes. The cultures were maintained at 37°C in a 7% CO_2 humidified atmosphere, and supernatant fluid was harvested every four days and replenished. RT activity of the supernatant fluid was assessed as previously described by Spira et al. [15].

FEc1 Cell Line

The FEC1 cell line is an EBV transformed B cell line prepared in our laboratory from a seropositive sooty mangabey. This line was established by immortalizing B cells from the PBMC of the sooty mangabey (FEc) by incubation with a preparation of EBV produced from the B95-8 cell line (ATCC, Rockville, MD). For the first ten tissue culture passages, these EBV transformed mangabey cells produced anti-SIV antibodies; however, antibody production by these cells has since ceased. The cells were CD4 transfected with the gene that codes for the human CD4 molecule and with a neomycin resistance gene, using a Moloney virus vector kindly provided by Dr. Richard Morgan, NIH, NHLBI. Selection for cells in which the CD4 gene was integrated was accomplished by placing the cells in media containing neomycin. Expression of CD4 was confirmed by flow microfluorometric (FMF) analysis, using fluorescein conjugated Leu-3a.

CD8 suppression of RT in the FEc1 cell line was performed as follows. To 0.5×10^6 FEc1 cells were added 2×10^6 PHA blasts prepared from the PBMC from the mangabey (Fec). The cultures were maintained in a final volume of 2 ml of media containing 10 units/ml rIL-2. On day zero, 20 μl of SIV/SMM or 100 μl of 10 × HIV-2 (courtesy of NIH, AIDS Research and Reference Reagent Program) was added to each culture; on day 2 the cultures were centrifuged, and the supernatant fluid was removed and replaced in order to remove any excess virus. On day 7 the supernatant fluids were harvested for RT activity.

Media

The media used throughout was RPMI 1640 supplemented with 100 units/ml of penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% fetal bovine serum (all from Gibco, Grand Island, NY). rIL-2 was a kind gift from Hoffman LaRoche Laboratories (Nutley, NJ).

RESULTS

CD8-Mediated Suppression of RT Activity

The ability of $CD8^+$ cells from SIV-infected rhesus macaques to inhibit the replication of exogenously added SIV in vitro was previously demonstrated [6]. As seen in Figure 1, we have been able to reproduce these results in that $CD8^+$ cells from the SIV/SMM infected rhesus were able to clearly inhibit the replication of the exogenously added SIV/SMM. Likewise, the $CD8^+$ cells from the uninfected rhesus were not able to suppress viral replication. Furthermore, as shown in Figure 1, the $CD8^+$ cells from the seropositive mangabey were also able to inhibit the replication of SIV. Thus, $CD8^+$ T cells from the chronically infected yet asymptomatic sooty mangabeys have the ability to inhibit RT activity in vitro.

SIV is easily isolated in vitro from cells derived from sooty mangabeys. Normally, this is accomplished by co-culturing these mangabey cells with human PHA blasts. It is possible that these xenogeneic cultures may abrogate CD8-mediated suppression of viral replication. We wanted to determine if $CD8^+$ cells were able to inhibit the replication of endogenous virus found in the PBMC of mangabeys. Thus, we measured endogenous RT activity in mangabey PBMC in the absence of human cells. Figure 2, which shows the results of one such experiment, is representative of data from four additional experiments. Whole PHA blasts or CD8 depleted PHA blasts were cultured as described in the Materials and Methods section; however, in this case, no

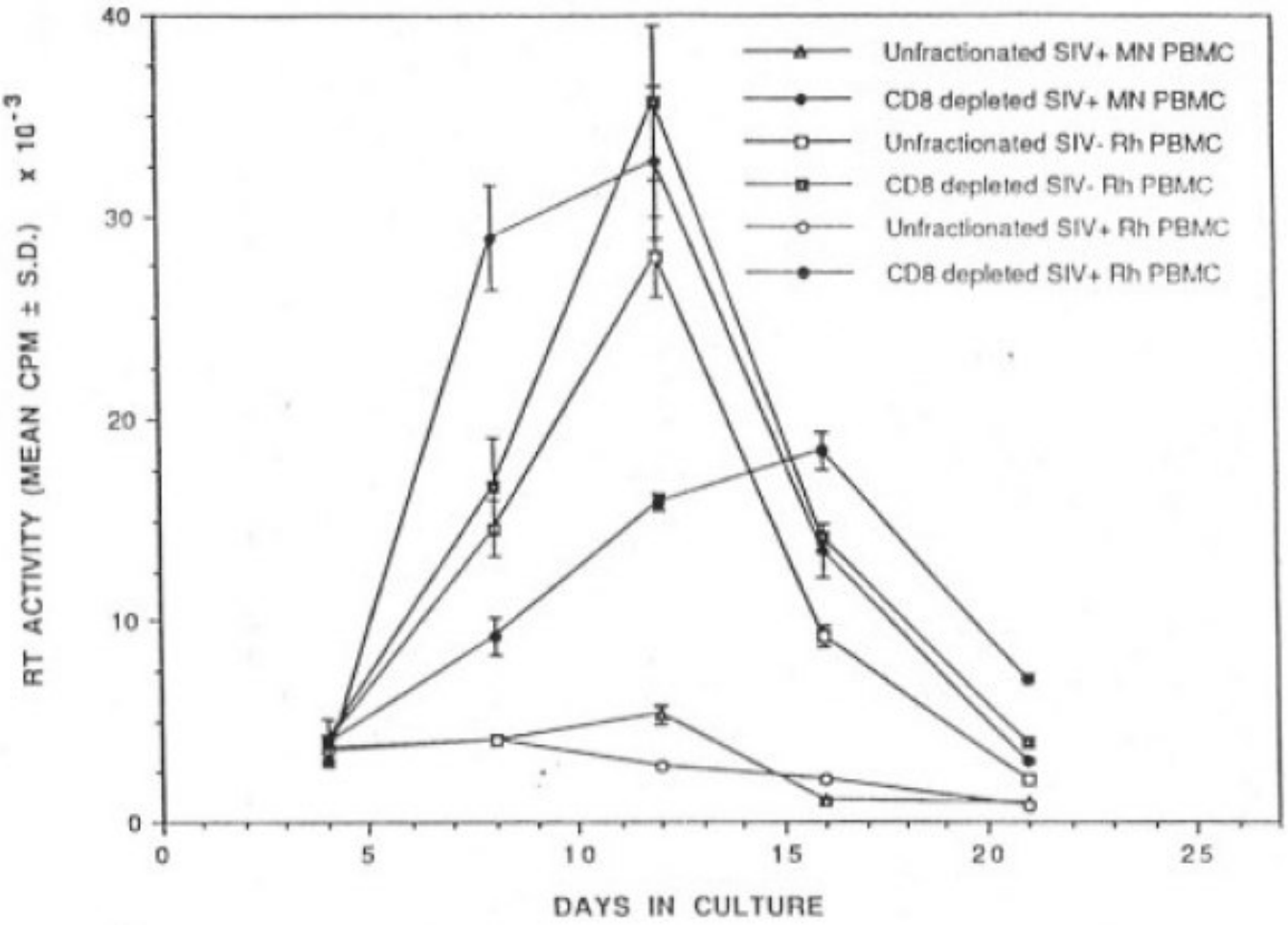

Fig. 1. Inhibition of RT activity by $CD8^+$ cells. PBMC from a seropositive mangabey were cultured in the presence of 0.2% PHA for three days. An aliquot of cells from each of these cultures was CD8 depleted. Exogenous SIV/SMM was added to each of the samples and cultured for 21 days. RT activity was monitored every four days. The data is expressed as the mean of triplicate cultures ± SD.

exogenous virus was added to the culture. As seen in Figure 2, the RT activity was higher in the cultures which were CD8 depleted, indicating that mangabey $CD8^+$ T cells have the ability to inhibit the replication of virus which is found endogenously in the PBMC of mangabeys.

CD8-Mediated Suppression of Viral Replication in an EBV Transformed CD4 Expressing Cell Line

The fact that $CD8^+$ cells from infected sooty mangabeys have the ability to inhibit viral replication suggests that perhaps this immune mechanism may play an important role in vivo. Since the mangabeys remain chronically infected with SIV, they provide a unique opportunity to study the mechanisms behind this phenomenon. EBV transformed B cells from an SIV infected mangabey were established in our laboratory. These cells were trans-

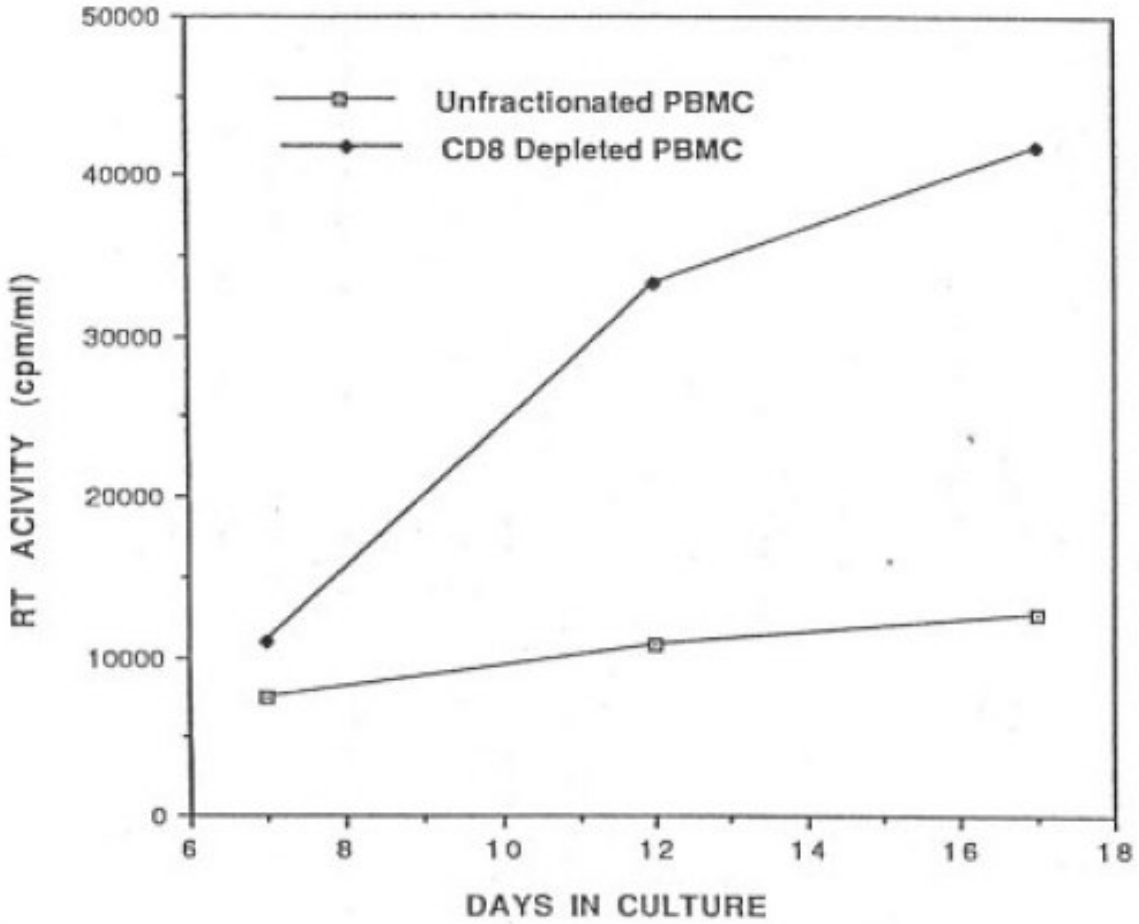

Fig. 2. Inhibition of endogenous virus replication by mangabey $CD8^+$ cells. PBMC from a seropositive mangabey were cultured in the presence of 0.2% PHA for three days. An aliquot of cells from this culture was CD8 depleted. RT activity from the whole PBMC culture and the CD8 depleted culture was monitored on days 7, 12, and 17. The data shown in representative of four additional experiments performed at the same time.

fected with the human CD4 gene. The detailed characterization of these cells is currently in progress. Figure 3 shows that these cells are expressing the CD4 molecule on their surface. Although it appears that most of the cells are expressing the CD4 gene, the overall mean density of expression of this cell surface marker is low. Currently, we are in the process of subcloning these cells in order to isolate a high density CD4 expressing clone. Nonetheless, as seen in Figure 4, these cells are able to replicate both SIV and HIV-2. Furthermore, the addition of autologous $CD8^+$ cells inhibits not only SIV/SMM but also HIV-2 replication in this cell line. This suggests that the epitopes involved in the recognition of the target cells for CD8-mediated suppression are shared by both SIV and HIV-2 infected cells. In light of the genomic homology between HIV-2 and SIV at the DNA level, these data are not surprising [3]. In the future we hope to exploit this autologous $CD4^+$ cell

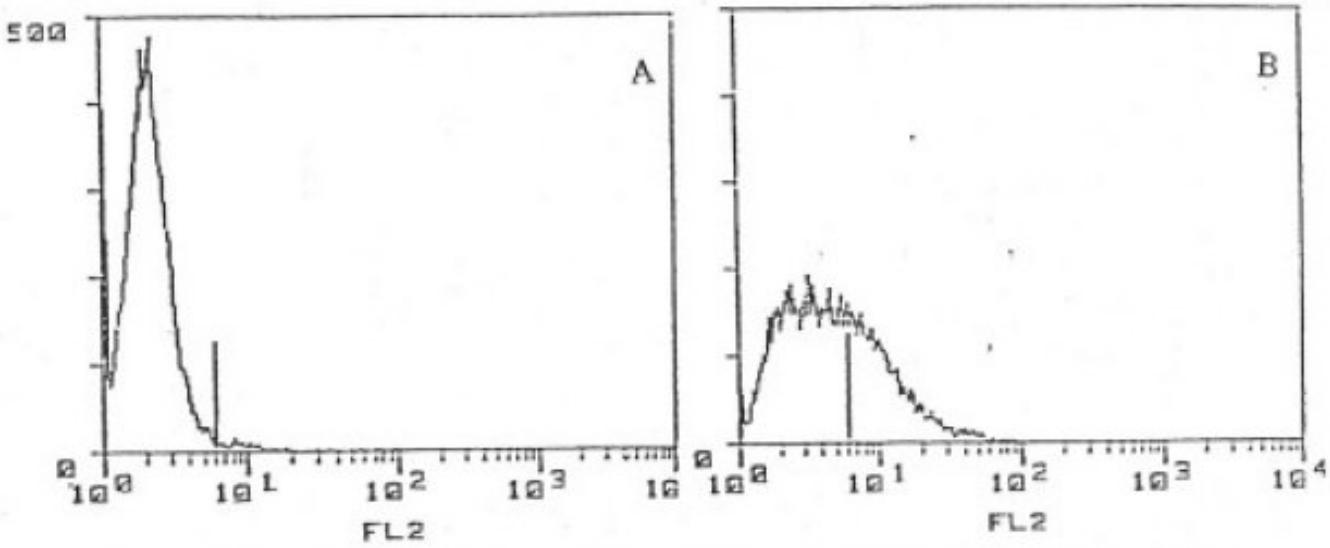

Fig. 3. Expression of CD4 on the surface of the FEc1 cell line. FEc1 cells were incubated with either fluorescinated goat anti mouse Ig (A) or fluorescinated Leu-3a (B) and analyzed by FMF. Although the density of the CD4 molecule on the surface of the cells is low, the entire peak in B appears to shift to the right (as compared with the negative control in A), indicating expression of CD4 on most of the cells.

line in order to further characterize the antigen specificity and mechanism of CD8-mediated suppression and investigate CTL activity in SIV infected mangabeys.

DISCUSSION

CD8-mediated suppression of viral replication in HIV-infected human subjects, as well as SIV-infected rhesus macaques, has previously been demonstrated [6,22]; however, the importance of this mechanism is tempered by the fact that infected human subjects and rhesus monkeys invariably die of AIDS. Mangabeys, on the other hand, are chronically infected with SIV, without any clinical consequences. Thus, the finding that $CD8^+$ cells from mangabeys can inhibit viral replication suggests that perhaps there is some in vivo relevance to these findings. This is particularly true since we have previously documented the prominent role of $CD8^+$ cells in the immune system of sooty mangabeys [11,14]. In addition, the in vivo frequency of circulating $CD8^+$ cells in the PBMC of a majority of rhesus or pigtailed macaques experimentally infected with SIV/SMM drops precipitously prior to the death of the animals. The mechanisms behind the abrogation of CD8-mediated immunity in rhesus and humans have yet to be elucidated. Certainly, it is not inconceivable that autoimmunity may play a role in such a

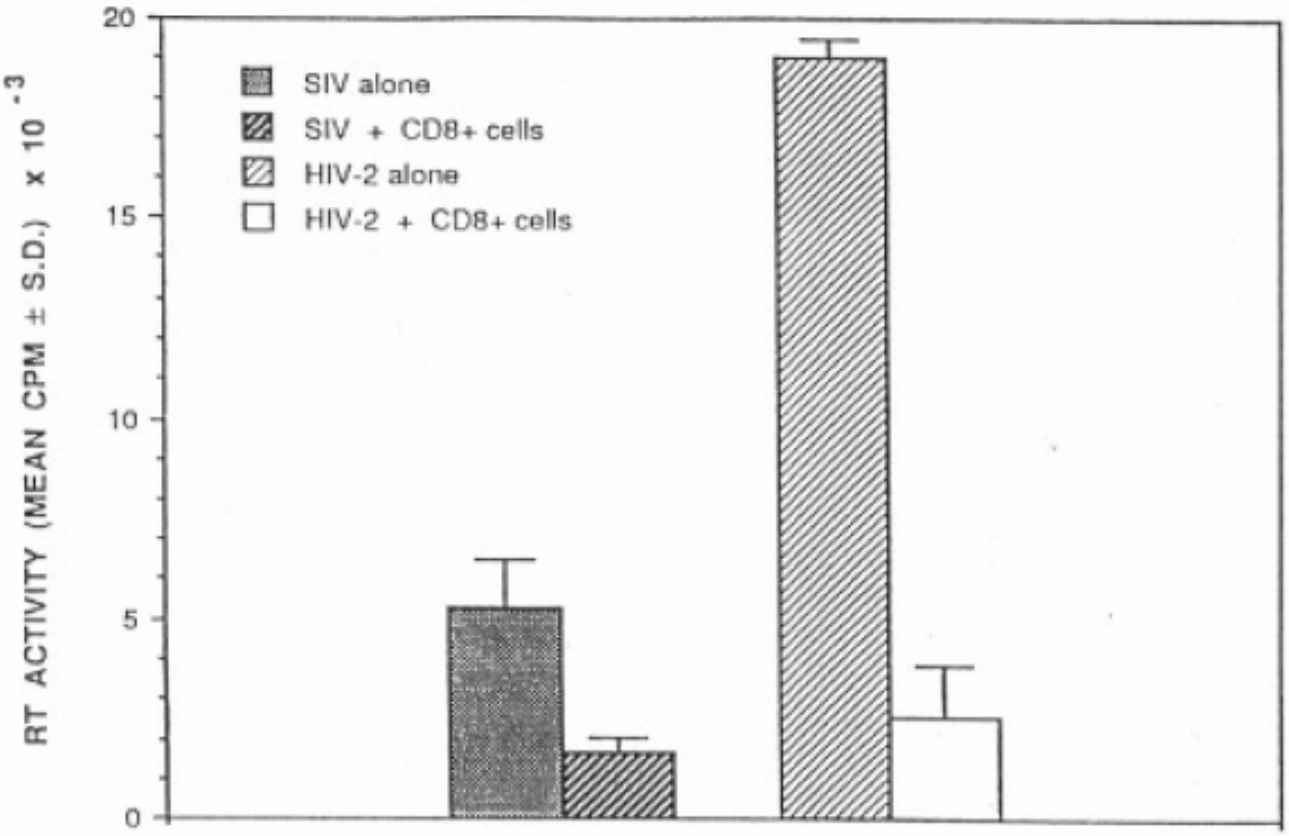

Fig. 4. Inhibition of SIV/SMM and HIV-2 replication in FEC1 cells by autologous $CD8^+$ cells. PBMC from the seropositive mangabey FEC were cultured in the presence of 0.2% PHA for three days. Aliquots of these cells were added to cultures containing FEC1 cells and either HIV-2 or SIV/SMM. RT activity was determined after seven days in culture. The data is expressed as the mean of triplicate cultures ± SD.

process. Indeed, antilymphocyte antibodies in the serum of AIDS patients have been described [7]. If such is the case, however, then how do mangabeys avoid such a fate? Perhaps it is the chronicity of the natural infection of SIV in mangabeys that leads to the development of tolerance to the autoimmune-producing viral epitopes. Mangabeys may actually acquire SIV infection at or before birth, which would lead to tolerance.

The specificity and molecular mechanisms of CD8-mediated suppression of viral replication have yet to be elucidated. To address this issue, our laboratory has established an EBV transformed cell line from an SIV infected mangabey in our colony. The transfection of these cells with the human CD4 gene makes available an autologous cell line which is SIV/SMM replication-competent. It is reasoned that FEc1 cell line or similar strategies will help to dissect the mechanism involved in this phenomenon. The data presented in this report show that autologous $CD8^+$ cells are able to inhibit the replication

of SIV in these cells. Furthermore, the antigen specificity of CD8-mediated suppression extends to HIV-2. Currently, experiments are in progress to test whether this antigen crossreactivity also holds true for the evolutionarily more distant HIV-1. In addition, we plan to use the FEc1 cell line to examine the role of $CD8^+$ cells on viral regulatory molecules. That is, do $CD8^+$ cells mediate suppression at the level of the induction of virus replication?

Phenotypic data may indicate that CD8-mediated suppression is classical CTL activity [18]. In the mangabey, NK and LAK activity is mediated by cells that do not express conventional NK and LAK cell surface markers [14]. Thus, we do not rule out the possibility that immunosurveillance may be involved. It may be that mangabeys have developed an immunosurveillance system which relies more heavily on a population of NK cells (which are normally found to a lesser extent in humans and rhesus macaques). Infection with HIV and SIV may enhance the frequency of these cells, which, in addition to exhibiting conventional NK activity, are able to inhibit viral replication. This might explain the high frequency of activated $CD8^+$ cells in the PBMC of mangabeys. The possibility that CTL activity is the basis of this inhibition of virus replication in vitro is certainly important to consider and is being actively pursued in our laboratory. Attempts are also being made to use the SIV infected FEc1 cell line to measure SIV-specific CTL and CMI in the PBMC of the sooty mangabey, FEc.

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